ENDOGENOUS AND EXOGENOUS GLUCOCORTICOIDS IN OBESITY AND STRESS-RELATED DISEASES

NESUT SAVAŞ

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Endogenous and Exogenous Glucocorticoids in Obesity and Stress-Related Diseases

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in obesitas en stressgerelateerde ziekten

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Chapter 1

General Introduction

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Oxford: Academic Press; 2019.

Obesity in the Clinic Room: Diagnostics First, Followed by Effective Treatment (Obesitas in de spreekkamer)
van der Valk E.S., Savas M., Burgerhart J.S., de Vries M., van den Akker E.L.T., van Rossum E.F.C.
Ned Tijdschr Geneeskd 2017; 161:D2310.

Stress and Obesity: Are There More Susceptible Individuals?
van der Valk E.S., Savas M., van Rossum E.F.C.
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A Comprehensive Diagnostic Approach to Detect Underlying Causes of Obesity in Adults
van der Valk E.S., van den Akker E.L.T., Savas M., Kleinendorst L., Visser J.A., Van Haelst M.M., Sharma A.M., van Rossum E.F.C.
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General Introduction

"*Experiments on rats show that if the organism is severely damaged by acute non-specific nocuous agents (…) a typical syndrome appears, the symptoms of which are independent of the nature of the damaging agent or the pharmacological type of the drug employed, and represent rather a response to damage as such.*"
- Hans Selye (*Nature*, 1936)

The discovery of the general stress response and its effects in organisms is typical for many great findings in that serendipity was of the essence. Endocrinologist Hans Selye recurrently injected rats with a cow ovarian extract to find new female sex hormones. Upon pathological examination, he discovered that the animals had developed enlarged adrenal glands, and gastrointestinal ulcers whereas immunological tissues as the thymus, spleen, and lymph glands had become smaller [(1)]. Subsequent experiments in which the rats were exposed to other (non-hormonal) agents or conditions such as cold or excessive involuntary exercise yielded however similar findings. This led to the hypothesis that organisms exert a non-specific response to diverse stimuli which he labelled as the "general adaptation syndrome" [(1)]. These "non-specific nocuous agents" would later be termed "stressors" whereas the typical syndrome is nowadays known as the stress response. This response is mediated by a hormonal symphony orchestrated by the hypothalamus-pituitary-adrenal (HPA) axis. Upon activation, it induces the secretion of glucocorticoids from the adrenal glands which in turn can directly or indirectly affect practically every element of an organism.

1. Glucocorticoids

1.1 Endogenous glucocorticoids

The secretion of glucocorticoids is under neurohormonal control of the HPA axis (see Figure 1). This class of steroid hormones, with the stress hormone cortisol being the most important, are produced in the adrenal glands. The primary signal for its secretion is generated in specialized neuronal cells in the paraventricular nucleus of the hypothalamus. These cells secrete among others the corticotropin-releasing hormone (CRH) which is transported down to the anterior pituitary gland to stimulate the secretion of adrenocorticotropic hormone (ACTH). From there, ACTH enters the circulation to start its journey with the adrenal glands as its main destination. As it hits the adrenal glands it finally results in the synthesis and secretion of glucocorticoids from cells in the zona fasciculata located in the adrenal cortex. Negative feedback of glucocorticoids on the hypothalamus and pituitary gland reduces the secretion of CRH and ACTH in order to facilitate termination of glucocorticoid secretion [(2)]. Glucocorticoids are essential in mental

and physical health giving their involvement in reproduction, metabolism, inflammation, cognition, and many other processes. Its relevance also becomes evident giving the presence of the glucocorticoid receptor in many tissues [3]. This widespread engagement makes glucocorticoids highly suitable effectors by default in regulating adaption to changing situations.

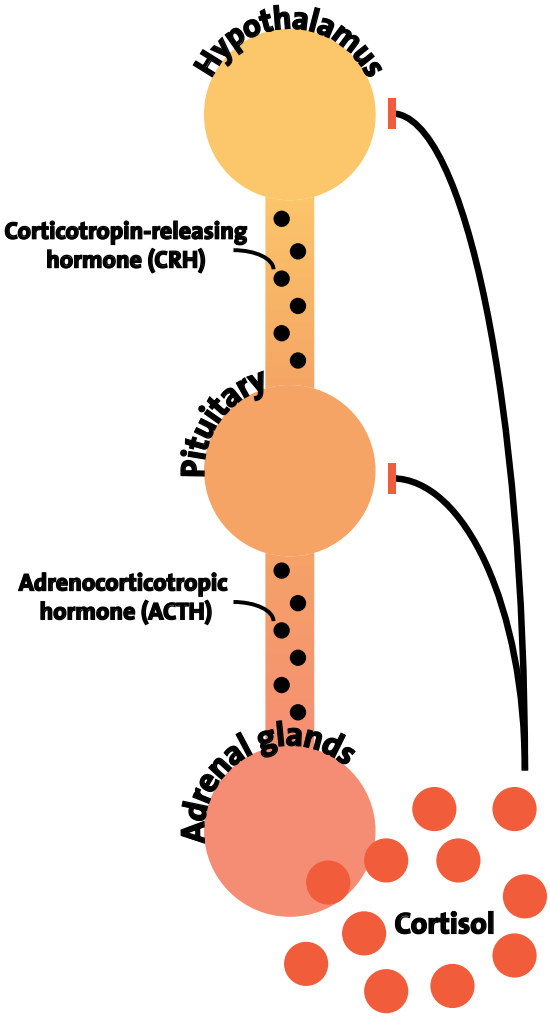

Figure 1: The hypothalamus-pituitary-adrenal axis.

The secretion of glucocorticoids, cortisol as the most important hormone, is under the control of the hypothalamus-pituitary-adrenal (HPA) axis. The primary signal originates from the hypothalamus and is executed by secretion of the corticotrophin-releasing hormone which subsequently prompts secretion of adrenocorticotrophic hormone (ACTH) from the adenohypophysis (i.e. anterior pituitary of the pituitary gland). ACTH subsequently stimulates the adrenal glands to secrete cortisol which in turn inhibits the hypothalamus and pituitary gland and thus negatively influences the HPA axis activation.

1.2 Glucocorticoid receptor

The glucocorticoid action does in essence dependent on the activation of the glucocorticoid receptor. This nuclear receptor is encoded by the *NR3C1* gene located on chromosome 5 which consists of one noncoding and eight coding exons (see Figure 2). Inactive glucocorticoid receptors remain in the cytoplasm coupled in a complex of heat shock proteins and kinases. Activation of the receptor occurs with binding to glucocorticoids after which it translocates to the nucleus. There it can up- and downregulate the transcription of certain genes. These transactivational and transrepressional effects are carried out in various ways including binding to specific glucocorticoid response elements and interaction with other transcription factors [4]. In addition to genomic actions, glucocorticoids can also induce rapid nongenomic effects through changes in intracellular signaling and other mechanisms [5].

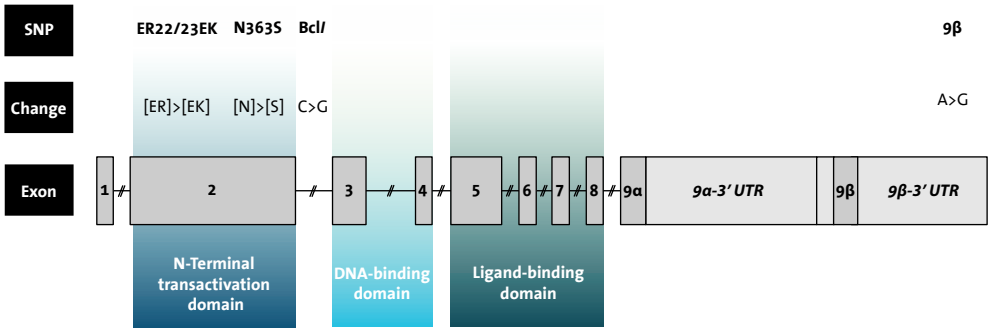

Figure 2: The glucocorticoid receptor and functional single nucleotide polymorphisms.

The BclI and N363S polymorphisms of the NR3C gene are associated with relatively increased glucocorticoid sensitivity, whereas the ER22/23EK and 9β variants are linked to relative glucocorticoid resistance. Missense mutations occur with the N363S and ER22/23EK polymorphisms where the amino acid asparagine (N) is changed to serine (S) in the former and arginine (R) to lysine (K) in the latter. Abbreviations: *A*, nucleotide adenine; *C*, nucleotide cytosine; *G*, nucleotide guanine; *SNP*, single nucleotide polymorphism; *UTR*, untranslated region. *Adapted from Savas and van Rossum. Encyclopedia of Endocrine Diseases, Second Edition, 2019.*

Increased transactivational activity is generally held responsible for cardiometabolic adverse events [6] as is often observed in patients with autonomous overproduction of glucocorticoids (i.e. Cushing's syndrome). The transrepressional activities of glucocorticoids are on the other hand accountable for immunosuppressive and anti-inflammatory effects of glucocorticoids [6]. Many glucocorticoid receptor polymorphisms have been found in the past decades. A handful of functional variants has however been linked to altered glucocorticoid receptor sensitivity and eventually distinct glucocorticoid effects.

1.2.1 Glucocorticoid receptor hypersensitive variants

The most abundant polymorphism involves an intronic nucleotide change in intron 2 and has been linked to an increased glucocorticoid sensitivity. Carriers of this Bcl*I* variant were more likely to have central adiposity [7,8], insulin resistance [9], and major depressive disorder [10] which is in common with higher glucocorticoid action. Another hypersensitive variant concerns the N363S polymorphism which results in a missense mutation due to a nucleotide change in exon 2. Earlier studies have shown an increased transactivational activity with isolated peripheral blood mononuclear cells [11] corresponding to clinical observations as enhanced cortisol suppression with low-dose 0.25 mg dexamethasone suppression test [12] and cardiometabolic features as increased body mass index [12,13] and dyslipidemia [14].

1.2.2 Glucocorticoid receptor resistant variants

Two functional polymorphisms have been associated with a relative glucocorticoid resistance. The ER22/22EK variant concerns two linked nucleotide changes in adjacent codons resulting in the change of the second amino acid from arginine to lysine. This yields glucocorticoid receptors with less transactivating activity [15] as observed with functional assays [11]. Another nucleotide change in exon 9β increases the stability of the splice variant glucocorticoid receptor β [16], which acts as a dominant-negative inhibitor of the active receptor isoform, and decreases the transrepressional glucocorticoid activity. The ER22/23EK and 9β polymorphisms have been linked to a smaller waist circumference [17,18] and beneficial metabolic profile [18,19]. Moreover, individuals with the 9β polymorphisms were found to have elevated inflammatory markers as well as increased incidence of cardiovascular disease possibly due to a pro-inflammatory status [20].

1.3 Glucocorticoid measurements

Glucocorticoids are secreted and can be quantified in different bodily fluids. Secretion of cortisol follows a circadian and pulsatile rhythm with the lowest levels at midnight and the highest concentrations in the early morning [21]. Besides the natural fluctuations, physical and mental stressors [22] as well as conditions like poorly controlled diabetes mellitus, polycystic ovary syndrome, or excessive alcohol consumption can also alter cortisol levels [23]. Blood, saliva, and urine have traditionally been used as matrices for cortisol assessment. The current guideline of the Endocrine Society for diagnosis of Cushing's syndrome recommend screening with dexamethasone suppression test, late-night salivary cortisol, and 24-hr urinary free cortisol measurements [24]. These tests are convenient in the assessment of relatively short-term cortisol exposure with periods ranging from seconds to minutes (with serum and saliva) and hours to days (with urine) depending on the duration of sample collection (see Figure 3). Diagnostic screening usually

also requires repeated testing and can be compromised by several factors such as patient compliance (e.g. collection of urine output, intake of dexamethasone), and interference with other commonly used drugs [24]. Moreover, physical or mental disabilities or living remote from the clinic site could also complicate the diagnostic process.

An increasing amount of research is being conducted regarding scalp hair as a novel instrument for cortisol assessment. Scalp hair grows with approximately one cm per month which provides the opportunity to assess long-term cortisol exposure over months to years depending on hair sample length [25]. It can easily be collected at every moment and stored at room temperature without the need to impose patients to certain instructions or limitations. Hair cortisol concentrations have been investigated in a wide range of clinical settings concerning not only patients with Cushing's syndrome [26], but also obesity [27], mental health disorders [28], and many other stress-related diseases [29]. Earlier studies performed immunoassays to assess hair cortisol concentrations [30], nowadays we can quantify cortisol as well as the inactive variant cortisone in scalp hair with high sensitivity and specificity by using state-of-the-art liquid chromatography-tandem mass spectrometry (LC-MS/MS) [31].

Exogenous glucocorticoids

Glucocorticoids induce potent anti-inflammatory and immunomodulatory effects as was also noticed in the experimental rats of Selye. The first reported application of exogenous glucocorticoid, isolated from bovine adrenal glands, was in a young female with rheumatoid arthritis at the Mayo Clinic in 1948 [32]. Since then the development of synthetic corticosteroids as well as their user range has shown a remarkable evolution.

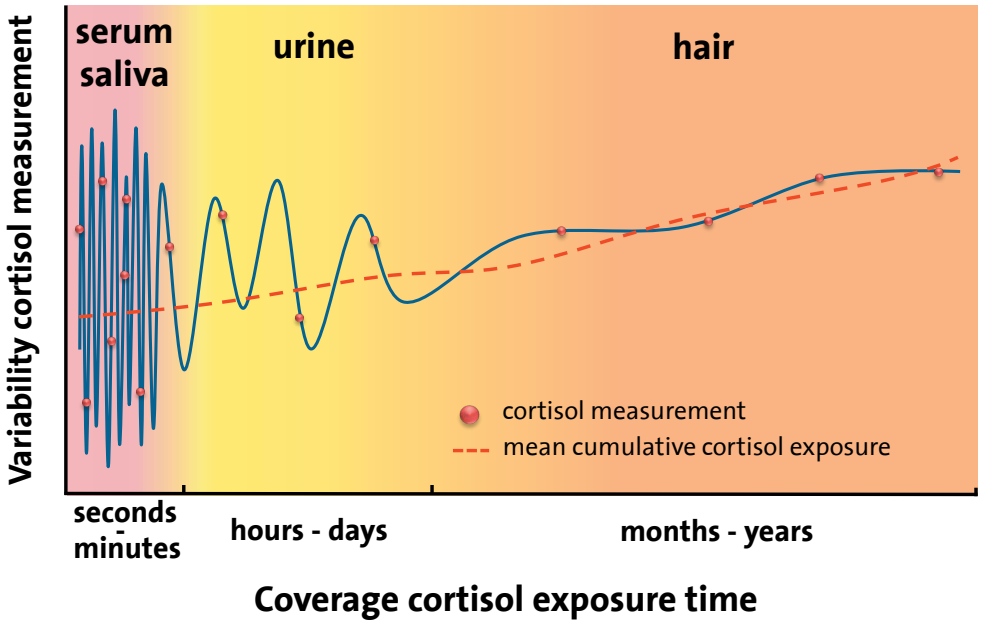

Figure 3: Impression of the variability and coverage of traditional matrices and scalp hair for assessment of cortisol concentrations.

The red dotted line represents the actual increase in cortisol concentrations over time in a fictional situation. Scalp hair cortisol analysis allows assessment of cortisol changes over a prolonged period of time whereas the traditional matrices (serum, saliva, and urine) cover a short time window of cortisol exposure.

Numerous synthetic corticosteroid compounds have been developed over time with different glucocorticoid and mineralocorticoid activity as well as duration of action. The availability of corticosteroids in different administration forms makes them a valuable addition in the treatment of a broad spectrum of inflammatory diseases. The systemic administration forms are applied by oral and parenteral route, whereas the local corticosteroids can be delivered by inhalation, nasal sprays, dermal applications, ocular and otological droplets, and various other ways. In the Netherlands, there were more than 13 million corticosteroid formulations prescribed last year according to data from the Dutch National Health Care Institute [33]. Some longitudinal population-based studies and large cohort studies are showing an increasing number of corticosteroid users over time. The prevalence of long-term (at least three months) systemic corticosteroid use in the United Kingdom increased with one third from 0.6% to 0.8% over a time span of twenty years [34]. Inhaled corticosteroids were for instance used by 1.1% and o.8% in respectively the United Kingdom and The Netherlands in the early 90s and this proportion of users rose to 1.7% and 1.4% in 1996 [35].

1.5 Adverse effects of glucocorticoids

The immune-dampening effects of corticosteroids are much appreciated in many inflammatory diseases. Use of corticosteroids is unfortunately often accompanied by adverse effects which are often not limited to specific tissues. The downside of supraphysiological exposure to glucocorticoids becomes clear with endogenous Cushing's syndrome. These patients are prone to develop cardiometabolic disturbances, cardiovascular events, neuropsychiatric pathologies, and a wide range of other comorbidities [36]. Endogenous Cushing's syndrome has however an extremely low incidence of less than five in million individuals annually. The main cause of Cushing's syndrome is the administration of exogenous glucocorticoids. Serious adverse events of corticosteroids occur when the agents enter the circulation as it ensues with the use of systemic formulations. Systemic availability of exogenous glucocorticoids suppresses the HPA axis which could lead to decreased glucocorticoid secretion and eventually adrenal gland atrophy and adrenal insufficiency. The relatively high potency and long half-life of the synthetic variants make this sequela even more likely [37]. A large meta-analysis demonstrated that users of oral or intra-articular corticosteroids had approximately 50% risk of developing adrenal insufficiency [38]. Systemic corticosteroid users also are more likely to develop serious Cushingoid-like features such as abdominal adiposity, diabetes mellitus, hypertension, dyslipidemia, mood disorders, and osteoporosis [39]. Interestingly, the most frequently observed adverse event was corticosteroid-induced lipodystrophy (i.e. abnormal accumulation of fat mass such as moon face) [40] and weight gain [41]. From patient's perspective, these were also reported as the most distressing event [40] and were mainly experienced as very bothersome [41]. Concerning serious corticosteroid-related adverse events, the focus has predominantly been put on the systemic forms although the majority of the prescriptions are for local corticosteroid types. One possible reason for this may be that health care providers assume that local corticosteroids can only induce 'minor' local adverse events as they do not enter the circulation. The previously mentioned meta-analysis showed however that users of locally applied corticosteroids, especially of the inhaled forms, also have a significantly increased risk of adrenal insufficiency hinting at systemic availability of these types [38]. It would therefore be of great clinical importance to assess whether the use of local corticosteroids is indeed associated with major corticosteroid-related adverse events.

2. Obesity

The World Health Organization has ranked overweight and obesity in the top 5 leading risk factors for mortality worldwide and as the third most important health risk in high-income countries [42]. The global prevalence of overweight and obesity has shown an impressive increase in the past decades [43] and is currently

responsible for more deaths than underweight [42]. Unhealthy nutrition and physical inactivity are commonly referred to as the obvious causes of obesity. Reversing obesity would seem relatively simple based on this, however, in practice patients with obesity do not always lose (sufficient) weight and/or maintain weight loss with a healthy lifestyle. The etiology of obesity is complex and includes weight-inducing as well as weight-maintaining factors covering a broad range of topics such as lifestyle, genetics, and hormonal composition. An attempt to categorize the main factors involved in obesity are listed in Figure 4.

cause	clinical signs and symptoms	examples
Lifestyle	• Unhealthy food intake • Lack of exercise • Average sleep <7 hours • Disturbed sleep • Snoring/apnea • Shift work • Alcohol abuse • Stress • Smoking cessation • Sociocultural background • Meal timing • Sedentary lifestyle	• Hypercaloric intake • Lack of exercise • Nocturnal eating • Obstructive sleep apnea • Repeated (very) low caloric diets with yoyo effect
Mental disorders	• Severe repeated binge-eating with or without inadequate compensation behavior • Depressive complaints	• Binge-eating disorder • Bulimia nervosa • Depression • Other specified feeding and eating disorders
Medication	• Weight increase related to initiation or dose increase of potentially weight-inducing drug(s)	• Anticonvulsants • Antidepressants • Antipsychotics • (Local) corticosteroids • Beta blockers • Diabetes drugs
Endocrine	• Acne • Hirsutism • Irregular menses • Acanthosis nigricans • Erectile dysfunction • Post-pregnancy • Menopause • Bradycardia • Muscle weakness • Cushingoid features • History of radiotherapy or severe head trauma	• Polycystic ovary syndrome • Hypogonadism • Post-pregnancy weight retention • Menopause • (Cyclic) Cushing's syndrome • Hypothyroidism • Growth hormone deficiency
(Mono-)genetic or syndromic	• Young age of onset • Hyperphagia • Red hair • Hypopigmentation • Exteme weight difference between family members • Dysmorphic features • Developmental delay • Autism or attention deficit disorder • Short stature • (Poly-)syndactyly • Retinal abnormalities • Severe myopia • Congenital deafness • Nephropathy	Defect or deficiency: • Melanocortin 4 receptor • Leptin (receptor) • Proopiomelanocortin • Prohormone convertase-1 • Prader-Willi syndrome • Bardet-Biedl syndrome • Albright syndrome • 16p11.2 deletion
Hypothalamic	• Cranial radiotherapy/ surgery • Head trauma • Neurological abnormalities • Hyperphagia • Decreased vision	• Post-radiation therapy • Post-surgery • Hypothalamic tumor • Malformation

Figure 4: Overview of potential causes of obesity.

Adapted from van der Valk et al. Obes Rev. 2019;20(6):795-804.

Obesity is often associated with metabolic syndrome components such as hypertension, dyslipidemia, and impaired glucose tolerance or diabetes mellitus. It has been proposed that glucocorticoid excess may play a role in the development of obesity since these comorbidities are also often observed in patients with hypercortisolism [44]. Previous studies investigating individuals with obesity have indeed found increased cortisol concentrations with traditional assessments of

short-term cortisol values [45,46]. Moreover, we reported significantly higher long-term cortisol concentrations, as measured in scalp hair, in individuals with obesity when compared to normal weight and overweight subjects [47] with a substantial part having levels even far above the applied cut-off for normal range values. The underlying etiology, direction, and magnitude of the association remain a topic of ongoing research, but it is a fact that individuals with obesity often encounter a multitude of physiological and psychological stressors that could induce and/or maintain a hypercortisolistic state. A thorough evaluation of potential weight-inducing and weight-maintaining factors should hence be a key principle in tackling obesity.

3. Stress-related diseases

Comorbidities of glucocorticoid excess are not limited to patients with Cushing's syndrome or obesity. Since activation of the stress system depends on stressors rather than specific conditions, it is possible that other chronic diseases, life-events, or ongoing stressful circumstances (e.g. work- or school-related) also activate the HPA axis and lead to higher secretion of cortisol. It would therefore be plausible that these individuals could develop glucocorticoid-related symptoms depending on genetic factors as well as various stress-related aspects such as the duration, intensity, and personal coping abilities. Since many of the serious comorbidities manifest after prolonged exposure to elevated glucocorticoid levels, hair cortisol analysis could provide more understanding of whether there is such an association in specific diseases and conditions of interest.

Aims and outline of thesis

Glucocorticoids are essential for survival and adaptation to changing situations. Too much glucocorticoids can however yield a variety of symptoms and comorbidities. In this thesis, we aimed to investigate the role of endogenous and exogenous glucocorticoids in various stress-related diseases with a special focus on obesity. We additionally evaluated the diagnostic accuracy of scalp hair glucocorticoids in the screening of Cushing's syndrome as well as the clinical application in obesity and stress-related conditions.

In **chapter 2** we assessed the prevalence of a comprehensive set of potential weight-inducing and weight-maintaining factors including the use of exogenous glucocorticoids in a cohort of adults with obesity. **Chapter 3** describes our findings from a systematic evaluation for systemic and local corticosteroid use in subjects with obesity compared to non-obese controls from two independent cohorts. In **chapter 4** we investigated the relationship between the use of different corticosteroid types with anthropometric features and metabolic syndrome in

the general population. We further zoomed into this association on the level of glucocorticoid receptor polymorphisms in **chapter 5**. In a population-based study, we also assessed the link between exogenous glucocorticoid use and the presence of mood and anxiety disorders as well as executive cognitive functioning as described in **chapter 6.** With respect to scalp hair glucocorticoids, we performed a multicenter, international, prospective, case-control study to investigate the diagnostic efficacy of scalp hair cortisol and cortisone in the screening of endogenous Cushing's syndrome of which the results are presented in **chapter 7**. Giving the link between glucocorticoids and adiposity, we assessed the association between scalp hair cortisol and cortisone with anthropometrics and body composition parameters in **chapter 8**. Here we also present the results of our prospective longitudinal combined lifestyle intervention with cognitive behavioral therapy in individuals with obesity in which we among others investigated whether weight loss was associated with changes in scalp hair glucocorticoids and if use of systemic corticosteroids could impact the efficacy of the intervention. We further assessed the link between scalp hair cortisol with cardiometabolic and psychological outcomes in patients with Turner syndrome and erythropoietic protoporphyria in respectively **chapter 9** and **chapter 10**. We also performed a prospective comparative cohort study to investigate school-related stress as induced by raising performance standards in first-year medical students. **Chapter 11** describes the difference in academic performance as well as psychological and biological stress levels in these students. In **chapter 12** we have investigated potential school-related stress in children entering third grade. We measured long-term cortisol exposure in scalp hair before and after school entry and assessed whether changes were mediated by individual characteristics as temperament, academic skills, and executive functioning. In the final **chapter 13**, we performed functional magnetic resonance imaging to assess the link between long-term cortisol exposure as quantified with scalp hair cortisol and neural correlates in trauma-exposed female police officers with and without posttraumatic stress disorder.

References

1. Selye H. A Syndrome produced by Diverse Nocuous Agents. *Nature*. 1936;138(3479):32-32.
2. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med*. 1995;332(20):1351-1362.
3. Dezso Z, Nikolsky Y, Sviridov E, Shi W, Serebriyskaya T, Dosymbekov D, Bugrim A, Rakhmatulin E, Brennan RJ, Guryanov A, Li K, Blake J, Samaha RR, Nikolskaya T. A comprehensive functional analysis of tissue specificity of human gene expression. *BMC Biol*. 2008;6:49.
4. Gross KL, Cidlowski JA. Tissue-specific glucocorticoid action: a family affair. *Trends Endocrinol Metab*. 2008;19(9):331-339.
5. Song IH, Buttgereit F. Non-genomic glucocorticoid effects to provide the basis for new drug developments. *Mol Cell Endocrinol*. 2006;246(1-2):142-146.
6. Stahn C, Lowenberg M, Hommes DW, Buttgereit F. Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. *Mol Cell Endocrinol*. 2007;275(1-2):71-78.
7. Rosmond R, Chagnon YC, Holm G, Chagnon M, Perusse L, Lindell K, Carlsson B, Bouchard C, Bjorntorp P. A glucocorticoid receptor gene marker is associated with abdominal obesity, leptin, and dysregulation of the hypothalamic-pituitary-adrenal axis. *Obes Res*. 2000;8(3):211-218.
8. Buemann B, Vohl MC, Chagnon M, Chagnon YC, Gagnon J, Perusse L, Dionne F, Despres JP, Tremblay A, Nadeau A, Bouchard C. Abdominal visceral fat is associated with a BclI restriction fragment length polymorphism at the glucocorticoid receptor gene locus. *Obes Res*. 1997;5(3):186-192.
9. Weaver JU, Hitman GA, Kopelman PG. An association between a Bc1I restriction fragment length polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women. *J Mol Endocrinol*. 1992;9(3):295-300.
10. van Rossum EF, Binder EB, Majer M, Koper JW, Ising M, Modell S, Salyakina D, Lamberts SW, Holsboer F. Polymorphisms of the glucocorticoid receptor gene and major depression. *Biol Psychiatry*. 2006;59(8):681-688.
11. Russcher H, Smit P, van den Akker EL, van Rossum EF, Brinkmann AO, de Jong FH, Lamberts SW, Koper JW. Two polymorphisms in the glucocorticoid receptor gene directly affect glucocorticoid-regulated gene expression. *J Clin Endocrinol Metab*. 2005;90(10):5804-5810.
12. Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, Grobbee DE, Brinkmann AO, De Jong FH, Lamberts SW. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. *J Clin Endocrinol Metab*. 1998;83(1):144-151.
13. Lin RC, Wang WY, Morris BJ. High penetrance, overweight, and glucocorticoid receptor variant: case-control study. *BMJ*. 1999;319(7221):1337-1338.

14. Lin RC, Wang XL, Morris BJ. Association of coronary artery disease with glucocorticoid receptor N363S variant. *Hypertension*. 2003;41(3):404-407.
15. Russcher H, van Rossum EF, de Jong FH, Brinkmann AO, Lamberts SW, Koper JW. Increased expression of the glucocorticoid receptor-A translational isoform as a result of the ER22/23EK polymorphism. *Mol Endocrinol*. 2005;19(7):1687-1696.
16. Derijk RH, Schaaf MJ, Turner G, Datson NA, Vreugdenhil E, Cidlowski J, de Kloet ER, Emery P, Sternberg EM, Detera-Wadleigh SD. A human glucocorticoid receptor gene variant that increases the stability of the glucocorticoid receptor beta-isoform mRNA is associated with rheumatoid arthritis. *J Rheumatol*. 2001;28(11):2383-2388.
17. van Rossum EF, Voorhoeve PG, te Velde SJ, Koper JW, Delemarre-van de Waal HA, Kemper HC, Lamberts SW. The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. *J Clin Endocrinol Metab*. 2004;89(8):4004-4009.
18. Syed AA, Irving JA, Redfern CP, Hall AG, Unwin NC, White M, Bhopal RS, Weaver JU. Association of glucocorticoid receptor polymorphism A3669G in exon 9beta with reduced central adiposity in women. *Obesity (Silver Spring)*. 2006;14(5):759-764.
19. van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, Grobbee DE, de Jong FH, van Duyn CM, Pols HA, Lamberts SW. A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. *Diabetes*. 2002;51(10):3128-3134.
20. van den Akker EL, Koper JW, van Rossum EF, Dekker MJ, Russcher H, de Jong FH, Uitterlinden AG, Hofman A, Pols HA, Witteman JC, Lamberts SW. Glucocorticoid receptor gene and risk of cardiovascular disease. *Arch Intern Med*. 2008;168(1):33-39.
21. Clow A, Hucklebridge F, Stalder T, Evans P, Thorn L. The cortisol awakening response: more than a measure of HPA axis function. *Neurosci Biobehav Rev*. 2010;35(1):97-103.
22. Dickerson SS, Kemeny ME. Acute stressors and cortisol responses: a theoretical integration and synthesis of laboratory research. *Psychol Bull*. 2004;130(3):355-391.
23. Chabre O. The difficulties of pseudo-Cushing's syndrome (or "non-neoplastic hypercortisolism"). *Ann Endocrinol (Paris)*. 2018;79(3):138-145.
24. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab*. 2008;93(5):1526-1540.
25. Sauve B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clin Invest Med*. 2007;30(5):E183-191.
26. Hodes A, Meyer J, Lodish MB, Stratakis CA, Zilbermint M. Mini-review of hair cortisol concentration for evaluation of Cushing syndrome. *Expert Rev Endocrinol Metab*. 2018;13(5):225-231.
27. Jackson SE, Kirschbaum C, Steptoe A. Hair cortisol and adiposity in a population-based sample of 2,527 men and women aged 54 to 87 years. *Obesity (Silver Spring)*. 2017;25(3):539-544.

28. Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, van Rossum EF. Hair cortisol, stress exposure, and mental health in humans: a systematic review. *Psychoneuroendocrinology*. 2013;38(8):1220-1235.
29. Wester VL, van Rossum EF. Clinical applications of cortisol measurements in hair. *Eur J Endocrinol*. 2015;173(4):M1-10.
30. Manenschijn L, Koper JW, Lamberts SW, van Rossum EF. Evaluation of a method to measure long term cortisol levels. *Steroids*. 2011;76(10-11):1032-1036.
31. Noppe G, de Rijke YB, Dorst K, van den Akker EL, van Rossum EF. LC-MS/MS-based method for long-term steroid profiling in human scalp hair. *Clin Endocrinol (Oxf)*. 2015;83(2):162-166.
32. Saenger AK. Discovery of the wonder drug: from cows to cortisone. The effects of the adrenal cortical hormone 17-hydroxy-11-dehydrocorticosterone (Compound E) on the acute phase of rheumatic fever; preliminary report. Mayo Clin Proc 1949;24:277-97. *Clin Chem*. 2010;56(8):1349-1350.
33. The National Health Care Institute: Diemen, The Netherlands. Genees- en hulpmiddelen Informatie Project (GIP) databank. Revised October 2019. https://www.gipdatabank.nl.
34. Fardet L, Petersen I, Nazareth I. Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. *Rheumatology (Oxford)*. 2011;50(11):1982-1990.
35. van Staa TP, Cooper C, Leufkens HG, Lammers JW, Suissa S. The use of inhaled corticosteroids in the United Kingdom and the Netherlands. *Respir Med*. 2003;97(5):578-585.
36. Pivonello R, Isidori AM, De Martino MC, Newell-Price J, Biller BM, Colao A. Complications of Cushing's syndrome: state of the art. *Lancet Diabetes Endocrinol*. 2016;4(7):611-629.
37. Nicolaides NC, Pavlaki AN, Maria Alexandra MA, Chrousos GP. Glucocorticoid Therapy and Adrenal Suppression. 2000.
38. Broersen LH, Pereira AM, Jorgensen JO, Dekkers OM. Adrenal Insufficiency in Corticosteroids Use: Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2015;100(6):2171-2180.
39. Fardet L, Kassar A, Cabane J, Flahault A. Corticosteroid-induced adverse events in adults: frequency, screening and prevention. *Drug Saf*. 2007;30(10):861-881.
40. Fardet L, Flahault A, Kettaneh A, Tiev KP, Genereau T, Toledano C, Lebbe C, Cabane J. Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion. *Br J Dermatol*. 2007;157(1):142-148.
41. Curtis JR, Westfall AO, Allison J, Bijlsma JW, Freeman A, George V, Kovac SH, Spettell CM, Saag KG. Population-based assessment of adverse events associated with long-term glucocorticoid use. *Arthritis Rheum*. 2006;55(3):420-426.
42. World Health Organization. Global health risks: mortality and burden of disease attributable to selected major risks. Geneva: World Health Organization.
43. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF, Abraham JP, Abu-Rmeileh NM, Achoki T, AlBuhairan FS, Alemu ZA,

Alfonso R, Ali MK, Ali R, Guzman NA, Ammar W, Anwari P, Banerjee A, Barquera S, Basu S, Bennett DA, Bhutta Z, Blore J, Cabral N, Nonato IC, Chang JC, Chowdhury R, Courville KJ, Criqui MH, Cundiff DK, Dabhadkar KC, Dandona L, Davis A, Dayama A, Dharmaratne SD, Ding EL, Durrani AM, Esteghamati A, Farzadfar F, Fay DF, Feigin VL, Flaxman A, Forouzanfar MH, Goto A, Green MA, Gupta R, Hafezi-Nejad N, Hankey GJ, Harewood HC, Havmoeller R, Hay S, Hernandez L, Husseini A, Idrisov BT, Ikeda N, Islami F, Jahangir E, Jassal SK, Jee SH, Jeffreys M, Jonas JB, Kabagambe EK, Khalifa SE, Kengne AP, Khader YS, Khang YH, Kim D, Kimokoti RW, Kinge JM, Kokubo Y, Kosen S, Kwan G, Lai T, Leinsalu M, Li Y, Liang X, Liu S, Logroscino G, Lotufo PA, Lu Y, Ma J, Mainoo NK, Mensah GA, Merriman TR, Mokdad AH, Moschandreas J, Naghavi M, Naheed A, Nand D, Narayan KM, Nelson EL, Neuhouser ML, Nisar MI, Ohkubo T, Oti SO, Pedroza A, Prabhakaran D, Roy N, Sampson U, Seo H, Sepanlou SG, Shibuya K, Shiri R, Shiue I, Singh GM, Singh JA, Skirbekk V, Stapelberg NJ, Sturua L, Sykes BL, Tobias M, Tran BX, Trasande L, Toyoshima H, van de Vijver S, Vasankari TJ, Veerman JL, Velasquez-Melendez G, Vlassov VV, Vollset SE, Vos T, Wang C, Wang X, Weiderpass E, Werdecker A, Wright JL, Yang YC, Yatsuya H, Yoon J, Yoon SJ, Zhao Y, Zhou M, Zhu S, Lopez AD, Murray CJ, Gakidou E. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384(9945):766-781.

44. Bjorntorp P, Rosmond R. Obesity and cortisol. *Nutrition*. 2000;16(10):924-936.
45. Al-Safi ZA, Polotsky A, Chosich J, Roth L, Allshouse AA, Bradford AP, Santoro N. Evidence for disruption of normal circadian cortisol rhythm in women with obesity. *Gynecol Endocrinol*. 2018;34(4):336-340.
46. Vicennati V, Pasqui F, Cavazza C, Garelli S, Casadio E, di Dalmazi G, Pagotto U, Pasquali R. Cortisol, energy intake, and food frequency in overweight/obese women. *Nutrition*. 2011;27(6):677-680.
47. Wester VL, Staufenbiel SM, Veldhorst MA, Visser JA, Manenschijn L, Koper JW, Klessens-Godfroy FJ, van den Akker EL, van Rossum EF. Long-term cortisol levels measured in scalp hair of obese patients. *Obesity (Silver Spring)*. 2014;22(9):1956-1958.

Chapter 2

Extensive Phenotyping for Potential Weight-Inducing Factors in an Outpatient Population With Obesity

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Abstract

Background: *Obesity has been associated with miscellaneous weight-inducing determinants. A comprehensive assessment of known obesity-related factors other than diet and physical activity within one cohort is currently lacking.*

Objectives: *To assess the prevalence of potential contributors to obesity and self-reported triggers for marked weight gain in an adult population with obesity and between obesity classes.*

Methods: *In this observational cohort study, we assessed 408 persons with obesity (aged 41.3 ± 14.2 years, BMI 40.5 ± 6.2 m2) visiting our obesity clinic. They were evaluated for use of weight-inducing drugs, hormonal abnormalities, menarcheal age, (high) birth weight, sleep deprivation, and obstructive sleep apnea syndrome (OSAS). We additionally assessed self-reported triggers for marked weight gain and performed genetic testing in patients suspected of genetic obesity.*

Results: *Nearly half of the patients were using a potentially weight-inducing drug, which was also the most reported trigger for marked weight gain. For the assessed hormonal conditions, a relatively high prevalence was found for hypothyroidism (14.1%), polycystic ovary syndrome (12.0%), and male hypogonadism (41.7%). A relatively low average menarcheal age (12.6 ± 1.8 years) was reported, whereas there was a high prevalence of a high birth weight (19.5%). Sleep deprivation and OSAS were reported in, respectively, 14.5 and 13.7% of the examined patients. Obesity class appeared to have no influence on the majority of the assessed factors. Of the genetically analyzed patients, a definitive genetic diagnosis was made in 3 patients (1.9%).*

Conclusions: *A thorough evaluation of patients with obesity yields a relatively high prevalence of various potentially weight-inducing factors. Diagnostic screening of patients with obesity could therefore benefit these patients by potentially reducing the social stigma and improving the outcomes of obesity treatment programs by tackling, where possible, the weight-inducing factors in advance.*

Introduction

In the last decades, there has been an evident increase worldwide in the prevalence of obesity [1]. This ominous trend brings along many health problems given the strong associations between adiposity and noncommunicable diseases. In addition to metabolic and cardiovascular diseases, obesity carries an increased risk of various cancer types, depression, and other illnesses compromising the quality of life.

The multifactorial etiology of obesity makes it difficult to find a long-lasting solution. Dietary composition and reduced physical activity, also acclaimed as the "big two" by Keith et al. [2], have always been of major concern regarding the epidemic and treatment of obesity. However, this approach tends to undervalue other factors also described to contribute to or at least maintain obesity. For instance, various genetic alterations have been found to induce obvious monogenic (e.g., melanocortin 4 receptor [MC4R] and pro-opiomelanocortin [POMC] mutations) or syndromic forms of obesity (e.g., Prader-Willi and Bardet-Biedl syndromes) or have been linked to non-syndromic obesity in which the onset and severity depend on interaction with the environment. Other relatively more prevalent factors that increase the risk of obesity are, for example, an early age at menarche, [3] a high birth weight, [4, 5] and various hormonal causes such as hypothyroidism, (endogenous or exogenous) Cushing's syndrome, and hypothalamic abnormalities [6]. Additionally, there are also diverse potentially modifiable weight-inducing factors such as the use of obesogenic drugs [7], and diminished sleep duration [8, 9], or factors for which the direction of association has not yet been fully understood or is bidirectional (e.g., low testosterone levels [10], polycystic ovary syndrome [PCOS] [11], and obstructive sleep apnea syndrome [OSAS] [12]).

Most experimental and observational studies regarding obesity usually highlight one particular factor. One of the targets of our multidisciplinary referral center for obesity is to systematically evaluate and identify those factors that could induce and/or maintain excess body weight in adults. Hence, the main purpose of this study was to extensively phenotype and assess multiple potential weight-inducing factors, as mentioned above, within our total obese cohort and stratified by adult obesity classes. Our secondary objectives were to evaluate the relationship with self-reported triggers for marked weight gain and to assess the yield of targeted genetic screening for obesity.

Participants and Methods

Study Population

Patients with obesity were referred to our academic obesity center CGG for assessment of potential contributing factors to adiposity. After registration, a comprehensive standardized medical questionnaire was sent to the patients for a more thorough evaluation of, among other things, their medical history, drug use, family history, and other factors as assessed here. We assessed data of patients who were seen at the outpatient clinic between June 2011 and August 2016. After excluding individuals who had a BMI below 30.0 at the time of the clinic visit or insufficient data, a total of 408 patients with obesity were included in the current study.

Sociodemographic Factors

Weight, height, and blood pressure were measured during the site visit. BMI was computed by dividing weight (kg) by height (m^2). Nationality was determined according to Statistics Netherlands [13]. The highest attendant education level was coded as follows: low (i.e., no education, primary education, or special education), middle (i.e., secondary education or vocational studies), or high (i.e., higher professional education or university education).

Assessment of Potential Weight-Inducing Factors

Medical history and drug use were assessed using the referral letter of the primary care provider and completed medical questionnaires and were subsequently confirmed and further detailed during the outpatient clinic visit.

Currently used drugs were assessed for potential weight-inducing adverse events. Accordingly, we compiled a list of drugs which were previously reported to be associated with weight gain (Table 1) [7, 14–21]. For exploratory purposes, we additionally included drugs which were less frequently been reported as weight-inducing (e.g., antihistamines and proton pump inhibitors) as compared to the well-established obesogenic drugs. Hormonal contraceptives, other than medroxyprogesterone, were not included due to the controversy about their weight-altering effects [22].

Thyroid function was categorized into the following 4 groups based on the availability of both medical history and current thyroid function measurements: (1) euthyroid (i.e., no history of a thyroid disorder and normal thyroid function tests), (2) hypothyroidism (including all patients who were previously or newly diagnosed with hypothyroidism and patients who underwent thyroidectomy; these patients were subdivided into groups of patients who were currently inadequately

Table 1: Overview of drugs described to be associated with weight gain.[7,14-21]

Drug class	Drug name	
Anticonvulsants	Carbamazepine Gabapentin	Pregabalin Valproic acid
Antidepressants	Amitriptyline Citalopram Clomipramine Clovoxamine Desipramine Doxepin Duloxetine Escitalopram Fluoxetine Fluvoxamine	Imipramine Maprotiline Mirtazapine Nortriptyline Paroxetine Phenelzine Sertraline Tranylcypromine Trimipramine
Antihistamines	Astemizole Cetirizine Cyproheptadine	Diphenhydramine Fexofenadine (Des)loratadine
Antipsychotics	Aripiprazole Chlorpromazine Clozapine Fluphenazine Haloperidol Lithium Olanzapine Paliperidone	Perphenazine Quetiapine Risperidone Thioridazine Thiothixene Trifluoperazine (Ziprasidone)[a]
Corticosteroids		
Diabetes drugs	Insulin Sulfonylurea • Chlorpropamide • Glibenclamide[b] • Glimepiride • Glipizide	Thiazolidinediones • Troglitazone • Pioglitazone • Rosiglitazone
Hypertension drugs	Alpha-blockers • Clonidine • Prazosin • Terazosin Beta-blockers • Atenolol • Metoprolol • Propranolol	Calcium channel blockers • Flunarizine • Nisoldipine Centrally acting agents • Methyldopa
Proton pump inhibitors	Lansoprazole Omeprazole	Rabeprazole
Others	Leuprolide acetate Medroxyprogesterone	Pizotifen Protease inhibitor

[a] Ziprasidone is reported to both induce weight gain [7] as weight loss [15]; current use of ziprasidone was not observed in our sample; [b]Also known as "glyburide" in the United States.

treated [increased thyroid-stimulating hormone (TSH) levels], patients who were adequately treated or had a resolved hypothyroidism [normal TSH and free thyroxine (FT4) levels], patients who were overtreated [lowered TSH levels], and

patients with other thyroid function test results), (3) subclinical hypothyroidism, and (4) other thyroid states. For this, blood samples were drawn for determination of, among other things, TSH and FT4 as part of our routine lab measurements.

PCOS was classified as previously diagnosed if the patient indicated having been investigated earlier and the diagnosis was established. For screening purposes, patients suspected to have PCOS were referred to a specialized gynecological outpatient clinic. Clinically suspected patients who were not (yet) further investigated or for whom the results were still pending due to investigations elsewhere were classified as a separate category.

In male patients without testosterone replacement therapy, total testosterone and sex hormone-binding globulin (SHBG) were measured if necessary to determine male hypogonadism. Due to the association of SHBG with body weight, we calculated non-SHBG-bound testosterone according to de Ronde et al. [23]. Male hypogonadism was defined as non-SHBG testosterone levels lower than 2 SD from the mean according to the corresponding age category as noted by de Ronde et al. [23], with patients younger than 40 years belonging to the youngest category.

Subjects were also evaluated for age at menarche (years), self-reported birth weight (g), and average sleep duration (h/night). With respect to OSAS, we referred suspected cases to an otolaryngologist and classified the patients using the same format as for PCOS.

Assessment of Marked Weight Gain

In order to also evaluate subjective reasons for weight gain, we assessed self-reported data about potential causes of any previous period of marked weight gain. For this purpose, we assessed reasons other than unhealthy eating behaviors and/or physical inactivity and categorized these as related to: health, psychosocial stress, work, pregnancy, drug use, substance cessation, and other causes.

Genetic Analysis

A genetic test was performed in a selection of 162 patients (39.7%). They fulfilled the criteria for requesting this analysis based on clinical suspicion of syndromic obesity (e.g., early-onset obesity with dysmorphic features/congenital malformations with or without an intellectual deficit, behavior problems, hyperphagia, and/or a striking family history), had intractable obesity despite a healthy lifestyle and repeated weight-loss attempts without other potential secondary causes, or had an insufficient response or a non-response to our intensive combined lifestyle treatment programs. Targeted diagnostic DNA sequencing of 52 obesity-related

genes, including 3 genes related to type 2 diabetes mellitus (Table 2), covering protein coding exons and flanking splice site consensus sequences, was performed at the ISO15189 accredited genome diagnostics department of UMC Utrecht. DNA was enriched using an Agilent SureSelectXT custom enrichment assay (ELID#0561501) followed by next-generation sequencing on an Applied Biosystems 5500XL SOLiD sequencer at a minimum of 100× median coverage. Horizontal coverage of the targeted sequence at >15× was >95%. The poorly captured fourth exon of the POMC gene (transcript NM_001035256.1) was analyzed via the Sanger sequencing method to reach >99% coverage for this gene (primer sequences are available on request).

Table 2: Gene panel for syndromic and nonsyndromic obesity analysis.

Gene name					
ALMS1	*BDNF*	*KIDINS220*	*MKS1*	*PTEN*	*TUB*
ARL6	*CCDC28B*	*LEP*	*MRAP2*	*SIM1*	*WDPCP*
BBS1	*CEP290*	*LEPR*	*NDN*	*SNRPD2*	
BBS2	*CRHR2*	*LZTFL1*	*NTRK2*	*SNRPN*	
BBS4	*FLOT1*	*MAGEL2*	*PAX6*	*SPG11*	
BBS5	*G6PC*	*MC3R*	*PCK1*	*TBX3*	
BBS7	*GNAS*	*MC4R*	*PCSK1*	*THRB*	
BBS9	*IRS1*[a]	*MCHR1*	*PHF6*	*TMEM67*	
BBS10	*IRS2*[a]	*MKKS*	*POMC*	*TRIM32*	
BBS12	*IRS4*[a]	*MKRN3*	*PRKAR1A*	*TTC8*	

[a] These genes were analysed for the purpose of evaluating co-morbidity risk (type 2 diabetes mellitus).

Statistical Analysis

IBM SPPS Statistics version 21 (IBM Corp., Armonk, NY, USA) was used for statistical analyses. Age at menarche was assessed continuously. For exploratory purposes, we also evaluated the prevalence of precocious menarche (i.e., younger than 9 years). Sleep duration and birth weight were assessed both as continuous and as categorical variables (i.e., <6.0, 6.0–8.0, and ≥8.0 h/night for sleep duration; <4,000 g and ≥4,000 g [i.e., high birth weight] for birth weight). In order to compare the differences in outcomes by severity of obesity, we analyzed our cohort using the following 3 BMI classes according to the WHO classification of adult obesity [24]: class I obesity for BMI between 30.00 and 34.99, class II for BMI between 35.00 and 39.99, and class III for BMI ≥40.00. Crude between-group differences in categorical variables were tested using a x2 test or Fisher's exact test, and for continuous variables ANOVA or the Kruskal-Wallis test was used when appropriate. The Mantel-Haenszel test for trend was performed to assess trends in prevalence numbers across the obesity classes. Logistic regression models and ANCOVA were used for between-group analyses with adjustments for sex and/or age as indicated. For all tests, $p < 0.05$ was considered statistically significant.

Results

Sample Characteristics

The general characteristics for the entire group and stratified by the 3 classes of adult obesity are provided in Table 3. About half of our cohort was classified as having class III obesity. No differences were found between classes with regard to sociodemographic factors.

Table 3: Descriptive characteristics of the study sample.

	Subjects, *n*	Overall	Adult obesity classes[a]		
			I (N=69)	II (N=144)	III (N=195)
Age, *years*	**408**	41.3 (±14.2)	39.8 (±14.5)	41.0 (±13.7)	42.1 (±14.5)
Sex, *female*	**408**	308 (76%)	48 (70%)	109 (76%)	151 (77%)
BMI, *kg/m²*	**408**	40.5 (±6.2)	33.1 (±1.4)	37.5 (±1.5)	45.4 (±5.2)
Blood pressure	**396**				
Systolic, *mmHg*		140 (±18)	137 (±16)	138 (±16)	142 (±19)
Diastolic, *mmHg*		81 (±13)	79 (±13)	82 (±12)	82 (±13)
Nationality	**408**				
Native		295 (72.3%)	49 (71.0%)	111 (77.1%)	135 (69.2%)
Western background		24 (5.9%)	3 (4.3%)	11 (7.6%)	10 (5.1%)
Non-western background		89 (21.8%)	17 (24.6%)	22 (15.3%)	50 (25.6%)
Education level	**361**				
Low		20 (5.5%)	2 (3.5%)	6 (4.7%)	12 (6.9%)
Middle		197 (54.6%)	26 (45.6%)	69 (53.5%)	102 (58.3%)
High		144 (39.9%)	29 (50.9%)	54 (41.9%)	61 (34.9%)
Menarcheal age, years	**301**	12.0 (11.0-15.0)	12.0 (10.9-15.1)	12.5 (11.0-14.0)	12.0 (11.0-15.0)
Sleeping, hours/night	**311**	7.1 (±1.4)	7.1 (±1.4)	7.1 (±1.3)	7.1 (±1.4)
<6.0 hours/night		45 (14.5%)	8 (17.0%)	18 (16.2%)	19 (12.4%)
6.0-8.0 hours/night		170 (54.7%)	22 (46.8%)	59 (53.2%)	89 (58.2%)
≥8.0 hours/night		96 (30.9%)	17 (36.2%)	34 (30.6%)	45 (29.4%)
Birth weight, grams	**272**	3402 (±744)	3423 (±698)	3361 (±805)	3428 (±711)
<4000 grams		219 (80.5%)	37 (80.4%)	87 (83.7%)	95 (77.9%)
≥4000 grams		53 (19.5%)	9 (19.6%)	17 (16.3%)	27 (22.1%)

Data are shown as numbers (frequency), mean (±SD), and median (10^{th}-90^{th} percentile). [a]Obesity classes are classified as according to the WHO classification of adult obesity [24], i.e. class I for BMI between 30.00-34.99 kg/m², class II for BMI between 35.00-39.99 kg/m², and class III for adults with a BMI equal to or greater than 40.00 kg/m². Abbreviation: *BMI*, body mass index.

Potentially Weight-Inducing Factors

Overall, 48.0% of the patients were using any potentially weight-inducing drug at the time of the clinic visit. Corticosteroids (local and systemic) were the most used weight-inducing drugs (23.8%), followed by proton pump inhibitors (11.3%), antihistamines (8.6%), antidepressants (8.3%), hypertension drugs (8.3%), diabetes drugs (5.9%), anticonvulsants (2.5%), antipsychotics (2.0%), and other drugs (0.7%) such as medroxyprogesterone (0.5%) and protease inhibitors (0.2%). Except for

proton pump inhibitors, the 3 obesity classes did not differ in use of any of the potentially weight-inducing drugs (Fig. 1).

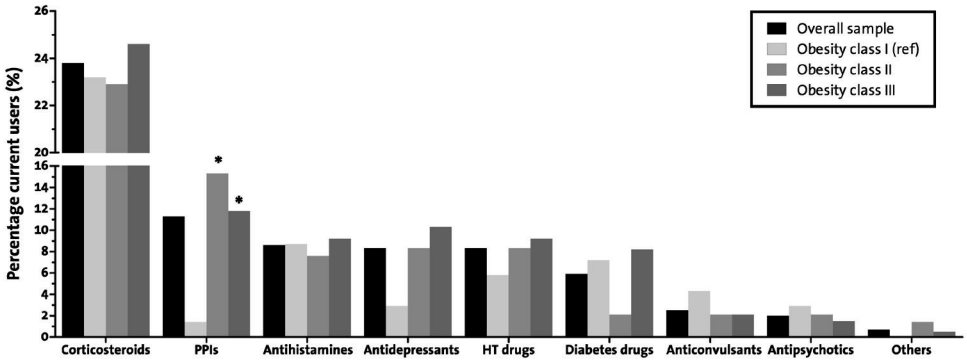

Figure 1: Current use of potentially weight-inducing drugs (Table 1) by subjects with obesity in the overall group and stratified by obesity class (i.e., class I, BMI = 30.00–34.99; class II, BMI = 35.00–39.99; and class III, BMI ≥40.00).

Between-group analyses, with class I as the reference group, were performed with logistic regression analyses with adjustments for sex and age. * $p < 0.05$. *HT*, hypertension; *PPI*, proton pump inhibitor.

The majority of the patients had no history of any thyroid disorder in combination with normal thyroid hormone test results (Table 4). Five hypothyroid patients (10.0% of the hypothyroid group) were undertreated with thyroxine analogs. A new diagnosis of hypothyroidism and subclinical hypothyroidism was made in, respectively, 2 (0.6%) and 9 (2.5%) of the screened cases. No significant differences were noted in prevalence rates of (subclinical) hypothyroidism between the 3 obesity classes.

Table 4: Thyroid status in outpatients with obesity.

	Overall (N=354)	Adult obesity classes[a]		
		I (N=69)	II (N=144)	III (N=195)
Euthyroid	280 (79.1%)	43 (71.7%)	100 (82.0%)	137 (79.7%)
Hypothyroidism	50 (14.1%)	13 (21.7%)	12 (9.8%)	25 (14.5%)
Adequately treated or resolved	31 (8.8%)	8 (13.3%)	6 (4.9%)	17 (9.9%)
Inadequately treated	5 (1.4%)	1 (1.7%)	2 (1.6%)	2 (1.2%)
Overtreated	10 (2.8%)	4 (6.7%)	3 (2.5%)	3 (1.7%)
Undiagnosed	2 (0.6%)	0 (0.0%)	1 (0.8%)	1 (0.6%)
Others[b]	2 (0.6%)	0 (0.0%)	0 (0.0%)	2 (1.2%)
Subclinical hypothyroidism	15 (4.2%)	1 (1.7%)	7 (5.7%)	7 (4.1%)
Previously diagnosed	6 (1.7%)	1 (1.7%)	3 (2.5%)	2 (1.2%)
Undiagnosed	9 (2.5%)	0 (0.0%)	4 (3.3%)	5 (2.9%)
Others[c]	9 (2.5%)	3 (5.0%)	3 (2.5%)	3 (1.7%)

Data are shown as number (frequency).

[a] Obesity classes are classified as according to the WHO classification of adult obesity [24], i.e. class I for BMI between 30.00-34.99 kg/m^2, class II for BMI between 35.00-39.99 kg/m^2, and class III for adults with a BMI equal to or greater than 40.00 kg/m^2;

[b] Includes one patient with hypothyroidism during block and replace therapy for Graves's disease and one patient with untreated hypothyroidism in history with recent altered thyroid hormone tests suspected of amiodarone-induced thyrotoxicosis;

[c] Includes nine patients with no known thyroid disorder in the past, but laboratory testing showed a subclinical hyperthyroidism (n=2), normal TSH with lowered FT4 values (n=6), and normal TSH with increased FT4 levels (n=1).

Thirty female patients (9.7%) presented with PCOS at the first visit. After consultation, 17 (5.5%) women were additionally suspected of having PCOS. The diagnosis could be confirmed in 7 women, yielding a prevalence rate of PCOS in our female obese sample of at least 12.0% given the fact that some were evaluated elsewhere or decided not to undergo any further investigation at that moment (Fig. 2a). A higher obesity class was associated with a lower PCOS prevalence rate (20.8, 14.7, and 7.3% from the lowest to the highest class), but this did not reach statistical significance after adjustment for age.

Thirty-six male patients, aged 44.0 ± 14.7 years, had their total testosterone and SHBG levels measured. Non-SHBG-bound testosterone levels showed an incremental decrease across the obesity classes ($p = 0.035$, adjusted for age). Hypogonadism was present in 41.7% of the investigated men, with prevalences ranging from 20.0 (1/5) to 47.1 (8/17) and 42.9% (6/14) in the consecutive classes. No novel cases in endogenous Cushing's syndrome or growth hormone deficiency were diagnosed. Obesity due to iatrogenic damage to the pituitary and/or the hypothalamus was suspected in 2 patients; one of whom had developed hyperphagia after excision of suprasellar craniopharyngioma and the other of whom gained substantial weight after undergoing surgery with adjuvant radiotherapy for a nonfunctioning pituitary macroadenoma.

The age at menarche was on average 12.6 ± 1.8 years with a median (10th to 90th percentile) of 12.0 (11.0–15.0) years (Table 3). Precocious menarche was present in 2 women with class III adult obesity. No significant differences were found between the classes and there were no differences by nationality.

The mean birth weight was 3,402 ± 744 g in the overall group (Table 3). The prevalence of a high birth weight was 19.5%. No significant between-group difference or trend was found among the obesity classes.

The average amount of sleep in the total obese group was 7.1 ± 1.4 h/night, which was consistent across classes. Sleep deprivation, defined as a sleep duration of less than 6 h, was reported in 14.5% of the outpatients (Table 3). The diagnosis of OSAS was confirmed in 45 outpatients (11.0%) prior to the clinic visit. After examinations for suspected OSAS, there were in total 56 outpatients (13.7%) with confirmed OSAS, excluding those who declined further assessments or decided to be investigated elsewhere (Fig. 2b). With regard to the obesity classes, there was a significant positive trend between OSAS and obesity class (4.4, 15.3, and 15.9% in the consecutive classes, respectively; p = 0.027, adjusted for sex and age).

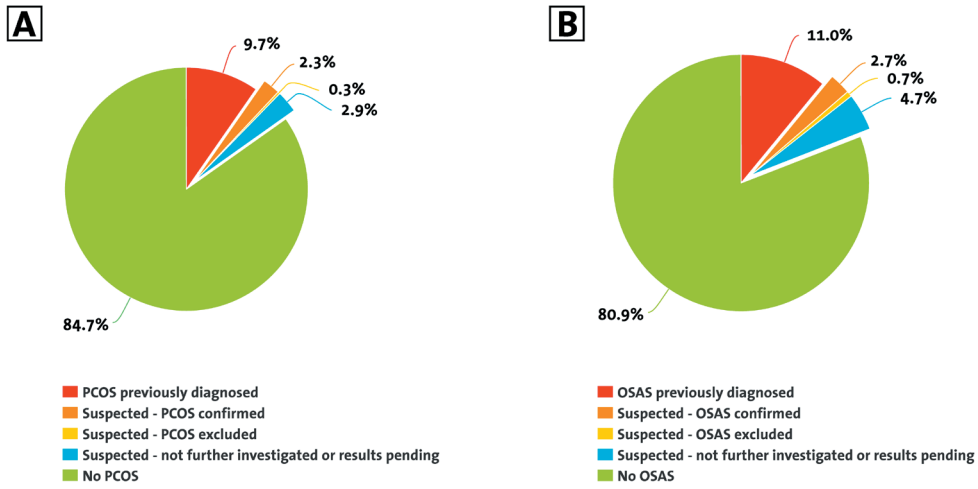

Figure 2: Prevalence rates of PCOS (a) and OSAS (b) in subjects with obesity.

The pulled slices represent the status of adult subjects with obesity suspected of having PCOS or OSAS after the clinic visit.

Self-Reported Causes of Marked Weight Gain

A total of 224 (54.9%) outpatients reported having experienced at least one moment of marked weight gain. The most common reported trigger was the use of medical drugs, which occurred in 69 out of 408 (16.9%) subjects in the total study population. Among these patients, weight gain was most frequently preceded by use of corticosteroids (33.3%), followed by use of antidepressants and/or antipsychotics (24.6%), hormonal contraceptives (23.2%), and other drugs (18.8%). A health-related cause (e.g., thyroid disorder, menopause, and joint disorder) was the second most reported precipitating factor (52/408; 12.8%) for marked weight gain. Other reported main causes were psychosocial stress (30/408; 7.4%; e.g., relationship difficulties or divorce, death of a relative, and overall increased perceived stress), pregnancy-related causes (29/408; 7.1%), substance cessation (15/408; 3.7%; stopped smoking or taking illicit drugs), and work-related factors (14/408; 3.4%; e.g., change to a sedentary work style, shift work, retirement or cessation of work for other reasons). Sixteen patients (3.9%) reported other reasons such as sleep deprivation, moving out of the parental home, or in vitro fertilization treatment, while 34 outpatients (8.3%) were unaware of a potential cause. A moment of marked weight gain was most frequently reported in patients with class II obesity (63.9%), which was significantly higher than in those with class III obesity (48.2%; $p = 0.003$, adjusted for sex and age) but not compared to class I obesity (55.1%).

Genetic Analysis

A definitive genetic diagnosis could be made in 3 female patients (1.9%) with class III obesity and this was based on known pathogenic mutations in the MC4R (n = 2) and POMC (n = 1) genes (Table 5). Likely pathogenic contributing genetic variants were identified in 9 patients (5.6%), and another 15 patients (9.3%) were shown to be carriers of mutations associated with autosomal recessive diseases probably conferring no or only a low increased risk for obesity. Two patients were identified as having a mutation in one of the screened comorbidity genes (i.e., IRS1 [n = 1] and IRS2 [n = 1]). Mutations were found to be more present in the higher obesity classes; however, inferential analysis for differences between classes was not performed due to low numbers.

Table 5: Genetic screening results for obesity-related genes in our population of subjects with obesity.

#	Gene	Genetic defect	Sex	Class[a]	Variation	Explanation
Definitive diagnosis of genetic obesity						
1	*MC4R*	Pathogenic	F	III	Heterozygous c.105C>A, p.(Tyr35*)	
2	*MC4R*	Pathogenic	F	III	Heterozygous c.105C>A, p.(Tyr35*)	
3	*POMC*	Pathogenic	F	III	Heterozygous c.662A>G, p.(Tyr221Cys)	
Possible diagnosis of genetic obesity or risk factor for obesity						
4	*LEPR*	VUS	F	II	Heterozygous c.1835G>A, p.(Arg612His)	Pathogenic heterozygous *LEPR* mutations are a known risk factor for obesity, segregation analysis needed. This mutation is described in patients with leptin receptor deficiency in combination with other pathogenic *LEPR* mutations.
5	*LEPR*	VUS	F	III	Heterozygous c.2963C>T, p.(Thr988Met)	Pathogenic heterozygous *LEPR* mutations are a known risk factor for obesity, segregation analysis needed.
6	*MRAP2* *CEP290*	Likely pathogenic Pathogenic	F	II	Heterozygous c.373C>T, p.(Arg125Cys) Heterozygous c.4723A>T, p.(Lys1575*)	*MRAP2* likely pathogenic variant, segregation analysis needed; *CEP290* carrier status autosomal recessive disease probably no or low risk factor for obesity.
7	*MRAP2*	Likely pathogenic	F	III	Heterozygous c.373C>T, p.(Arg125Cys)	Likely pathogenic variant, segregation analysis needed.
8	*MRAP2*	Likely pathogenic	F	III	Heterozygous c.520C>T, p.(Gln174*)	Likely pathogenic variant.
9	*PCK1* *PCK1*	VUS VUS	M	III	Heterozygous c.1397C>T, p.(Ala466Val) Heterozygous c.1628G>A, p.(Arg543Gln)	Segregation analysis showed that a skinny sib did not have any of the variants.
10	*POMC*	VUS	M	III	Heterozygous c.229T>G, p.(Tyr77Asp)	Pathogenic heterozygous *POMC* mutations are a known risk factor for obesity, segregation analysis identified the mutation in the obese brother, which makes pathogenicity more likely.
11	*SNRPN*	Likely pathogenic	F	I	Heterozygous c.193C>T, p.(Arg65Trp)	Likely pathogenic variant fitting with phenotype, but first described missense mutations, further segregation analysis and/or functional study needed.
12	*TBX3*	VUS	F	III	Heterozygous c.2177G>T, p.(Arg726Leu)	Autosomal dominant diseases, segregation analysis not yet performed.

No genetic explanation for obesity (yet): VUS or non-interpretable status						
13	*BBS1*	Pathogenic	F	I	Heterozygous c.1570_1572del, p.(Asn524del)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
14	*BBS1*	VUS	F	II	Heterozygous c.742C>A, p.(Pro248Thr)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
15	*BBS4*	VUS	M	II	Heterozygous c.137A>G, p.(Lys46Arg)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
16	*BBS5*	VUS	M	III	Heterozygous c.110T>C, p.(Ile37Thr)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
17	*BBS9*	VUS	F	III	Heterozygous c.1600A>G, p.(Ile534Val)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
18	*BBS12*	VUS	M	III	Heterozygous c.1367A>G, p.(Tyr456Cys)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
19	*BBS12*	Pathogenic	M	III	Heterozygous c.476C>T, p.(Pro159Leu)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
20	*CEP290*	Pathogenic VUS	F	II	Heterozygous c.6516del, p.(Lys2172fs) Heterozygous c.564T>G, p.(Asp188Glu)	No clinical features of Bardet-Biedl syndrome. Probably no or low risk factor for obesity.
21	*CEP290*	VUS VUS	F	III	Heterozygous c.7190T>C, p.(Leu2397Pro) Heterozygous c.7394_7395del, p.(Glu2465fs)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity. Variants in cis.
22	*PCK1*	VUS	F	III	Heterozygous c.1526C>T, p.(Pro509Leu)	Carrier status autosomal recessive disease, no known dominant phenotype.
23	*SPG11*	VUS	F	III	Heterozygous c.5121G>T, p.(Glu1707Asp)	Carrier status autosomal recessive disease, no known dominant phenotype.
24	*SPG11*	Pathogenic	F	III	Heterozygous c.5989_5992del, p.(Leu1997fs)	Carrier status autosomal recessive disease, no known dominant phenotype.
25	*TMEM67*	Pathogenic	F	II	Heterozygous c.958A>T, p.(Ser320Cys)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
26	*TMEM67*	Pathogenic	M	II	Heterozygous c.622A>T, p.(Arg208*)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
27	*WDPCP*	Pathogenic	F	II	Heterozygous c.160G>A, p.(Asp54Asn)	Carrier status autosomal recessive disease; probably no or low risk factor for obesity.
Mutations in comorbidity risk genes						
28	*IRS1*	VUS	F	I	Heterozygous c.1835A>G, p.(Tyr612Cys)	
29	*IRS2*	VUS	F	II	Heterozygous c.319G>T, p.(Ala107Ser)	

[a] Indicates the adult obesity classes as according to the WHO classification [24], i.e. class I for BMI between 30.00-34.99 kg/m^2, class II for BMI between 35.00-39.99 kg/m^2, and class III for adults with a BMI equal to or greater than 40.00 kg/m^2. Abbreviation: VUS, variant of unknown clinical significance.

Discussion

In the present study, we extensively phenotyped subjects with obesity for various factors potentially contributing to obesity, including genetic predispositions, and assessed self-reported causes of marked weight gain. Interestingly, we found that about half of the obese subjects were using one or more prescribed drugs associated with weight gain. The use of corticosteroids was particularly high in our sample, which was especially remarkable since drug use, in general, was reported as the most common triggering factor for marked weight increase. In this light, it is interesting to mention that we recently found strong associations between local corticosteroids (e.g., inhaled or nasal) and an increased BMI in a large population-based study [25].

Obesity is frequently associated with the onset or exacerbation of different comorbidities which often require a pharmacological intervention, such as hypertension, type 2 diabetes mellitus, gastroesophageal reflux disease, asthma, and depression. Unfortunately, some of these drugs may induce weight gain or complicate the process of weight loss. Interestingly, drug use was the most self-reported triggering factor for marked weight gain in our study population. Highest user rates were mainly found for corticosteroids, and we previously reported evident differences in recent use of these between obese and nonobese subjects [26]. It was notable that, besides corticosteroids and psychotropic drugs, also hormonal contraceptives were frequently suggested as a trigger for marked weight gain in women. However, a Cochrane review by Gallo et al. [22] could not establish a causal relationship between the popular combination contraceptives and weight gain. Unfortunately, the type of hormonal contraceptive used during periods of marked weight gain in our female users was largely unknown.

First-line assessment of obesity generally includes investigation of the more generally known weight-inducing disorders which are mainly of hormonal origin. This could explain why we only found few new cases of hypothyroidism and PCOS and none for Cushing's syndrome and growth hormone deficiency. With regard to the thyroid functioning, the prevalence rate of hypothyroidism was much higher compared to the rate in the general population [27, 28]. This may have attributed to weight gain over time and could perhaps still be a contributing factor in 10% of our hypothyroid patients who are (biochemically) undertreated [29]. Moreover, we found a high prevalence of PCOS as compared to previous findings in unselected populations [30, 31]. In men, we found a high percentage of new hypogonadism cases. The reverse causality between hypogonadism and obesity in men in combination with the lack of data on clinically related signs and symptoms complicates the contributing role of low testosterone with regard to obesity in our male

outpatients. Nevertheless, testosterone suppletion in obese hypogonadal men seems to be effective with regard to sustained weight loss in the long term [32] while, importantly, weight loss by itself can also increase testosterone levels [33].

With regard to menarche, we found a relatively younger median age of onset in comparison to the general Dutch female population (12.0 vs. 13.1 years) [34]; however, the reason for this difference is not necessarily clear. The direct link between age at menarche and adulthood obesity seems less evident and both are considered to be subsequent to a high childhood BMI and/or increased sexual maturation due to other factors [3], while a low socioeconomic status has also been suggested to give rise to both menarche at a young age and obesity in adult life [35].

A high weight at birth, which appears to have a differentiating capacity with regard to weight and BMI in adulthood, was 2-fold more common in our cohort when compared to the general population [36, 37]. Various studies have shown that adults but also children with a high birth weight have more adverse anthropometric and body compositional features in comparison to normal birth weight subjects, whereas no differences have been found for subjects with a low birth weight [5, 37]. Similar findings were also found in a comprehensive meta-analysis which showed a positive association between a high birth weight and adult obesity [4].

We found a relatively higher percentage of subjects with a self-reported sleep duration of less than 6 h/night when compared to numbers from the general population [8, 38]. In spite of differences in sleep requirement, such a short sleep duration is in general associated with a higher body weight and obesity [39]. In addition to the longer time awake increasing the opportunity for food consumption, it has been found that sleep deprivation can lead to derangement of appetite-regulating hormones (e.g., leptin, ghrelin, and cortisol) which can result in a greater appetite and a more energy-dense food intake [9, 40]. The interplay between sleep deprivation, OSAS, and obesity [41] further supports the importance of sufficient sleep for a healthy body weight.

Genetic analysis is relatively new in the diagnostic workup of non-syndromic obesity. We here identified a definite genetic cause for the obesity phenotype in 3 patients. The identified underlying molecular defects (MC4R and POMC mutations) could offer (future) personalized treatment programs for these patients [42, 43]. One could also hypothesize that certain mutations could possibly indicate a contraindication for certain obesity treatment options in cases with a negative response to treatment. For patients themselves, it is often a psychological burden to be blamed by society for not being able to lose weight. In our clinical experience,

a genetic diagnosis could perhaps aid in understanding and accepting why they show a different treatment response when compared to others and thereby increase the social support and decrease stigmatization. Our results, however, showed that the majority of the patients who received an abnormal result were carriers of a variant of unknown significance that could possibly contribute to the obesity phenotype. An individual combination of different variants of unknown significance (polygenic inheritance), each having a small effect on weight gain, might well add up to a larger obesity risk for such a person. Besides, most of these comprised the BBS gene mutations for which heterozygosity has been postulated as a risk factor in obesity [44]. Frequency analysis in an obesity cohort and segregation analysis in family members showed, however, that BBS carriage is probably not an important risk factor for obesity [45]. Future studies are needed to assess the clinical significance and confirm a possible association between obesity and the uncertain variants we identified here.

In the current study, we comprehensively and systematically evaluated various medical conditions as well as lifestyle, iatrogenic, and genetic factors related to weight gain in a single study population of subjects with obesity. Despite the distribution of the patients over all obesity classes, it is conceivable that our patients are more prone to underlying disorders in comparison to the general obese population given the setting of our clinic as an obesity expertise center. Unfortunately we did not have a control cohort and hence used literature based on general population data for comparison. Additionally, some of the data are self-reported and could hence be subject to recall bias. Furthermore, it should be emphasized that the number of genetic variations found may be underestimated since it does not include other genetic (syndromic) causes, chromosomal abnormalities (e.g., Turner syndrome), genomic microdeletions and duplications (e.g., 16p11.2 deletion syndrome), methylation abnormalities (e.g., Prader-Willi syndrome), uniparental disomy (e.g., UPD14), or triplet repeat expansions (e.g., fragile X syndrome) associated with obesity, as these are not detectable by the applied method. However, during our clinical assessment and physical examination we observed no signs or symptoms of these syndromes in this group of patients with obesity.

Conclusion

In conclusion, a thorough evaluation of potential weight-inducing factors in subjects with obesity showed especially a high prevalence of use of weight-inducing drugs and hormonal abnormalities, with virtually no differences across the distinct degrees of obesity. Drug use was additionally reported to be the most frequent trigger for marked weight gain, with corticosteroids being the

most prevalent culprit. Although we could only confirm a definite genetic obesity diagnosis in 2% of the patients who were offered a genetic test, a personalized treatment option can (perhaps) be offered in a future clinical trial setting. This exemplifies the importance of genetic analysis in the diagnostic workup of obesity in specific patient groups (e.g., patients with symptoms suggesting monogenetic or syndromal obesity). Future large prospective cohort studies should focus on the factors as assessed here but also on others that could contribute to adiposity or impede weight loss (e.g., brown fat activity, endocrine disruptors, ambient temperature, and maternal age [2, 46]). This in order to better understand the bidirectional relationship between most of the obesogenic factors and adiposity and to eventually decrease the social stigma surrounding obesity and optimize and develop more efficient and tailored weight loss interventions.

References

1 Collaboration NC; NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 coun- tries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet. 2016 Apr;387(10026):1377–96.

2 Keith SW, Redden DT, Katzmarzyk PT, Boggiano MM, Hanlon EC, Benca RM, et al. Putative contributors to the secular increase in obesity: exploring the roads less traveled. Int J Obes. 2006 Nov;30(11):1585–94.

3 Pierce MB, Leon DA. Age at menarche and adult BMI in the Aberdeen children of the 1950s cohort study. Am J Clin Nutr. 2005 Oct;82(4):733–9.

4 Yu ZB, Han SP, Zhu GZ, Zhu C, Wang XJ, Cao XG, et al. Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. Obes Rev. 2011 Jul;12(7):525–42.

5 Rillamas-Sun E, Sowers MR, Harlow SD, Randolph JF Jr. The relationship of birth weight with longitudinal changes in body composition in adult women. Obesity (Silver Spring). 2012 Feb;20(2):463–5.

6 Weaver JU. Classical endocrine diseases causing obesity. Front Horm Res. 2008;36:212–28.

7 Sheehan AH. Weight gain. In: Tisdale JE, Miller DA, editors. Drug-induced Diseases: Prevention, Detection, and Management. 2nd ed. Bethesda, MD, USA: American Society of Health-System Pharmacists; 2010. pp. 629–42.

8 Kohatsu ND, Tsai R, Young T, Vangilder R, Burmeister LF, Stromquist AM, et al. Sleep duration and body mass index in a rural population. Arch Intern Med. 2006 Sep;166(16):1701–5.

9 Taheri S, Lin L, Austin D, Young T, Mignot E. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med. 2004 Dec;1(3):e62.

10 Tsai EC, Boyko EJ, Leonetti DL, Fujimoto WY. Low serum testosterone level as a predictor of increased visceral fat in Japanese-American men. Int J Obes Relat Metab Disord. 2000 Apr;24(4):485–91.

11 Gambineri A, Pelusi C, Vicennati V, Pagotto U, Pasquali R. Obesity and the polycystic ovary syndrome. Int J Obes Relat Metab Disord. 2002 Jul;26(7):883–96.

12 Romero-Corral A, Caples SM, Lopez-Jimenez F, Somers VK. Interactions between obesity and obstructive sleep apnea: implications for treatment. Chest. 2010 Mar;137(3):711–9.

13 Alders M. Classification of the population with a foreign background in The Netherlands: Statistic Netherlands, paper for the conference „The measure and mismeasure of populations. The statistical use of ethnic and racial categories in multicultural societies". Paris: CERI; 2001.

14 Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, et al.; Endocrine Society. Pharma- cological management of obesity: an endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2015 Feb;100(2):342–62.

15 Bray GA, Ryan DH. Medical therapy for the patient with obesity. Circulation. 2012 Apr;125(13):1695–703.
16 Malone M. Medications associated with weight gain. Ann Pharmacother. 2005 Dec;39(12):2046–55.
17 Cheskin LJ, Bartlett SJ, Zayas R, Twilley CH, Allison DB, Contoreggi C. Prescription medications: a modifiable contributor to obesity. South Med J. 1999 Sep;92(9):898–904.
18 Leslie WS, Hankey CR, Lean ME. Weight gain as an adverse effect of some commonly prescribed drugs: a systematic review. QJM. 2007 Jul;100(7):395–404.
19 Breum L, Fernstrom MH. Drug-induced obesity; International textbook of obesity. Hoboken: Wiley; 2002. p. 269–81.
20 Yoshikawa I, Nagato M, Yamasaki M, Kume K, Otsuki M. Long-term treatment with proton pump inhibitor is associated with undesired weight gain. World J Gastroenterol. 2009 Oct;15(38):4794–8.
21 Ratliff JC, Barber JA, Palmese LB, Reutenauer EL, Tek C. Association of prescription H1 antihistamine use with obesity: results from the National Health and Nutrition Examination Survey. Obesity (Silver Spring). 2010 Dec; 18(12):2398–400.
22 Gallo MF, Lopez LM, Grimes DA, Carayon F, Schulz KF, Helmerhorst FM. Combination contraceptives: effects on weight. Cochrane Database Syst Rev. 2014 Jan;(1):CD003987.
23 de Ronde W, van der Schouw YT, Pierik FH, Pols HA, Muller M, Grobbee DE, et al. Serum levels of sex hormone- binding globulin (SHBG) are not associated with lower levels of non-SHBG-bound testosterone in male newborns and healthy adult men. Clin Endocrinol (Oxf). 2005 Apr;62(4):498–503.
24 Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i–xii.
25 Savas M, Muka T, Wester VL, van den Akker EL, Visser JA, Braunstahl GJ, et al. Associations between systemic and local corticosteroid use with metabolic syndrome and body mass index. J Clin Endocrinol Metab. 2017 Oct; 102(10):3765–74.
26 Savas M, Wester VL, Staufenbiel SM, Koper JW, van den Akker EL, Visser JA, et al. Systematic evaluation of corticosteroid use in obese and non-obese individuals: A multi-cohort study. Int J Med Sci. 2017 Jun;14(7):615–21.
27 Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC. The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. J Clin Endocrinol Metab. 2014 Mar;99(3):923–31.
28 Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum tsh, t(4), and thyroid antibodies in the united states population (1988 to 1994): national health and nutrition examination survey (nhanes iii). J Clin Endocrinol Metab. 2002 Feb;87(2):489–99.
29 Fox CS, Pencina MJ, D'Agostino RB, Murabito JM, Seely EW, Pearce EN, et al. Relations of thyroid function to body weight: cross-sectional and longitudinal observations in a community-based sample. Arch Intern Med. 2008 Mar;168(6):587–92.

30 Asunción M, Calvo RM, San Millán JL, Sancho J, Avila S, Escobar-Morreale HF. A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. J Clin Endocrinol Metab. 2000 Jul;85(7):2434–8.

31 Azziz R, Woods KS, Reyna R, Key TJ, Knochenhauer ES, Yildiz BO. The prevalence and features of the polycystic ovary syndrome in an unselected population. J Clin Endocrinol Metab. 2004 Jun;89(6):2745–9.

32 Saad F, Yassin A, Doros G, Haider A. Effects of long-term treatment with testosterone on weight and waist size in 411 hypogonadal men with obesity classes I-III: observational data from two registry studies. Int J Obes. 2016 Jan;40(1):162–70.

33 Corona G, Rastrelli G, Monami M, Saad F, Luconi M, Lucchese M, et al. Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. Eur J Endocrinol. 2013 May; 168(6):829–43.

34 Talma H, Schönbeck Y, van Dommelen P, Bakker B, van Buuren S, Hirasing RA. Trends in menarcheal age between 1955 and 2009 in the Netherlands. PLoS One. 2013 Apr;8(4):e60056.

35 Stöckl D, Döring A, Peters A, Thorand B, Heier M, Huth C, et al. Age at menarche is associated with prediabetes and diabetes in women (aged 32-81 years) from the general population: the KORA F4 Study. Diabetologia. 2012 Mar;55(3):681–8.

36 Apfelbacher CJ, Loerbroks A, Cairns J, Behrendt H, Ring J, Krämer U. Predictors of overweight and obesity in five to seven-year-old children in Germany: results from cross-sectional studies. BMC Public Health. 2008 May;8(1):171.

37 Hirschler V, Bugna J, Roque M, Gilligan T, Gonzalez C. Does low birth weight predict obesity/overweight and metabolic syndrome in elementary school children? Arch Med Res. 2008 Nov;39(8):796–802.

38 Krueger PM, Friedman EM. Sleep duration in the United States: a cross-sectional population-based study. Am J Epidemiol. 2009 May;169(9):1052–63.

39 Chaput JP, Després JP, Bouchard C, Tremblay A. The association between sleep duration and weight gain in adults: a 6-year prospective study from the Quebec Family Study. Sleep. 2008 Apr;31(4):517–23.

40 Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet. 1999 Oct;354(9188):1435–9.

41 Beccuti G, Pannain S. Sleep and obesity. Curr Opin Clin Nutr Metab Care. 2011 Jul;14(4):402–12.

42 Kühnen P, Clément K, Wiegand S, Blankenstein O, Gottesdiener K, Martini LL, et al. Proopiomelanocortin defi- ciency treated with a melanocortin-4 receptor agonist. N Engl J Med. 2016 Jul;375(3):240–6.

43 Danielsson P, Janson A, Norgren S, Marcus C. Impact sibutramine therapy in children with hypothalamic obesity or obesity with aggravating syndromes. J Clin Endocrinol Metab. 2007 Nov;92(11):4101–6.

44 Croft JB, Swift M. Obesity, hypertension, and renal disease in relatives of Bardet-Biedl syndrome sibs. Am J Med Genet. 1990 May;36(1):37–42.
45 Kleinendorst L, Massink MP, Cooiman MI, Savas M, van der Baan-Slootweg OH, Roelants RJ, et al. Genetic obesity: next-generation sequencing results of 1230 patients with obesity. J Med Genet. 2018 Sep;55(9):578– 86.
46 Harms M, Seale P. Brown and beige fat: development, function and therapeutic potential. Nat Med. 2013 Oct; 19(10):1252–63.

Chapter 3

Systematic Evaluation of Corticosteroid Use in Obese and Non-Obese Individuals: A Multi-Cohort Study

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Abstract

Background: *Although the use of corticosteroids has been linked to high incidence of weight gain, no data are available concerning the differences in corticosteroid use between a diverse obese population and non-obese individuals. The main purpose of this study was to systematically explore the use of corticosteroids in obese subjects compared to non-obese controls. In addition, we also explored self-reported marked weight gain within obese subjects.*

Methods: *Two hundred seventy-four obese outpatients (median [range] BMI: 40.1 kg/m^2 [30.5-67.0]), and 526 non-obese controls (BMI: 24.1 kg/m^2 [18.6-29.9]) from two different Dutch cohort studies were included. Corticosteroid use at the time of clinic or research site visit for up to the preceding three months was recorded in detail. Medical records and clinical data were evaluated with regard to age and body mass index in relation to corticosteroid use, single or multiple type use, and administration forms.*

Results: *Recent corticosteroid use was nearly twice as high for obese subjects than for non-obese controls (27.0% vs. 11.9% and 14.8%, both P<.001). Largest differences were found for use of local corticosteroids, in particular inhaled forms, and simultaneous use of multiple types. Marked weight gain was self-reported during corticosteroid use in 10.5% of the obese users.*

Conclusion: *Corticosteroid use, especially the inhaled agents, is higher in obese than in non-obese individuals. Considering the potential systemic effects of also local corticosteroids, caution is warranted on the increasing use in the general population and on its associations with weight gain*

Conclusions: *A thorough evaluation of patients with obesity yields a relatively high prevalence of various potentially weight-inducing factors. Diagnostic screening of patients with obesity could therefore benefit these patients by potentially reducing the social stigma and improving the outcomes of obesity treatment programs by tackling, where possible, the weight-inducing factors in advance.*

Introduction

Synthetic corticosteroids are invaluable in the treatment of a wide range of somatic disorders and have shown their value in many physically demanding conditions. Their different administration routes (e.g. topical, inhaled, nasal, ocular, intra-articular, oral, intra-venous) encourage the use of these medications in both local and systemic disorders in which their mitigating effect on inflammation and the immune system is desired. The widespread use of corticosteroids becomes obvious in national surveys since it is prescribed at least 5.8 million times annually in the 17 million-strong Dutch population [1], whereas in the United States prescription numbers reach over 40 million [2]. These numbers may even underestimate the total use when taking into account over the counter sale of corticosteroids and the use in alternative medicine, since some of the non-registered herbal creams have been found to contain potent corticosteroids [3, 4]. In regard to oral corticosteroids, its use substantially increased with thirty percent over the past two decades, with a prevalence of current use around 1% of the population [5-7]. For inhaled corticosteroids, the percentage of users even doubled between 1990 and 1997 in both the United Kingdom and the Netherlands [8].

In addition to their therapeutic effects, corticosteroids are well known to induce a variety of adverse effects affecting virtually all body systems [9, 10]. Corticosteroid users often experience endocrine and metabolic changes, in particular an increase in weight [11]. This is not surprising, since it is known that high cortisol levels can lead to increased appetite, (truncal) fat accumulation, and altered lipid and glucose metabolism [12-14]. Prolonged use, especially of oral corticosteroids, is notorious for inducing hypercortisolism related side effects and is archetypal for exogenous Cushing's syndrome [15]. However, those systemic side effects are not confined to systemic use, but were also found in local use of corticosteroids. In a recent meta-analysis Broersen et al. investigated different characteristics of corticosteroid use and their effects on adrenal suppression. They found that use of nearly all forms of corticosteroids resulted in an increased risk of adrenal insufficiency [16]. The highest numbers were found for intra-articular injections and oral use (absolute risk of 52.2% and 48.7%, respectively), while similar numbers were also found in patients using multiple administration forms, including combinations of only local corticosteroids. These results indirectly indicate that also local agents result in high systemic corticosteroid exposure and a subsequent suppression of the adrenal gland function due to negative feedback mechanisms, irrespective of the route of administration, and thus potentially lead to weight gain and its cardiometabolic derangements.

Although various studies have shown an increasing effect of corticosteroids on body mass index (BMI), it still remains unknown whether there is a difference in overall corticosteroid use or in use of particular administration forms between obese and non-obese in the general population. Based on the results of the above-mentioned meta-analysis [16] and given the fact that weight gain is one of the most common undesirable effects of corticosteroid use, we hypothesized an overall higher user rate in obese subjects. Hence, in the present study we systematically investigated the use of corticosteroids in an obese outpatient population in comparison to two independent non-obese control cohorts. Moreover, in the same obese population, we also specifically examined if marked weight gain could be correlated to corticosteroid use.

Subjects and Methods

Obese subjects

Two hundred eighty-two obese patients visiting the Obesity Center CGG of the Erasmus Medical Center (Rotterdam, The Netherlands) between June 2011 and September 2015 were initially included. Before visiting the outpatient clinic, which is a multidisciplinary referral center for diagnostic testing and tailored treatment of obesity, all patients were requested to complete an extensive questionnaire regarding factors related to their overweight. With this questionnaire, we obtained data on self-reported marked weight gain, including questions about whether the patient recalled a time period where they experienced a marked increase in weight, and if so, if they suspected any triggering factor for that. The questionnaire also included questions concerning current and previous medication use, including specific questions about the use of corticosteroids. Recent corticosteroid use was defined as use at the time of visit and/or in the preceding three months and was categorized as local (topical, inhaled, nasal, ocular, intra-articular) or systemic (oral/intra-venous) use and as single or multiple type (i.e., combinations of different administration routes) use. All completed questionnaires were scrutinized by experienced physicians and discussed with the patient at the clinic visit in order to avoid incomplete information or misinterpretation of the questions. These questionnaires and electronic medical records, including records of the visit, were also used to assess weight and height. BMI was computed by dividing weight (kg) by height squared (m^2). Patients in whom the time of corticosteroid use was unknown (N=8) were excluded from the analyses. Ethical approval was obtained for the present study.

Non-obese controls

In order to assess the use of corticosteroids in non-obese subjects, we included participants of two different Dutch cohort studies: the Lifelines and the Netherlands Study of Depression and Anxiety (NESDA) cohort.

The Lifelines cohort is a large population-based cohort study from the Northern Netherlands (www.lifelines.nl) [17]. Participants are observed over an extended period of time and are subjected to multiple moments of data and sample collection. One of the collection procedures requires the patients to complete a questionnaire about corticosteroid use in the past three months. For this study, we included a sample of 295 participants who had completed this self-report research questionnaire. In these persons, we assessed the same anthropometric features and corticosteroid-related characteristics (yes/no current corticosteroid use, types of administration forms, and single or multiple type use) as in the obese outpatients.

The other control cohort was recruited from NESDA, a large ongoing longitudinal cohort study among adult participants with a current or past psychopathological diagnosis together with healthy controls with no previous psychiatric diseases [18]. Here, we evaluated the clinical data and questionnaires of 355 psychiatrically healthy controls in whom the same research questionnaire as in the Lifelines cohort was collected [19]. In order to minimize recall bias with regard to corticosteroid use, we assessed both completed questionnaires and minutely detailed information about medication use that was checked during each visit at the research site.

For comparative analyses, we excluded participants with underweight (BMI<18.50) or obesity (BMI ≥30.00) from both Lifelines (control group I) and NESDA (control group II) cohorts, which resulted in the exclusion of respectively 60 (20.3%) and 61 (17.2%) subjects. From the latter group, also three healthy controls were excluded because of inconclusive data on corticosteroid use. Subsequently, a total number of 526 non-obese controls (control group I, N=235; control group II, N=291) were enrolled in this study. In order to investigate if there was a relationship between corticosteroid use and age and whether the numbers of recent users between obese and non-obese subjects differed with age, we analyzed the differences between both groups in weighted age-tertiles. This resulted in the classification of persons <36 years in the first tertile, 36-49 years in the second, and ≥50 years in the last tertile.

Statistical analysis

Statistical analysis was performed with IBM SPPS Statistics version 21 (IBM Corp., Armonk, NY) and GraphPad Prism version 5.01 (GraphPad Software Inc., La Jolla, CA) for Windows. Differences in demographic and clinical characteristics were analyzed using Chi-square tests and ANOVA's, when appropriate. Trend analysis for corticosteroid use in relation to age-tertiles was performed with the Cochran-Armitage test for trend. Logistic regression analyses were conducted for comparative analyses between the obese and the control groups and were adjusted for age and sex as indicated. P-values below 0.05 were considered to indicate statistical significance for all analyses.

Results

Baseline characteristics

The demographic and clinical characteristics of the three groups are summarized in Table 1. The average BMI in the obese group was 40.7±6.3 kg/m^2 versus 24.7±2.6 (control group I, P<.001) and 24.0±2.8 kg/m^2 (control group II, P<.001) in the non-obese cohorts. All groups consisted primarily of women, with percentages ranging from 64.9% (control group II) up to 75.2% (obese group). Obese participants were on average younger compared to control group II (41.5±14.3 vs. 46.7±14.9 years, P<.001) but were not different in age compared to control group I.

Table 1: Demographic and clinical characteristics of study participants.

	Obese	Non-obese			
		Control group I	P_{diff}	Control group II	P_{diff}
N	274	235		291	
Sex, n (%)			.347		.008
Male	68 (24.8)	67 (28.5)		102 (35.1)	
Female	206 (75.2)	168 (71.5)		189 (64.9)	
Age, years	41.5 (14.3)	42.0 (11.7)	.662	46.7 (14.9)	<.001
BMI, kg/m^2	40.7 (6.3)	24.7 (2.6)	<.001	24.0 (2.8)	<.001

Values are presented as number (percentage) or mean (SD). Differences were analyzed using Chi-square tests and ANOVA.

Corticosteroid use obese versus non-obese

In the obese group, 55.8% of all patients reported having used any form of corticosteroids at any time point. Among the obese subjects, 74/274 (27.0%) subjects were currently using or had used corticosteroids in the past three months. Among the recent users, the inhaled and nasal agents were most commonly used (Table 2). Asthma, hay fever/rhino(-sinusitis), and psoriasis were the main known indications for corticosteroid use (25.7%, 8.9%, and 7.9%; Table 3). Recent use of corticosteroids in the obese group was significantly higher compared to non-obese

from both control cohorts (11.9%, P<.001 [control group I] and 14.8%, P<.001 [control group II]; Figure 1).

Table 2: Recent use of different corticosteroid administration forms in obese and non-obese individuals.

	Obese (N=274)	Non-obese			
		Control group I (N=235)	P_{diff}	Control group I (N=291)	P_{diff}
Local	70 (25.5%)	27 (11.5%)	**<.001**	38 (13.1%)	**<.001**
Topical	21 (7.7%)	11 (4.7%)	.145	17 (5.8%)	.323
Inhaled	38 (13.9%)	7 (3.0%)	**<.001**	11 (3.8%)	**<.001**
Nasal	23 (8.4%)	12 (5.1%)	.147	15 (5.2%)	.173
Ocular	3 (1.1%)	0 (0.0%)	-	1 (0.3%)	.251
Intra-articular	3 (1.1%)	0 (0.0%)	-	0 (0.0%)	-
Systemic (oral/i.v.)	7 (2.6%)	2 (0.9%)	.180	8 (2.7%)	.631
Multiple types	17 (6.2%)	4 (1.7%)	**.015**	7 (2.4%)	**.038**

Values are presented as number (percentage). Differences in use of each corticosteroid administration form between obese patients and the control groups were analyzed separately using logistic regression analyses adjusted for sex and age. Abbreviation: *i.v.*, intra-venous.

Table 3: Indications for recent corticosteroid use in the obese group.

	Corticosteroid prescriptions (N=101)
Asthma, n (%)	26 (25.7)
Hay fever/rhino(-sinusitis), n (%)	9 (8.9)
Psoriasis, n (%)	8 (7.9)
Eczema, n (%)	7 (6.9)
COPD, n (%)	6 (5.9)
Nasal congestion, n (%)	3 (3.0)
Ocular diseases*, n (%)	3 (3.0)
Auto-immune diseases†, n (%)	2 (2.0)
Others‡, n (%)	12 (11.9)
Unknown, n (%)	25 (24.8)

Values are presented as number (percentage). *Includes iridocyclitis, scleritis, and uveitis; †Includes cerebral vasculitis and Crohn's disease; ‡Includes among others alopecia areata, nasal polyps, panhypopituitarism, and renal transplantation.

Dividing the control groups into two weight classes, i.e. "normal weight" (BMI 18.50–24.99) and "overweight" (BMI 25.00-29.99), and comparing these to the obese subjects still resulted in significant differences regarding the recent use of corticosteroids. Largest differences were observed between normal weight controls from both cohorts and the obese subjects (P<.001 [control group I] and P=.001 [control group II]; Figure 1).

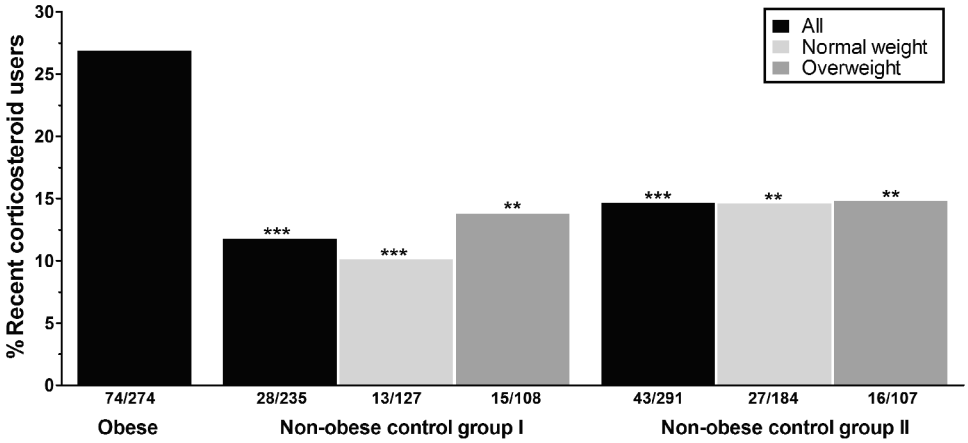

Figure 1: Recent corticosteroid use in obese and non-obese subjects.

Analyses between the obese group and the non-obese control groups as a whole (black bars), or stratified for two weight classes (light gray = normal weight, dark gray = overweight) are controlled for sex and age. All asterisks depict P-values for the comparisons with the obese group. **P<.01, ***P<.001.

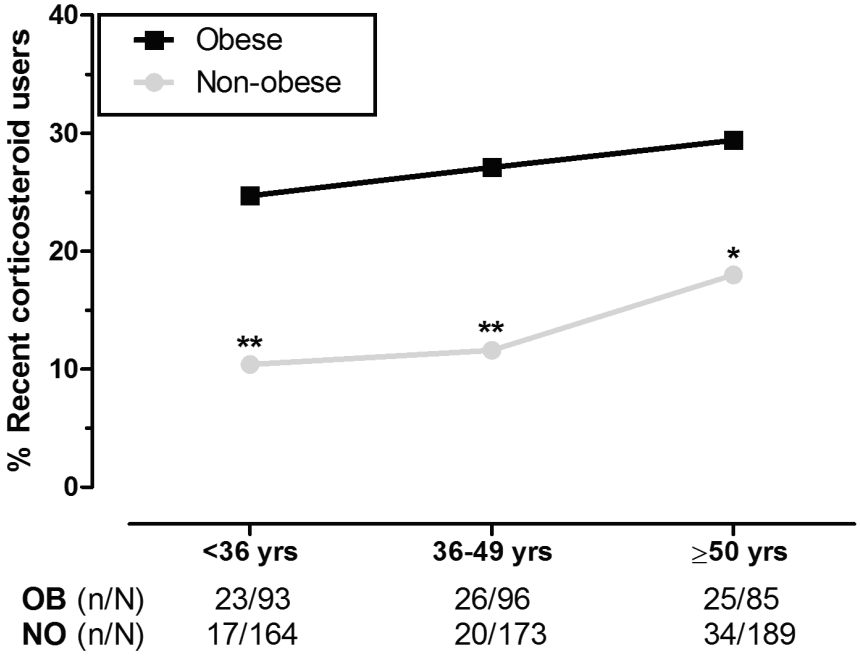

Figure 2: Relation between different age groups and use of corticosteroids.

The three age groups represent weighted age-tertiles of obese and the combined non-obese participants from both control groups. Logistic regression analyses between obese and non-obese age groups are adjusted for sex. *P<.05, **P<.01. Abbreviations: *OB*, obese; *NO*, non-obese.

With regard to age-tertiles, we found significantly higher corticosteroid use in obese subjects for each age group with the smallest difference in the oldest tertile (mean difference per tertile: 14.3%, P=.005 [first tertile], 15.5%, P=.001 [second tertile], and 11.4%, P=.039 [third tertile]; Figure 2). Separate trend analyses showed a significant trend in the non-obese group (x^2=4.520, P=.034) and no significance in the obese (x^2=0.679, P=.410).

Administration routes of corticosteroids

In the obese group, the use of local corticosteroids was significantly higher compared to both non-obese controls (25.5% vs. 11.5% [control group I] and 13.1% [control group II], both P<.001; Table 2). In addition, stratification for the different administration routes revealed significantly higher rates for inhaled corticosteroids in the obese subjects. There were, however, no differences in use of the other local corticosteroids or the systemic administration forms.

Use of multiple types of corticosteroids was present in 17 obese patients (6.2%). This was significantly higher than in the control groups I (1.7%, P=.015) and II (2.4%, P=.038). The majority of the multiple type users of both the obese and the non-obese groups were using at least one inhaled corticosteroid (88% and 73%, respectively). The combination of inhaled corticosteroids with at least one topical corticosteroid was most common in the obese group (47%), whereas in the non-obese controls inhaled forms were frequently combined with nasal corticosteroids (55%; Table 4).

Table 4: Combination of corticosteroids in users of multiple types of corticosteroids.

	Obese (N=17)	Non-obese (N=11)
Inhaled with topical, n (%)	5 (29)	1 (9)
Inhaled with nasal, n (%)	5 (29)	4 (36)
Inhaled with topical and nasal, n (%)	2 (12)	0 (0)
Inhaled with nasal and oral, n (%)	1 (6)	2 (18)
Topical with nasal, n (%)	0 (0)	2 (18)
Others, n (%)	4 (24)	2 (18)

Values are presented as number (percentage) within the group of multiple types users for the obese group and combined non-obese control group.

Marked weight gain

Of the obese subjects who reported recent or ever use of corticosteroids, 10.5% considered the use of corticosteroids as the underlying cause of a period of marked weight gain. The oral administration form was reported most frequently (12/16 subjects) as the triggering factor, followed by two patients who had previously received corticosteroid injections. Majority of the patients from the former administration form (67%) had used or were currently using prednisone for over 3 months continuously, two subjects had been prescribed prednisone for a short-term period (<3 months) and two patients had used it for an unknown duration.

Discussion

To the best of our knowledge, this is the first study to systematically examine corticosteroid use in a diverse sample of obese and non-obese individuals. Here, we have shown that the use of corticosteroids was significantly higher in obese outpatients when compared to non-obese subjects from two separate control groups. This finding was consistent across all age groups but became less evident in the oldest group. Higher rates of use were primarily found for the local corticosteroids, in particular for the inhaled administration forms. In addition, we also found that a significantly higher percentage of the obese individuals were simultaneously using multiple corticosteroid types in comparison to non-obese subjects. However, no differences were observed with respect to oral corticosteroid use.

Cushing's syndrome is most commonly induced by exogenous corticosteroid administration, typically attributed to (long-term) systemic corticosteroid use, and is frequently accompanied by weight gain [20, 21]. However, the increased risk of occurrence of adrenal insufficiency even with local administration forms [16] shows the importance of surveillance for systemic effects of all administration types. We found that more than half of our obese sample have used corticosteroids at any point in time and that their recent use more often involves multiple administration routes, with the latter been strongly linked to supraphysiological systemic levels of glucocorticoids (based on high absolute risk of adrenal insufficiency) [16]. These findings tend to support our hypothesis that local corticosteroid forms, as being the most common prescribed agents in our obese group, could eventually contribute to amongst others a higher weight and/or a more laborious weight loss. But given the nature of this study, it is not possible to demonstrate temporality and to infer a causal relationship between corticosteroid use and obesity.

Regardless of the fact that in this study we did not assess the effect of corticosteroids on weight gain, physicians should be vigilant for corticosteroid-

induced side effects in all patients gaining weight in a short period of time since approximately 10% of the marked weight gain in the ever corticosteroid users seemed to be preceded by corticosteroid use. In concordance with previous reports by Berthon et al, who showed that weight gain as a result of oral corticosteroids is unlikely in short-term users (<3 months) in contrast to long term users (≥3 months) [22, 23], majority of our corticosteroid induced marked weight gainers reported to have used corticosteroids for at least couple of months to several years. The cumulative exposure to corticosteroids seems therefore to be an essential factor in inducing weight changes. Since inhaled corticosteroids are generally prescribed for chronic conditions, and multiple type use most often includes inhaled agents, it is reasonable to hypothesize that these forms more gradually contribute to weight gain. The increasing prevalence of obesity [24] as well as increased corticosteroid use in the past decades [5, 8] additionally nourish the idea that corticosteroid use could be a substantial contributing factor for overall weight gain in the Western world. This is especially important given the fact that corticosteroids not only promote the accumulation of abdominal fat but also stimulate the appetite for high calorie "comfort" foods [12].

However, the cause-and-effect relationship between corticosteroid use and obesity seems to be bidirectional. Besides the well-known cardiometabolic diseases such as diabetes mellitus, dyslipidemia, and atherosclerosis, obesity has been linked to low-grade inflammation and various immune-mediated conditions [25, 26]. In the present study, we found that obese patients are using inhaled corticosteroids more frequently, which are mainly prescribed for asthma and chronic obstructive pulmonary disease (COPD). This is in line with literature where both conditions have been linked to higher BMI [27-29]. Interestingly, in a study with asthmatic obese patients, Van Huisstede et al. showed that weight loss after bariatric surgery was associated with improved asthma control and lower systemic inflammation markers [30]. Similar results were found in other studies in which weight loss was associated with less asthmatic symptoms and increased lung function [31, 32]. In addition, weight loss and lower BMI have also been associated with reduced disease severity or better therapeutic response in other immune-related disorders including psoriasis [33, 34], rheumatoid

arthritis [35, 36], and ankylosing spondylitis [37, 38]. This emphasizes the mentioned relationship between obesity and inflammation and could be an alternative reason for high corticosteroid use in our obese sample. Another plausible explanation would be that there is not a causal link between these parameters but that other factors, such as a low social-economic status and a pro-inflammatory genetic

profile, lead to both obesity and more inflammation subsequently requiring the use of corticosteroids.

Nevertheless, it still remains disputable which of the two directions, i.e. corticosteroid use preceding obesity or vice versa, prevails in clinical practice. Patients with COPD, for instance, commonly present with overweight or obesity [39]. Since corticosteroids are an important part of the medical treatment of COPD, it could be proposed that the overall high BMI in these patients is partly the result of corticosteroid use. Aside from the reverse causality between these characteristics, it would be advisable to screen all obese patients for corticosteroid use. In the case of corticosteroid use, one should reconsider if the use is still necessary and if so, whether an alternative treatment is available. The importance of this can be derived from a previous study in asthmatic obese patients for whom the diagnosis could not be confirmed in 41% of the cases after extensive pulmonary testing, although 23% of these patients were still currently using inhaled corticosteroids [40]. In these cases, ceasing of corticosteroids under medical supervision could potentially help in losing weight more easily. Otherwise, patients may succumb to a vicious cycle of weight gain, obesity-related comorbidities, and further corticosteroid need.

One of the strengths of the present study is the use of two different non-obese control groups and the fact that both the study group and the control groups are from the same country. Moreover, the same detailed questionnaire on corticosteroid use was administered in both non-obese cohorts.

An important study limitation worth noting is that information about the dose and duration of corticosteroid use was incomplete and hence not used in this study. Both components are known to play an important role in the accumulative exposure and induction of side effects in corticosteroid users [41]. Nevertheless, medical conditions requiring corticosteroids are most often of a chronic nature and demand corticosteroid use for a longer period of time or at least with frequent intervals. Moreover, various studies have shown that weight gain can also occur in response to relatively low doses of corticosteroids. In a study of more than two thousand long-term corticosteroid users, Curtis et al. have found that weight gain manifested in 70% of the low-dose systemic users and was indeed the most prevalent self-reported adverse event [11].

In conclusion, corticosteroid use is high in obese individuals who have been referred due to their obesity and common across all ages. High user rates were especially prevalent for inhaled corticosteroids and the simultaneous use of different administration forms. This warrants stricter monitoring of corticosteroid

use in obese as these medications can potentially induce weight gain and maintain excess weight. However, large longitudinal prospective cohort studies are needed to specifically determine the individual effect of the different corticosteroid administration forms on weight gain.

References

1. [Internet] The National Health Care Institute: Diemen, The Netherlands. Genees- en hulpmiddelen Informatie Project (GIP) databank. Revised November 2016. https://www.gipdatabank.nl/databank.asp
2. Hsiao CJ, Cherry DK, Beatty PC, Rechtsteiner EA. National Ambulatory Medical Care Survey: 2007 summary. Natl Health Stat Report. 2010: 1-32.
3. Keane FM, Munn SE, du Vivier AW, Taylor NF, Higgins EM. Analysis of Chinese herbal creams prescribed for dermatological conditions. BMJ. 1999; 318: 563-4.
4. Ramsay HM, Goddard W, Gill S, Moss C. Herbal creams used for atopic eczema in Birmingham, UK illegally contain potent corticosteroids. Archives of Disease in Childhood. 2003; 88: 1056-7.
5. Fardet L, Petersen I, Nazareth I. Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. Rheumatology (Oxford). 2011; 50: 1982-90.
6. van Staa TP, Leufkens HG, Abenhaim L, Begaud B, Zhang B, Cooper C. Use of oral corticosteroids in the United Kingdom. QJM. 2000; 93: 105-11.
7. Overman RA, Yeh JY, Deal CL. Prevalence of oral glucocorticoid usage in the United States: a general population perspective. Arthritis Care Res (Hoboken). 2013; 65: 294-8.
8. van Staa TP, Cooper C, Leufkens HG, Lammers JW, Suissa S. The use of inhaled corticosteroids in the United Kingdom and the Netherlands. Respir Med. 2003; 97: 578-85.
9. Buchman AL. Side effects of corticosteroid therapy. J Clin Gastroenterol. 2001; 33: 289-94.
10. Judd LL, Schettler PJ, Brown ES, Wolkowitz OM, Sternberg EM, Bender BG, et al. Adverse consequences of glucocorticoid medication: psychological, cognitive, and behavioral effects. Am J Psychiatry. 2014; 171: 1045-51.
11. Curtis JR, Westfall AO, Allison J, Bijlsma JW, Freeman A, George V, et al. Population-based assessment of adverse events associated with long-term glucocorticoid use. Arthritis Rheum. 2006; 55: 420-6.
12. Fardet L, Feve B. Systemic glucocorticoid therapy: a review of its metabolic and cardiovascular adverse events. Drugs. 2014; 74: 1731-45.
13. Vegiopoulos A, Herzig S. Glucocorticoids, metabolism and metabolic diseases. Mol Cell Endocrinol. 2007; 275: 43-61.
14. Peckett AJ, Wright DC, Riddell MC. The effects of glucocorticoids on adipose tissue lipid metabolism. Metabolism. 2011; 60: 1500-10.
15. Saag KG, Koehnke R, Caldwell JR, Brasington R, Burmeister LF, Zimmerman B, et al. Low dose long-term corticosteroid therapy in rheumatoid arthritis: an analysis of serious adverse events. Am J Med. 1994; 96: 115-23.
16. Broersen LH, Pereira AM, Jorgensen JO, Dekkers OM. Adrenal Insufficiency in Corticosteroids Use: Systematic Review and Meta-Analysis. J Clin Endocrinol Metab. 2015; 100: 2171-80.

17. Scholtens S, Smidt N, Swertz MA, Bakker SJ, Dotinga A, Vonk JM, et al. Cohort Profile: LifeLines, a three-generation cohort study and biobank. Int J Epidemiol. 2015; 44: 1172-80.
18. Penninx BW, Beekman AT, Smit JH, Zitman FG, Nolen WA, Spinhoven P, et al. The Netherlands Study of Depression and Anxiety (NESDA): rationale, objectives and methods. Int J Methods Psychiatr Res. 2008; 17: 121-40.
19. Staufenbiel SM, Penninx BW, de Rijke YB, van den Akker EL, van Rossum EF. Determinants of hair cortisol and hair cortisone concentrations in adults. Psychoneuroendocrinology. 2015; 60: 182-94.
20. Shibli-Rahhal A, Van Beek M, Schlechte JA. Cushing's syndrome. Clin Dermatol. 2006; 24: 260-5.
21. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet. 2006; 367: 1605-17.
22. Berthon BS, Gibson PG, McElduff P, MacDonald-Wicks LK, Wood LG. Effects of short-term oral corticosteroid intake on dietary intake, body weight and body composition in adults with asthma - a randomized controlled trial. Clin Exp Allergy. 2015; 45: 908-19.
23. Berthon BS, MacDonald-Wicks LK, Wood LG. A systematic review of the effect of oral glucocorticoids on energy intake, appetite, and body weight in humans. Nutr Res. 2014; 34: 179-90.
24. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014; 384: 766-81.
25. Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol. 2015; 3: 207-15.
26. Kanneganti TD, Dixit VD. Immunological complications of obesity. Nat Immunol. 2012; 13: 707-12.
27. Camargo CA, Jr., Weiss ST, Zhang S, Willett WC, Speizer FE. Prospective study of body mass index, weight change, and risk of adult-onset asthma in women. Arch Intern Med. 1999; 159: 2582-8.
28. Ronmark E, Andersson C, Nystrom L, Forsberg B, Jarvholm B, Lundback B. Obesity increases the risk of incident asthma among adults. Eur Respir J. 2005; 25: 282-8.
29. Franssen FM, O'Donnell DE, Goossens GH, Blaak EE, Schols AM. Obesity and the lung: 5. Obesity and COPD. Thorax. 2008; 63: 1110-7.
30. van Huisstede A, Rudolphus A, Castro Cabezas M, Biter LU, van de Geijn GJ, Taube C, et al. Effect of bariatric surgery on asthma control, lung function and bronchial and systemic inflammation in morbidly obese subjects with asthma. Thorax. 2015; 70: 659-67.

31. Maniscalco M, Zedda A, Faraone S, Cerbone MR, Cristiano S, Giardiello C, et al. Weight loss and asthma control in severely obese asthmatic females. Respir Med. 2008; 102: 102-8.
32. Stenius-Aarniala B, Poussa T, Kvarnstrom J, Gronlund EL, Ylikahri M, Mustajoki P. Immediate and long term effects of weight reduction in obese people with asthma: randomised controlled study. BMJ. 2000; 320: 827-32.
33. Jensen P, Zachariae C, Christensen R, Geiker NR, Schaadt BK, Stender S, et al. Effect of weight loss on the severity of psoriasis: a randomized clinical study. JAMA Dermatol. 2013; 149: 795-801.
34. Gisondi P, Del Giglio M, Di Francesco V, Zamboni M, Girolomoni G. Weight loss improves the response of obese patients with moderate-to-severe chronic plaque psoriasis to low-dose cyclosporine therapy: a randomized, controlled, investigator-blinded clinical trial. Am J Clin Nutr. 2008; 88: 1242-7.
35. Heimans L, van den Broek M, le Cessie S, Siegerink B, Riyazi N, Han KH, et al. Association of high body mass index with decreased treatment response to combination therapy in recent-onset rheumatoid arthritis patients. Arthritis Care Res (Hoboken). 2013; 65: 1235-42.
36. Ottaviani S, Gardette A, Tubach F, Roy C, Palazzo E, Gill G, et al. Body mass index and response to infliximab in rheumatoid arthritis. Clin Exp Rheumatol. 2015; 33: 478-83.
37. Ottaviani S, Allanore Y, Tubach F, Forien M, Gardette A, Pasquet B, et al. Body mass index influences the response to infliximab in ankylosing spondylitis. Arthritis Res Ther. 2012; 14: R115.
38. Gremese E, Bernardi S, Bonazza S, Nowik M, Peluso G, Massara A, et al. Body weight, gender and response to TNF-alpha blockers in axial spondyloarthritis. Rheumatology (Oxford). 2014; 53: 875-81.
39. O'Donnell DE, Ciavaglia CE, Neder JA. When obesity and chronic obstructive pulmonary disease collide. Physiological and clinical consequences. Annals of the American Thoracic Society. 2014; 11: 635-44.
40. van Huisstede A, Castro Cabezas M, van de Geijn GJ, Mannaerts GH, Njo TL, Taube C, et al. Underdiagnosis and overdiagnosis of asthma in the morbidly obese. Respir Med. 2013; 107: 1356-64.
41. McDonough AK, Curtis JR, Saag KG. The epidemiology of glucocorticoid-associated adverse events. Curr Opin Rheumatol. 2008; 20: 131-7.

Chapter 4

Associations Between Systemic and Local Corticosteroid Use With Metabolic Syndrome and Body Mass Index

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Abstract

Context: *Use of systemic corticosteroids (CSs) may induce adverse cardiometabolic alterations, potentially leading to obesity and metabolic syndrome (MetS). Although evidence is accumulating that local CSs have considerable systemic effects, their effects on cardiometabolic factors in the general population remain unclear.*

Objective: *To investigate the association between overall CS use and specific CS types with MetS, body mass index (BMI), and other cardiometabolic traits.*

Design: *Cross-sectional cohort study.*

Setting: *General population from the northern Netherlands.*

Participants: *A total of 140,879 adult participants in the population-based Lifelines Cohort Study.*

Main Outcome Measures: *BMI, waist circumference, systolic and diastolic blood pressure, fasting metabolic serum parameters, and a comprehensive set of potential confounding factors.*

Results: *In women, overall, systemic, and local CS use was associated with higher odds of having MetS. Among local female users, only nasal (odds ratio [OR], 1.20 [95% confidence interval (CI), 1.06 to 1.36]) and inhaled CSs [OR, 1.35 (95% CI, 1.24 to 1.49)] users were more likely to have MetS. In men, no association was found between overall and specific CS use and presence of MetS. Use of local-only CSs in women, specifically inhaled CSs in both sexes, was associated with higher BMI.*

Conclusions: *Use of local CSs, particularly inhaled types, as well as systemic CSs, was associated with higher likelihood of having MetS, higher BMI, and other adverse cardiometabolic traits, especially among women. Because the inhaled agents are the main group of prescribed CSs, this might be a substantial risk to public health in case of a yet-to-be-proven causal relationship.*

Introduction

Synthetic glucocorticoids, also known as corticosteroids (CSs), are widely used potent anti-inflammatory drugs with multiple indications and many administration forms used for both systemic and local disorders [1]. Due to the increased prevalence of diseases frequently requiring CS therapy, prescriptions of CSs have increased markedly in the last decades [2, 3]. There are increasing concerns that use of systemic administration forms can lead to supraphysiological glucocorticoid exposure and induce adverse cardiometabolic changes such as obesity, diabetes, dyslipidemia, and hypertension, all of which are components of the metabolic syndrome (MetS) [4, 5]. The relationship between high glucocorticoid exposure and induction of various cardiometabolic alterations has been consistently reported in patients with Cushing syndrome who frequently develop these adverse metabolic changes during the course of the disease [6]. Because of these known adverse events, systemic CS users are generally well-monitored after starting treatment [5], in contrast to users of the various local administration forms in whom systemic absorption is usually less expected. However, a recent meta-analysis suggests that local CSs may also be associated with an increased systemic glucocorticoid exposure exemplified by the increased risk of adrenal insufficiency in users of local types [7]. Because many of the CS users are often prescribed a local administration form, it could be hypothesized that use of local CSs is a contributing factor to MetS and obesity in the general population. Nevertheless, most studies on this topic have been focused on systemic CS therapies [4], and evidence regarding the effect of local CS use on MetS and its components in the general population is scarce. Hence, we assessed the associations between overall CS use and specific CS types with MetS, body mass index (BMI), and other cardiometabolic risk factors in a large population-based cohort study.

Methods

Study design and population

Lifelines is a multidisciplinary, prospective, population-based cohort study examining, in a unique three-generation design, the health and health-related behaviors of 167,729 persons living in the northern Netherlands [8]. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical, and psychological factors that contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics. For this study, we included baseline information on 152,180 adult participants. Subjects with incomplete report on drug use, nonfasting laboratory blood values, or missing information on any of the MetS components or BMI were excluded from the analyses, which resulted in a total study sample size of 140,879 participants. Informed consent and ethical

approval of the study protocol were obtained according to the principles of the Declaration of Helsinki and in accordance with the research code of the University Medical Center Groningen.

Assessment of drug use

Drug use was evaluated with a self-reported questionnaire and a visual drug container inspection. All prescribed drugs were coded according to the World Health Organization Anatomical Therapeutic Chemical (ATC) classification system. Concurring with the ATC methodology, we classified CSs into the following categories of administration forms: systemic (i.e., oral and parenteral, including intra-articular injections), topical (i.e., dermatological), nasal, inhaled, otological, ocular, intestinal, local oral, hemorrhoidal, and gynecological forms. The last three forms were combined as "other CSs" due to their low prescription numbers. For assessment of the presence of MetS and its components, we assessed the use of antihypertensives, blood glucose–lowering drugs, and lipid-modifying drugs. We also determined the use of hormonal replacement therapy in women and the use of other exogenous sex hormones and potentially weight-inducing psychotropics [9, 10] in all subjects to adjust for their potential metabolic alterations (see Supplemental Table 1 for further details).

Measures of MetS risk factors

All measurements were done consistently following standardized operating protocols by trained technicians. Body weight (in kg) and height (in cm) were measured without shoes and accurately to the nearest half unit. BMI was calculated by dividing body weight by height in meters squared. Waist circumference (WC) was measured in an upright position and in the middle between the front edge of the lower ribs and the iliac crest. Blood pressure was measured 10 times with a 1-minute interval with an automatic blood pressure monitor (DinaMap Monitor; GE Health Care, Freiburg, Germany) and proper-sized cuff. The last two successive measurements most representative of resting blood pressure were used to calculate mean systolic blood pressure (SBP) and diastolic blood pressure (DBP). Blood samples were taken in the morning after an overnight fast and were processed for measurements on the same day. Measurements for triglycerides, high-density lipoprotein (HDL)-cholesterol, and glucose were performed on a Roche Modular P chemistry analyzer (Roche, Basel, Switzerland) by using enzymatic colorimetric and hexokinase methods. Data on BMI, WC, SBP, DBP, and fasting serum levels of triglycerides, HDL-cholesterol, and glucose were complete for all subjects.

Table 1: Descriptive characteristics of the study population.

	Women (N=82 443)			Men (N=58 436)		
	Non-CS users	CS users	P*diff*	Non-CS users	CS users	P*diff*
Numbers	72 832 (88.3%)	9611 (11.7%)		52 719 (90.2%)	5717 (9.8%)	
Age (years)	44.2 (13.0)	45.6 (13.4)	<.001	45.3 (13.1)	47.4 (13.6)	<.001
Ethnicity			.110			.003
Dutch native	68 707 (94.3%)	9028 (93.9%)		50 239 (95.3%)	5397 (94.4%)	
Others	4125 (5.7%)	583 (6.1%)		2480 (4.7%)	320 (5.6%)	
Education level						
No education	343 (0.5%)	70 (0.7%)		286 (0.5%)	40 (0.7%)	
Primary education	1562 (2.1%)	279 (2.9%)		1077 (2.0%)	169 (3.0%)	
Lower or preparatory vocational education	8471 (11.6%)	1258 (13.1%)		8282 (15.7%)	892 (15.6%)	
Lower general secondary education	11 141 (15.3%)	1513 (15.7%)		6000 (11.4%)	668 (11.7%)	
Intermediate vocational education for apprenticeship	21 879 (30.0%)	2839 (29.5%)	<.001	16 286 (30.9%)	1603 (28.0%)	<.001
Higher general secondary education or pre-university secondary education	7227 (9.9%)	890 (9.3%)		3730 (7.1%)	393 (6.9%)	
Higher vocational education	16 815 (23.1%)	2057 (21.4%)		12 600 (23.9%)	1443 (25.2%)	
University	3821 (5.2%)	467 (4.9%)		3699 (7.0%)	412 (7.2%)	
Other education	1573 (2.2%)	238 (2.5%)		759 (1.4%)	97 (1.7%)	
Smoking						
Non-smoker	35 502 (48.7%)	4779 (49.7%)	<.001	22 793 (43.2%)	2540 (44.4%)	<.001
Former smoker	22 356 (30.7%)	3151 (32.8%)		17 037 (32.3%)	2126 (37.2%)	
Current smoker	14 974 (20.6%)	1681 (17.5%)		12 889 (24.4%)	1051 (18.4%)	
Alcohol use						
None	20 308 (27.9%)	2935 (30.5%)		5652 (10.7%)	683 (11.9%)	
≤ 1 drink/day	39 501 (54.2%)	5016 (52.2%)	<.001	23 279 (44.2%)	2582 (45.2%)	.003
1-2 drinks/day	10 814 (14.8%)	1350 (14.0%)		15 348 (29.1%)	1579 (27.6%)	
>2 drinks/day	2209 (3.0%)	310 (3.2%)		8440 (16.0%)	873 (15.3%)	
Physical activity						
Inactive	4390 (6.0%)	639 (6.6%)	.042	2218 (4.2%)	227 (4.0%)	.078
Semi-active	32 837 (45.1%)	4265 (44.4%)		25 137 (47.7%)	2651 (46.4%)	
Norm-active	35 605 (48.9%)	4707 (49.0%)		25 364 (48.1%)	2839 (49.7%)	

Menstrual status						
Menstruating	45 678 (62.7%)	5556 (57.8%)	<.001	N/A	N/A	
Not menstruating	27 154 (37.3%)	4055 (42.2%)		N/A	N/A	
Comorbidities						
Diabetes mellitus	1588 (2.2%)	347 (3.6%)	<.001	1927 (3.7%)	254 (4.4%)	.003
Hypertension	15 451 (21.2%)	2602 (27.1%)	<.001	16 916 (32.1%)	2144 (37.5%)	<.001
Stroke	495 (0.7%)	72 (0.7%)	.438	476 (0.9%)	70 (1.2%)	.016
Coronary heart disease	564 (0.8%)	125 (1.3%)	<.001	1686 (3.2%)	237 (4.1%)	<.001
Cancer	3757 (5.2%)	544 (5.7%)	.038	1919 (3.6%)	280 (4.9%)	<.001
Osteoarthritis	5970 (8.2%)	1113 (11.6%)	<.001	2916 (5.5%)	405 (7.1%)	<.001
Chronic obstructive pulmonary disease	2071 (2.8%)	2371 (24.7%)	<.001	1623 (3.1%)	1469 (25.7%)	<.001
Asthma	3459 (4.7%)	3584 (37.3%)	<.001	2721 (5.2%)	1917 (33.5%)	<.001
Drug use*						
Antihypertensives	8643 (11.9%)	1649 (17.2%)	<.001	6809 (12.9%)	1011 (17.7%)	<.001
Blood glucose-lowering drugs	1126 (1.5%)	244 (2.5%)	<.001	1254 (2.4%)	181 (3.2%)	<.001
Lipid-modifying drugs	3555 (4.9%)	687 (7.1%)	<.001	4618 (8.8%)	642 (11.2%)	<.001
HRT, only female sex hormones	13 054 (17.9%)	1957 (20.4%)	<.001	N/A	N/A	
HRT, other sex hormones	824 (1.1%)	144 (1.5%)	.002	85 (0.2%)	30 (0.5%)	<.001
Psychotropics	4829 (6.6%)	868 (9.0%)	<.001	1870 (3.5%)	261 (4.6%)	<.001
Cardiometabolic traits						
Body mass index (kg/m^2)	25.7 (4.6)	26.7 (5.3)	<.001	26.4 (3.7)	26.7 (3.9)	<.001
Waist circumference (cm)	86.4 (12.1)	89.1 (13.4)	<.001	95.0 (10.8)	96.7 (11.5)	<.001
Systolic blood pressure (mm Hg)	121.9 (15.3)	123.5 (15.4)	<.001	130.3 (14.1)	131.1 (14.2)	<.001
Diastolic blood pressure (mm Hg)	71.7 (8.8)	72.0 (8.7)	<.001	76.4 (9.4)	77.2 (9.3)	<.001
Triglycerides (mmol/L)†,‡	1.02 (0.58)	1.08 (0.61)	<.001	1.40 (1.02)	1.40 (0.93)	.298
HDL-cholesterol (mmol/L)‡	1.62 (0.40)	1.61 (0.40)	.026	1.31 (0.32)	1.32 (0.33)	.011
Glucose (mmol/L)‡	4.89 (0.76)	4.96 (0.86)	<.001	5.18 (0.90)	5.20 (0.92)	.100
Metabolic syndrome	10 323 (14.2%)	1874 (19.5%)	<.001	11 020 (20.9%)	1348 (23.6%)	<.001

Data are provided as mean (SD) or numbers (%). *The ATC-codes for the included drugs are depicted in Supplemental Table 1; †Descriptive data shown for original untransformed data; ‡Values can be converted to conventional units (i.e. mg/dL) by dividing by the following conversion factors: 0.0113 for triglycerides, 0.0259 for HDL-cholesterol, and 0.0555 for glucose. Abbreviations: *CS*, corticosteroids; *HDL*, high density lipoprotein; *HRT*, hormone replacement therapy; *N/A*, not applicable.

Assessment of MetS

MetS was defined according to the joint interim statement criteria [11]. The diagnosis could be established if at least three of the following components were present: (1) WC ≥88 cm in women and ≥102 cm in men; (2) SBP ≥130 mm Hg, DBP ≥85 mm Hg, and/or use of antihypertensives in patients with known hypertension; (3) triglycerides ≥1.7 mmol/L and/or use of lipid-modifying drugs; (4) HDL-cholesterol <1.3 mmol/L in women and <1.0 mmol/L in men and/or use of lipid-modifying drugs; and (5) fasting serum glucose ≥5.6 mmol/L and/or use of blood glucose–lowering drugs.

Assessment of covariates

To adjust for factors that might influence the outcome of the analyses, we assessed data for various potential covariates. Ethnicity was grouped into two categories (i.e., Dutch natives and others) and was based on the reported country of birth of both parents. Education was based on the highest completed level and was classified as no education, primary education, lower or preparatory vocational education, lower general secondary education, intermediate vocational education or apprenticeship, higher general secondary education or preuniversity secondary education, higher vocational education, university, and others. Smoking was categorized under the following three statuses: nonsmokers (i.e., not smoked in the past month and never smoked for a full year), former smokers (i.e., stopped smoking, had not smoked in the past month but had smoked for a full year or more in the past), and current smokers (i.e., currently smoking or smoked in the past month). Alcohol use was based on self-reported drinking frequency of alcoholic beverages in the past month and the average amount per drinking day and was computed into categories of nonusers and users of up to one drink per day, one to two drinks per day, or more than two drinks per day. Physical activity was assessed by the reported average days per week of at least half an hour of doing odd jobs, gardening, bicycling, or exercises combined and classified into three categories: inactives (0 days per week), semiactives (1 to 4 days per week), and norm-actives (≥5 days per week). In women, we additionally assessed their menstrual status (yes/no currently menstruating) at the moment of inclusion.

Diabetes mellitus was defined according the definition of World Health Organization/International Diabetes Federation [12] and was deemed present in case of fasting serum glucose level ≥7.0 mmol/L and/or use of blood glucose–lowering drugs. Corresponding to the report of the Seventh Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [13], hypertension was defined as SBP ≥140 mm Hg, DBP ≥90 mm Hg, and/or use of antihypertensives. Cardiovascular diseases were assessed by self-

reported health questionnaire items and were defined as a history of stroke and/or coronary heart disease(s) (i.e., myocardial infarction, balloon angioplasty, and/or bypass surgery in the past). The other weight-related comorbidities [i.e., cancer, osteoarthritis, chronic obstructive pulmonary disease (COPD), and asthma] were all deemed present if the subject had indicated to be known with the diagnosis and, in the case of asthma, the diagnosis was confirmed by a doctor.

Statistical analysis

Crude differences in continuous variables were assessed with analysis of variance and in categorical variables with x^2 tests. Triglyceride levels were positively skewed and were therefore log10-transformed to achieve normal distribution. Initial inferential analyses showed strong interaction effect (P_{int} <0.001) for sex; hence we decided to stratify all analyses for women and men. We used multivariable logistic regression models to assess the relation of overall and specific CS use with the presence of MetS. Considering the multiple potential combinations of the five components required for the diagnosis, we also analyzed the association of CS use with each component separately and with all possible combinations. CS users were analyzed (1) by combining all types of CSs and (2) by differentiating between systemic users (i.e., systemic only or combined with local forms) and local-only users (i.e., any of the forms except the systemic). Further, we additionally performed analyses for single-type users and multiple administration forms, because the last has been previously shown to be associated with substantial risk of adrenal insufficiency (7). In the first model, the association between CS use (total and specified groups) and MetS was adjusted for age. In the second model, we concurrently adjusted for all covariates. We used analysis of covariances to assess the association of CS use with BMI and other cardiometabolic traits. Adjustments were done similarly as in the second logistic regression model, with additional corrections for diabetes mellitus, use of lipid-modifying drugs, and antihypertensive use. Data on the covariates were missing in <4% of the subjects, except for physical activity (5.9%), alcohol use (7.1%), and menstrual status (8.2%). Missing data were iteratively imputed in five imputation data sets by using the Markov Chain Monte Carlo method. All analyses were conducted two sided, with 0.05 as level of significance, and performed with IBM SPSS Statistics version 21.0.01 (IBM Corp., Armonk, NY).

Sensitivity analyses

Adjustment of the main analysis for menstrual status did likely not fully differentiate the effect of menopause on MetS diagnosis. Due to the expected higher prevalence of MetS in postmenopausal women and with increasing age, we repeated these analyses stratified for age below, and equal to, or above 50 years.

To explore the presence of confounding by indication, we additionally repeated the analysis in both sexes in subjects with and without osteoarthritis, asthma, and/or COPD. Moreover, because adiposity is evidently related to MetS and adverse cardiometabolic traits, we also stratified our main analysis by obesity levels (BMI <30.0 and ≥30.0 kg/m^2).

Results

Overall, 58.5% of the subjects were women and a total of 10.9% was currently using any form of CSs. All descriptive characteristics for both sexes and stratified for CS use are shown in Table 1. CS use was present in 11.7% and 9.8% of female and male subjects, respectively, and comprised predominantly the use of local-only administration forms (95.4% and 95.3%) and single-type users (81.9% and 84.8%). The most prescribed CSs in both single- and multiple-type users were inhaled, nasal, and topical agents, consecutively (Table 2). MetS was more common in men when compared with women. Both male and female CS users were more likely to have MetS when compared with nonusers, but the relative difference in women was much higher than in men (+5.3% vs +2.7%, P<0.001).

CS use and MetS diagnosis

Female CS users had higher likelihood of having MetS in comparison with nonusers, which remained statistically significant after full adjustments for covariates (odds ratio [OR], 1.24 [95% confidence interval (CI), 1.17 to 1.32], P<0.001; Table 3). Stratified analyses for systemic and local-only female CS users revealed increased odds for both group of users, with the strongest association in users of systemic agents [ORs, 1.68 (95% CI, 1.34 to 2.10) and 1.22 (95% CI, 1.14 to 1.30), both P<0.001, respectively]. The associations in female users of local-only CSs were mainly driven by subjects using nasal [OR, 1.20 (95% CI, 1.06 to 1.36), P=0.005] and inhaled CSs [OR, 1.35 (95% CI, 1.24 to 1.49), P<0.001]. In contrast, for men, there was no association between CS use, neither for systemic nor local-only use, and MetS.

CS use and MetS components

CS use in women was associated with significantly higher odds for each of the five MetS components and all of the possible combinations required for MetS diagnosis (Fig. 1). These findings were consistent for both users of systemic and local-only CSs, except for the reduced HDL-cholesterol component in the former group [OR, 1.20 (95% CI, 0.96 to 1.49), P=0.102]. In men, CS use was only associated with the elevated WC component [OR, 1.14 (95% CI, 1.06 to 1.21), P<0.001]. Considering administration route, the relation with WC component in men remained in local-only users [OR, 1.15 (95% CI, 1.07 to 1.23), P<0.001], whereas systemic CS use was

associated with decreased odds of having the elevated fasting glucose component [OR, 0.57 (95% CI, 0.41 to 0.80), P=0.001]. Moreover, in men, an inverse relation was found between systemic CS use and nearly all MetS combinations consisting of at least the HDL-cholesterol and fasting glucose components.

CS use and cardiometabolic traits

The associations between overall CS use and specific CS administration forms and types with cardiometabolic traits are presented in Fig. 2 (see also Supplemental Table 2 for adjusted mean differences).

Female CS users had higher BMI [+0.47 kg/m^2 (95% CI, 0.38 to 0.57)], WC [+1.38 cm (95% CI, 1.13 to 1.63)], SBP [+0.37 mm Hg (95% CI, 0.06 to 0.68)], and triglycerides [+0.007 log mmol/L (95% CI, 0.003 to 0.011)] when compared with nonusers. Similar findings together with nominally significant higher fasting serum glucose levels [+0.01 mmol/L (95% CI, 0.001 to 0.03)] were also present in users of local-only CSs. Systemic CS users, by contrast, had increased HDL-cholesterol [+0.09 mmol/L (95% CI, 0.06 to 0.13)] and decreased fasting serum glucose levels [–0.26 mmol/L (95% CI, –0.32 to –0.21)] in addition to an increased WC [+1.72 cm (95% CI, 0.66 to 2.79)] and triglycerides [+0.050 log mmol/L (95% CI, 0.033 to 0.068)]. Inhaled CS users also had higher BMI [+0.86 kg/m^2 (95% CI, 0.70 to 1.02)], WC [+2.43 cm (95% CI, 2.02 to 2.83)], SBP [+0.69 mm Hg (95% CI, 0.20 to 1.19)], and fasting serum glucose levels [+0.03 mmol/L (95% CI, 0.01 to 0.05)].

In men, local-only CS use was associated with a higher WC [+0.79 cm (95% CI, 0.51 to 1.08)] and DBP [+0.52 mm Hg (95% CI, 0.26 to 0.78)]. Systemic CS use was associated with higher HDL-cholesterol [+0.18 mmol/L (95% CI, 0.14 to 0.21)] and lower fasting serum glucose [–0.34 mmol/L (95% CI, –0.42 to –0.26)]. Of the different administration types, use of inhaled CSs in men was also associated with higher BMI [+0.25 kg/m^2 (95% CI, 0.09 to 0.41)], WC [+1.44 cm (95% CI, 0.97 to 1.90)], and SBP [+0.74 mm Hg (95% CI, 0.11 to 1.37)], in addition to higher DBP [+0.60 mm Hg (95% CI, 0.18 to 1.01)] and HDL-cholesterol [+0.02 mmol/L (95% CI, 0.01 to 0.04)].

Sensitivity analyses

Analyses stratified by menopause status in women, age, and presence of inflammatory diseases yielded nearly similar results with the main analyses (Supplemental Tables 3 and 4). Stratification by BMI did not change the results in men but revealed higher likelihood of having MetS in local-only users only in nonobese females, which was largely explained by the inhaled CS users (Supplemental Table 5).

Table 2: Corticosteroid use categorized by route of administration and number of types.

	Female corticosteroid users (N=9611)			Male corticosteroid users (N=5717)		
Administration route	**Local only use**	**Systemic use***		**Local only use**	**Systemic use***	
	9170 (95.4%)	441 (4.6%)		5451 (95.3%)	266 (4.7%)	
Number of types	**All users†**	**Single type use‡**	**Multiple type use§**	**All users†**	**Single type use‡**	**Multiple type use§**
Systemic corticosteroids	441 (4.6%)	311(3.9%)	130 (7.5%)	266 (4.7%)	192 (4.0%)	74 (8.5%)
Topical corticosteroids	2122 (22.1%)	1566 (19.9%)	556 (32.0%)	1428 (25.0%)	1124 (23.2%)	304 (35.1%)
Nasal corticosteroids	3566 (37.1%)	2201 (27.9%)	1365 (78.7%)	1965 (34.4%)	1321 (27.2%)	644 (74.3%)
Inhaled corticosteroids	4969 (51.7%)	3529 (44.8%)	1440 (83.0%)	2750 (48.1%)	2032 (41.9%)	718 (82.8%)
Otological corticosteroids	109 (1.1%)	61 (0.8%)	48 (2.8%)	59 (1.0%)	37 (0.8%)	22 (2.5%)
Ocular corticosteroids	134 (1.4%)	102 (1.3%)	32 (1.8%)	102 (1.8%)	89 (1.8%)	13 (1.5%)
Intestinal corticosteroids	88 (0.9%)	66 (0.8%)	22 (1.3%)	38 (0.7%)	32 (0.7%)	6 (0.7%)
Others • Hemorrhoidal corticosteroids • Local oral corticosteroids • Gynaecological corticosteroids	75 (0.8%) 40 (0.4%) 35 (0.4%) 0 (0.0%)	40 (0.5%) 23 (0.3%) 17 (0.2%) 0 (0.0%)	35 (2.0%) 17 (1.0%) 18 (1.0%) 0 (0.0%)	49 (0.9%) 32 (0.6%) 17 (0.3%) 0 (0.0%)	23 (0.5%) 14 (0.3%) 9 (0.2%) 0 (0.0%)	26 (3.0%) 18 (2.1%) 8 (0.9%) 0 (0.0%)
Total users	9611 (100.0%)	7876 (81.9%)	1735 (18.1%)	5717 (100.0%)	4850 (84.8%)	867 (15.2%)

Values are provided as numbers (%). *Includes also subjects using systemic corticosteroids in combination with local forms. †,‡,§ Percentages between brackets indicate the proportion of users within the group of total corticosteroid users, single type users, and multiple type users respectively.

Table 3: Odds ratios (95% confidence interval) for the association between corticosteroid use and metabolic.

	Women (N=82 443)				Men (N=58 436)			
			Metabolic syndrome				*Metabolic syndrome*	
	N	MetS*	Model 1	Model 2	N	MetS*	Model 1	Model 2
Total corticosteroid use	9611	1874 (19.5%)	1.38 (1.30 to 1.46)***	1.24 (1.17 to 1.32)***	5717	1348 (23.6%)	1.05 (0.98 to 1.12)	1.00 (0.93 to 1.08)
• Local only use	9170	1731 (18.9%)	1.35 (1.27 to 1.44)***	1.22 (1.14 to 1.30)***	5451	1265 (23.2%)	1.04 (0.97 to 1.12)	1.00 (0.93 to 1.08)
• Systemic use	441	143 (32.4%)	1.97 (1.59 to 2.45)***	1.68 (1.34 to 2.10)***	266	83 (31.2%)	1.15 (0.88 to 1.52)	0.97 (0.72 to 1.30)
Multiple type use	1735	341 (19.7%)	**1.40 (1.24 to 1.59)*** **	**1.26 (1.10 to 1.44)*** **	867	209 (24.1%)	1.03 (0.88 to 1.22)	0.93 (0.78 to 1.10)
Single type use	7876	1533 (19.5%)	**1.38 (1.29 to 1.47)*** **	**1.24 (1.16 to 1.32)*** **	4850	1139 (23.5%)	1.05 (0.98 to 1.13)	1.01 (0.94 to 1.10)
• Systemic corticosteroid(s)	311	101 (32.5%)	**1.96 (1.51 to 2.53)*** **	**1.74 (1.33 to 2.27)*** **	192	52 (27.1%)	0.97 (0.69 to 1.35)	0.88 (0.62 to 1.26)
• Topical corticosteroid(s)	1566	218 (13.9%)	0.98 (0.84 to 1.15)	0.98 (0.84 to 1.15)	1124	214 (19.0%)	0.86 (0.73 to 1.00)	0.90 (0.77 to 1.06)
• Nasal corticosteroid(s)	2201	331 (15.0%)	**1.18 (1.04 to 1.33)** **	**1.20 (1.06 to 1.36)** **	1321	262 (19.8%)	0.97 (0.84 to 1.11)	1.00 (0.86 to 1.16)
• Inhaled corticosteroid(s)	3529	840 (23.8%)	**1.66 (1.52 to 1.80)*** **	**1.35 (1.24 to 1.49)*** **	2032	559 (27.5%)	**1.21 (1.09 to 1.34)*** **	1.08 (0.96 to 1.21)
• Otological corticosteroid(s)	61	11 (18.0%)	1.22 (0.61 to 2.41)	1.14 (0.57 to 2.31)	37	13 (35.1%)	1.76 (0.86 to 3.62)	1.55 (0.71 to 3.38)
• Ocular corticosteroid(s)	102	18 (17.6%)	0.96 (0.57 to 1.64)	0.93 (0.54 to 1.60)	89	25 (28.1%)	1.13 (0.70 to 1.84)	1.26 (0.77 to 2.07)
• Intestinal corticosteroid(s)	66	11 (16.7%)	1.14 (0.58 to 2.24)	0.91 (0.44 to 1.86)	32	9 (28.1%)	1.22 (0.54 to 2.76)	1.09 (0.45 to 2.63)
• Other corticosteroid(s)	40	3 (7.5%)	0.37 (0.11 to 1.24)	0.32 (0.09 to 1.11)	23	5 (21.7%)	1.04 (0.38 to 2.86)	1.12 (0.40 to 3.12)

In model 1, associations were adjusted for age. In model 2, additional adjustments were performed for ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e. stroke and/or coronary heart disease), other comorbidities (i.e. cancer, osteoarthritis, COPD, and/or asthma), use of potentially weight-inducing psychotropics, hormonal replacement therapy (only female sex hormones (only in women), and other sex hormones (in both sexes)), and menstrual status (only in women). Non-CS users were taken as reference group for all analyses. Abbreviation: MetS, metabolic syndrome. **P<0.010, ***P<0.001. *Numbers and percentages of subjects with metabolic syndrome diagnosis are given for the corresponding group of corticosteroid users. Prevalences of MetS in the group of female and male non-corticosteroid users were 14.2% and 20.9%, respectively.

Discussion

Overall, we found that use of local CSs is associated with MetS, especially in women in the general population. Moreover, users of local CSs in both men and women had more adverse cardiometabolic traits when compared with nonusers. Among the various local CSs, the strongest associations were found in users of inhaled administration forms.

It is unclear why CS use is associated with the presence of MetS in women but not in men. Sex-differences in side effects of CS use have been reported previously, with women being more susceptible [14–16]. Emerging evidence shows that CSs are associated with a decrease in bone mineral density [14, 15] and increased rate of skin bruising in women but not in men [16]. CS-induced lipodystrophy is also more common in women than in men and is associated with hypercholesterolemia, hypertriglyceridemia, and insulin resistance [17–19]. Sex differences exist in drug absorption, distribution, metabolism, and elimination, and therefore men and women might differ in their response to drug treatment [20]. Furthermore, women use inhaled CSs more often than men and have a higher reported adherence and positive attitude in regard to their medication [21]. Moreover, administration of CSs reduces the levels of sex hormones, including estrogen and testosterone, which have sex-specific cardiometabolic effects [22–25]. Also, high glucocorticoid exposure is well known to induce visceral fat accumulation [6, 26], which is recognized as a key driver of metabolic alterations [26]. Given the sexual dimorphism in fat distribution, with women having a more gynoid fat deposition, changes in fat differences due to exogenously administered CSs may be more obvious in women.

The strongest relation between local CS use and both increased presence of MetS and adverse cardiometabolic traits was found in inhaled CS users. Previous studies have assessed the safety of inhaled CSs by investigating the risk on various systemic adverse events other than MetS and found, for example, a higher risk for cataract formation [27], loss of bone mineral density [14, 15, 28], and cutaneous atrophy [29]. These and our findings correspond to the general hypothesis that inhaled CSs can induce serious systemic effects. Despite several small, prospective trials demonstrating systemic absorption of inhaled CSs [30–32], large and long-term randomized, placebo-controlled trials in CS-naive subjects focusing on cortisol-related metabolic effects are currently lacking. Nevertheless, the pharmacological characteristics of inhaled CSs have been extensively studied and support the hypothesis that these agents possess a high potential to induce systemic alterations [33–35]. It is known, for example, that the largest proportion of the inhaled dose (i.e., around 50% to 90%) is deposited in the oropharyngeal area, swallowed, and eventually absorbed in the gut as it is for the systemic variants. Besides, a fraction of the inhaled CSs will be

deposited in the lungs and directly absorbed into the circulation without being subjected to the presystemic metabolism of the liver [33, 34].

The distribution of the different types of inhaled CSs in this study were similar in both sexes and consisted predominantly of agents containing budesonide or fluticasone (Supplemental Fig. 1), which bind to the glucocorticoid receptor with an affinity of 9.4 and 18.0 times greater, respectively, than dexamethasone [33, 35]. Moreover, a relatively high fraction of these two agents is unbound when present in the circulation, in contrast to the more recently developed CSs (e.g., ciclesonide and mometasone furoate) [33, 35]. These and other factors such as particle size, lipophilicity, and clearance rate, as well as the type of inhaler device, determine the net amount of systemic availability and the potential for systemic adverse events in inhaled CS users [33–35]. Additionally, most of the inhaled users were using combination agents of CSs with beta-agonists, with the latter also being related to metabolic alterations [36]. It would therefore be conceivable that part of the increased MetS difference is due to the systemic availability of these agents. However, after full adjustment for covariates relevant to MetS as an outcome, we found rather similar likelihoods for users of only inhaled CSs with and without beta-agonists in both sexes (Supplemental Table 6).

In the current study, we additionally demonstrated an increased likelihood for MetS in women using only nasal CSs. The prescription pattern of the nasal CSs differed slightly from the inhaled forms in our sample, with fluticasone and mometasone furoate comprising the majority of the agents being used (Supplemental Fig. 2). These agents can, just as the inhaled forms, be absorbed directly into the circulation by local uptake in the nasopharynx or via the gastrointestinal tract after transportation by the nasociliary mucosa and hence theoretically exert systemic effects [34, 37]. However, both agents are considered to have very low systemic bioavailability of <1% with nasal administration [37] and have previously been shown not to evidently alter the hypothalamic-pituitary-adrenal axis function even when regularly administered or in high doses [38–40]. Because the main indications for nasal and inhaled forms (i.e., allergic rhinitis and asthma) are often present alongside [41], the effects of nasal CSs could perhaps be overestimated by prior use of inhaled CSs.

The relevance of this work could be put in context with the results of a previous large observational study by Souverein et al. [42] showing that users of systemic CSs, including also systemic with inhaled CS users, have increased risk for ischemic heart disease and heart failure events. Similar results were also shown in other large studies in which use of CSs was found to be associated with higher risk of

cardiovascular events [43, 44]. This was especially evident in the proportion of the CS users who eventually developed an iatrogenic Cushing syndrome, who were found to have higher risk in comparison with both nonusers and CS users not developing a Cushing-like phenotype [45]. Given the fact that from the different administration forms our findings were especially evident in users of systemic and inhaled CSs (both agents with high potential to enter the bloodstream) and because patients with Cushing syndrome are known to have increased cardiovascular disease risk [6], our results strengthen the hypothesis that these users could also be at risk for MetS complications.

There are several strengths of our present study. This is, to our knowledge, the first population-based study to examine the association between overall and specific use of CSs and the presence of MetS and its components. High-quality information about exposure and well-characterized participants are other strengths of the current investigation. Furthermore, the large sample size allowed us to perform several subgroup analyses. However, there are several limitations that need to be taken into account. First, the cross-sectional design does not allow us to address the temporality of the observed associations. Therefore, we cannot draw any conclusions with regard to the causality of the observations. Second, we cannot rule out that confounding by indication may be present. However, analysis restricted to nonobese participants and inflammatory diseases confirmed the findings in the general population. Although we corrected for a broad range of confounding factors in our analysis, we cannot exclude the possibility of residual confounding because of the observational study design.

Conclusions

Use of local CSs, particularly inhaled types, as well as systemic CSs was associated with higher likelihood of having MetS, higher BMI, and other adverse cardiometabolic traits, especially among women. Because the inhaled CSs are the main group of prescribed CSs, this might be a substantial risk to public health. Further studies are needed to confirm these findings and evaluate the direction of causality and mechanisms behind these associations.

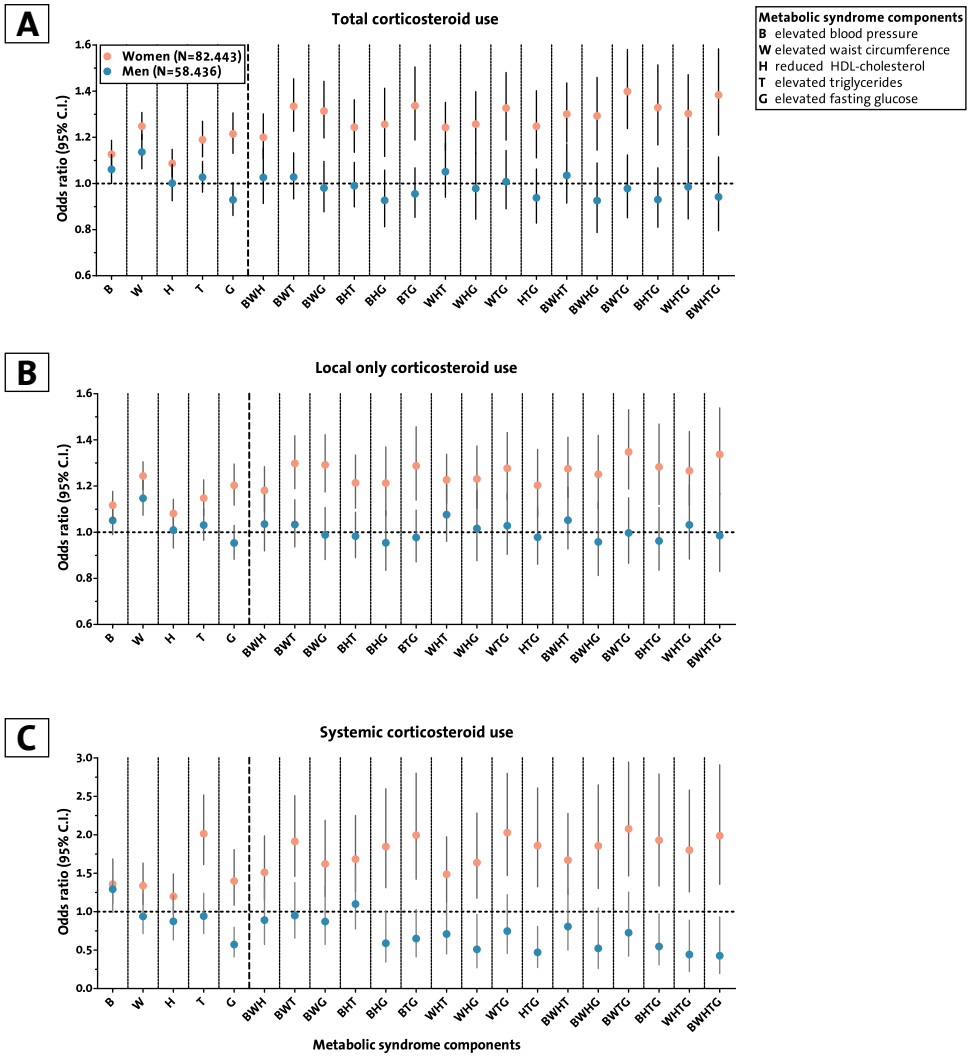


Figure 1: Associations between CS use and MetS components. The associations (OR with 95% CI) between CS use and the five MetS components separately and combined in (A) all CS users and stratified for (B) local-only CS users and (C) systemic CS users.

All analyses are adjusted for age, ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e., stroke and/or coronary heart disease), other comorbidities (i.e., cancer, osteoarthritis, COPD, and/or asthma), use of potentially weight-inducing psychotropics, HRT [only female sex hormones (in women) and other sex hormones (in both sexes)], and menstrual status (in women). Non-CS users were taken as reference group for all analyses. HRT, hormonal replacement therapy.

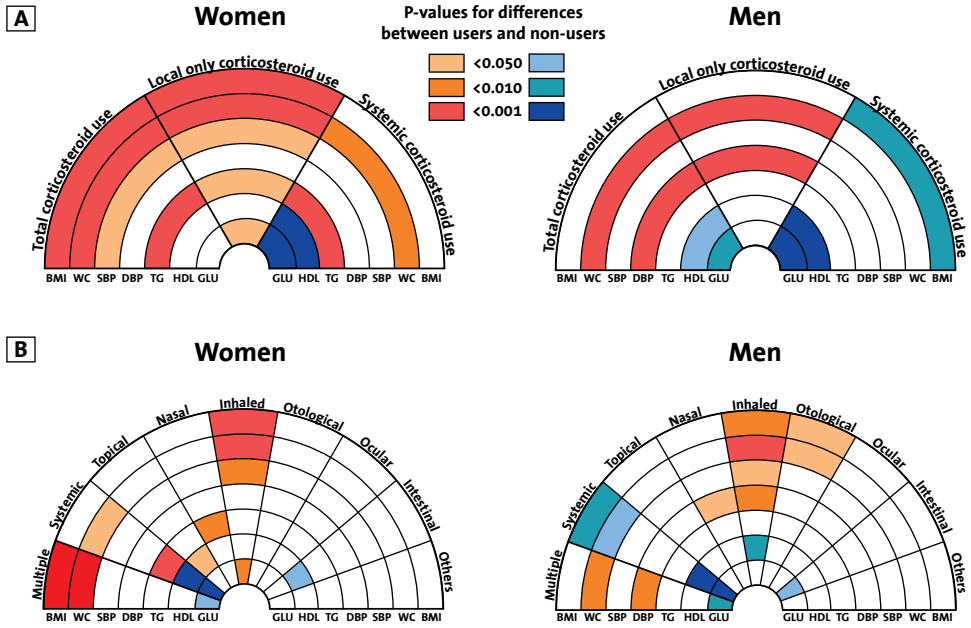


Figure 2: CS use and differences in cardiometabolic traits. Red tints indicate unfavorable differences, whereas the blue tints signify favorable differences in cardiometabolic traits between users and nonusers of CSs (see Supplemental Table 2 for adjusted mean differences).

The associations are shown for (A) the main CS users groups and specified for (B) the multiple-type and the various single-type users in both sexes. All analyses are adjusted for age, ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e., stroke and/or coronary heart disease), other comorbidities (i.e., cancer, osteoarthritis, COPD, and/or asthma), diabetes mellitus, use of potentially weight- inducing psychotropics, use of lipid-modifying drugs, use of antihypertensives, HRT [only female sex hormones (in women) and other sex hormones (in both sexes)], and menstrual status (in women). Non-CS users were taken as reference group for all analyses. GLU, fasting plasma glucose; HRT, hormonal replacement therapy; TG, triglycerides.

References

1. Swartz SL, Dluhy RG. Corticosteroids: clinical pharmacology and therapeutic use. Drugs. 1978;16(3):238–255.
2. Fardet L, Petersen I, Nazareth I. Prevalence of long-term oral glucocorticoid prescriptions in the UK over the past 20 years. Rheumatology (Oxford). 2011;50(11):1982–1990.
3. van Staa TP, Cooper C, Leufkens HG, Lammers JW, Suissa S. The use of inhaled corticosteroids in the United Kingdom and the Netherlands. Respir Med. 2003;97(5):578–585.
4. Fardet L, Fe`ve B. Systemic glucocorticoid therapy: a review of its metabolic and cardiovascular adverse events. Drugs. 2014;74(15): 1731–1745.
5. Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn ED, Leigh R, Brown JP, Cohen A, Kim H. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy Asthma Clin Immunol. 2013;9(1): 30.
6. Newell-Price J, Bertagna X, Grossman AB, Nieman LK. Cushing's syndrome. Lancet. 2006;367(9522):1605–1617.
7. Broersen LH, Pereira AM, Jørgensen JO, Dekkers OM. Adrenal insufficiency in corticosteroids use: systematic review and meta- analysis. J Clin Endocrinol Metab. 2015;100(6):2171–2180.
8. Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, Ormel J, Wolffenbuttel BH. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. Eur J Epidemiol. 2008;23(1):67–74.
9. Sheehan AH. Weight gain. In: Tisdale JE, Miller DA, eds. Drug- Induced Diseases: Prevention, Detection, and Management. Bethesda, MD: American Society of Health-System Pharmacists; 2010:629–642.
10. Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, Ryan DH, Still CD; Endocrine Society. Pharma- cological management of obesity: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2015;100(2): 342–362.
11. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC Jr; In- ternational Diabetes Federation Task Force on Epidemiology and Prevention; Hational Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Ath- erosclerosis Society; International Association for the Study of Obesity. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009;120(16):1640–1645.

12. World Health Organization. Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia: report of a WHO/IDF consultation. Available at: http://apps.who.int/iris/bitstream/10665/ 43588/1/9241594934_eng.pdf. Accessed 26 November, 2016.
13. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, Jones DW, Materson BJ, Oparil S, Wright JT Jr, Roccella EJ; National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure; National High Blood Pressure Education Program Coordinating Committee. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA. 2003; 289(19):2560–2572.
14. Packe GE, Douglas JG, McDonald AF, Robins SP, Reid DM. Bone density in asthmatic patients taking high dose inhaled beclome- thasone dipropionate and intermittent systemic corticosteroids. Thorax. 1992;47(6):414–417.
15. Marystone JF, Barrett-Connor EL, Morton DJ. Inhaled and oral corticosteroids: their effects on bone mineral density in older adults. Am J Public Health. 1995;85(12):1693–1695.
16. Mak VHF, Melchor R, Spiro SG. Easy bruising as a side-effect of inhaled corticosteroids. Eur Respir J. 1992;5(9):1068–1074.
17. Fardet L, Cabane J, Kettaneh A, Lebbe´ C, Flahault A. Corticosteroid- induced lipodystrophy is associated with features of the metabolic syndrome. Rheumatology (Oxford). 2007;46(7):1102–1106.
18. Fardet L, Flahault A, Kettaneh A, Tiev KP, Ge´ne´reau T, Tole´dano C, Lebbe´ C, Cabane J. Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion. Br J Dermatol. 2007;157(1):142–148.
19. Fardet L, Cabane J, Lebbe´ C, Morel P, Flahault A. Incidence and risk factors for corticosteroid-induced lipodystrophy: a prospective study. J Am Acad Dermatol. 2007;57(4):604–609.
20. Soldin OP, Mattison DR. Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet. 2009;48(3): 143–157.
21. Sundberg R, Tore´n K, Franklin KA, Gislason T, Omenaas E, Svanes C, Janson C. Asthma in men and women: treatment adherence, anxiety, and quality of sleep. Respir Med. 2010;104(3):337–344.
22. Crilly RG, Marshall DH, Nordin BE. Metabolic effects of corti- costeroid therapy in postmenopausal women. J Steroid Biochem. 1979;11(1B):429–433.
23. Kim C, Halter JB. Endogenous sex hormones, metabolic syndrome, and diabetes in men and women. Curr Cardiol Rep. 2014;16(4): 467.
24. Fitzgerald RC, Skingle SJ, Crisp AJ. Testosterone concentrations in men on chronic glucocorticosteroid therapy. J R Coll Physicians Lond. 1997;31(2):168–170.

25. Carson TE, Daane TA, Lee PA, Tredway DR, Wallin JD. Effect of intramuscular triamcinolone acetonide on the human ovulatory cycle. Cutis. 1977;19(5):633–637.
26. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their re- lation to the metabolic syndrome. Endocr Rev. 2000;21(6):697–738.
27. Cumming RG, Mitchell P, Leeder SR. Use of inhaled corticosteroids and the risk of cataracts. N Engl J Med. 1997;337(1):8–14.
28. Wong CA, Walsh LJ, Smith CJ, Wisniewski AF, Lewis SA, Hubbard R, Cawte S, Green DJ, Pringle M, Tattersfield AE. Inhaled corti- costeroid use and bone-mineral density in patients with asthma. Lancet. 2000;355(9213):1399–1403.
29. Capewell S, Reynolds S, Shuttleworth D, Edwards C, Finlay AY. Purpura and dermal thinning associated with high dose inhaled corticosteroids. BMJ. 1990;300(6739):1548–1551.
30. Wilson AM, McFarlane LC, Lipworth BJ. Effects of low and high doses of inhaled flunisolide and triamcinolone acetonide on basal and dynamic measures of adrenocortical activity in healthy vol- unteers. J Clin Endocrinol Metab. 1998;83(3):922–925.
31. Clark DJ, Lipworth BJ. Adrenal suppression with chronic dosing of fluticasone propionate compared with budesonide in adult asth- matic patients. Thorax. 1997;52(1):55–58.
32. Derom E, Van Schoor J, Verhaeghe W, Vincken W, Pauwels R. Systemic effects of inhaled fluticasone propionate and budesonide in adult patients with asthma. Am J Respir Crit Care Med. 1999; 160(1):157–161.
33. Derendorf H, Nave R, Drollmann A, Cerasoli F, Wurst W. Rele- vance of pharmacokinetics and pharmacodynamics of inhaled corticosteroids to asthma. Eur Respir J. 2006;28(5):1042–1050.
34. Lipworth BJ, Jackson CM. Safety of inhaled and intranasal cortico- steroids: lessons for the new millennium. Drug Saf. 2000;23(1):11–33.
35. Winkler J, Hochhaus G, Derendorf H. How the lung handles drugs: pharmacokinetics and pharmacodynamics of inhaled corticoste- roids. Proc Am Thorac Soc. 2004;1(4):356–363.
36. Abramson MJ, Walters J, Walters EH. Adverse effects of beta- agonists: are they clinically relevant? Am J Respir Med. 2003;2(4): 287–297.
37. Allen DB. Systemic effects of intranasal steroids: an endocrinologist's perspective. J Allergy Clin Immunol. 2000;106(4, Suppl):S179–S190.
38. Daley-Yates PT, Kunka RL, Yin Y, Andrews SM, Callejas S, Ng C. Bioavailability of fluticasone propionate and mometasone furoate aqueous nasal sprays. Eur J Clin Pharmacol. 2004;60(4):265–268.
39. Nayak AS, Settipane GA, Pedinoff A, Charous BL, Meltzer EO, Busse WW, Zinreich SJ, Lorber RR, Rikken G, Danzig MR, Nasonex Sinusitis G; Nasonex Sinusitis Group. Effective dose range of mometasone furoate nasal spray in the treatment of acute rhi- nosinusitis. Ann Allergy Asthma Immunol. 2002;89(3):271–278.

40. Patel D, Ratner P, Clements D, Wu W, Faris M, Philpot E. Lack of effect on adult and adolescent hypothalamic-pituitary-adrenal axis function with use of fluticasone furoate nasal spray. Ann Allergy Asthma Immunol. 2008;100(5):490–496.
41. Simons FE. Allergic rhinobronchitis: the asthma-allergic rhinitis link. J Allergy Clin Immunol. 1999;104(3 Pt 1):534–540.
42. Souverein PC, Berard A, Van Staa TP, Cooper C, Egberts AC, Leufkens HG, Walker BR. Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study. Heart. 2004;90(8):859–865.
43. Wei L, MacDonald TM, Walker BR. Taking glucocorticoids by prescription is associated with subsequent cardiovascular disease. Ann Intern Med. 2004;141(10):764–770.
44. Varas-Lorenzo C, Rodriguez LA, Maguire A, Castellsague J, Perez-Gutthann S. Use of oral corticosteroids and the risk of acute myocardial infarction. Atherosclerosis. 2007;192(2):376–383.
45. Fardet L, Petersen I, Nazareth I. Risk of cardiovascular events in people prescribed glucocorticoids with iatrogenic Cushing's syn-drome: cohort study. BMJ. 2012;345:e4928.

Supplemental Data

Supplemental Table 1: ATC codes for evaluated drugs.

Drug group	ATC-code			
Metabolic syndrome definition				
Antihypertensives	C02 C08	C03 C09	C04	C07
Blood glucose-lowering drugs	A10A	A10B		
Lipid-modifying drugs	C10A	C10B		
Other drugs				
Hormonal replacement therapy				
• Only female sex hormones	G03A	G03C	G03D	G03F
• Other sex hormones	G03B	G03E	G03H	G03X
Psychotropics*				
• Anticonvulsants	N03AF01	N03AG01	N03AX12	N03AX16
• Antidepressants	N06AA09 N06AB08 N06AB10 N06AA21 N06AF03	N06CA01 N06AA01 N06AB03 N06AX11 N06AB06	N06AB04 N06AA12 N06CA03 N06AA10 N06AF04	N06AA04 N06AX21 N06AA02 N06AB05 N06AA06
• Antipsychotics	N05AX12 N05AD01 N05AX13 N05AC02	N05AA01 N05AN N05AB03 N05AF04	N05AH02 N05AN01 N05AH04 N05AB06	N05AB02 N05AH03 N05AX08 N05AE04

*For each drug group of the psychotropics, we only assessed the substances which were likely to induce weight gain as an adverse event (1,2).

Supplemental Table 2: Adjusted mean differences in cardiometabolic traits between users and non-users of corticosteroids.

	Men (N=58 436)						
	BMI (kg/m²)	WC (cm)	SBP (mm Hg)	DBP (mm Hg)	TG (log mmol/L)[a]	HDL (mmol/L)	GLU (mmol/L)
Total corticosteroid use	+0.07 (-0.03 to 0.17)	**+0.72 (0.44 to 1.01)*** **	+0.25 (-0.14 to 0.63)	**+0.48 (0.22 to 0.73)*** **	0.000 (-0.007 to 0.006)	**+0.01 (0.002 to 0.02)***	**-0.03 (-0.05 to -0.01)** **
• Local only use	+0.10 (-0.002 to 0.20)	**+0.79 (0.51 to 1.08)*** **	+0.26 (-0.13 to 0.65)	**+0.52 (0.26 to 0.78)*** **	0.000 (-0.006 to 0.007)	+0.002 (-0.01 to 0.01)	-0.01 (-0.03 to 0.01)
• Systemic use	**-0.58 (-1.00 to -0.16)** **	-0.69 (-1.88 to 0.51)	-0.06 (-1.69 to 1.57)	-0.43 (-1.50 to 0.65)	-0.013 (-0.040 to 0.014)	**+0.18 (0.14 to 0.21)*** **	**-0.34 (-0.42 to -0.26)*** **
Multiple type use	+0.13 (-0.11 to 0.37)	**+1.05 (0.38 to 1.73)** **	+0.40 (-0.53 to 1.32)	**+0.87 (0.26 to 1.48)** **	-0.003 (-0.018 to 0.013)	+0.02 (-0.004 to 0.04)	**-0.06 (-0.11 to -0.02)** **
Single type use	+0.06 (-0.05 to 0.16)	**+0.67 (0.37 to 0.97)*** **	+0.22 (-0.19 to 0.63)	**+0.41 (0.14 to 0.68)** **	0.000 (-0.007 to 0.007)	**+0.01 (0.00 to 0.02)***	**-0.02 (-0.04 to -0.001)***
• Systemic corticosteroid(s)	**-0.76 (-1.26 to -0.27)** **	**-1.64 (-3.04 to -0.23)***	-0.27 (-2.18 to 1.65)	-0.40 (-1.67 to 0.86)	-0.023 (-0.055 to 0.009)	**+0.18 (0.13 to 0.22)*** **	**-0.36 (-0.45 to -0.27)*** **
• Topical corticosteroid(s)	-0.13 (-0.33 to 0.08)	+0.11 (-0.47 to 0.70)	-0.06 (-0.85 to 0.74)	+0.02 (-0.51 to 0.54)	+0.001 (-0.012 to 0.014)	-0.02 (-0.03 to 0.003)	+0.01 (-0.03 to 0.04)
• Nasal corticosteroid(s)	+0.02 (-0.18 to 0.21)	+0.33 (-0.22 to 0.87)	-0.20 (-0.94 to 0.54)	**+0.63 (0.14 to 1.11)***	+0.001 (-0.011 to 0.013)	-0.01 (-0.02 to 0.01)	+0.02 (-0.02 to 0.06)
• Inhaled corticosteroid(s)	**+0.25 (0.09 to 0.41)** **	**+1.44 (0.97 to 1.90)*** **	**+0.74 (0.11 to 1.37)***	**+0.60 (0.18 to 1.01)** **	-0.001 (-0.012 to 0.009)	**+0.02 (0.01 to 0.04)** **	-0.03 (-0.06 to 0.004)
• Otological corticosteroid(s)	**+1.32 (0.19 to 2.44)***	**+3.49 (0.30 to 6.67)***	+0.84 (-3.51 to 5.18)	-1.84 (-4.71 to 1.02)	+0.063 (-0.009 to 0.136)	-0.06 (-0.16 to 0.04)	+0.13 (-0.08 to 0.34)
• Ocular corticosteroid(s)	+0.26 (-0.46 to 0.99)	+1.14 (-0.92 to 3.20)	+0.08 (-2.73 to 2.88)	+1.19 (-0.66 to 3.03)	+0.013 (-0.034 to 0.059)	-0.02 (-0.08 to 0.05)	-0.07 (-0.21 to 0.07)
• Intestinal corticosteroid(s)	-0.36 (-1.57 to 0.84)	+0.31 (-3.12 to 3.74)	-0.92 (-5.59 to 3.76)	+0.07 (-3.01 to 3.15)	-0.025 (-0.103 to 0.053)	-0.02 (-0.13 to 0.08)	**-0.29 (-0.52 to -0.06)***
• Other corticosteroid(s)	+0.71 (-0.71 to 2.13)	+0.32 (-3.73 to 4.36)	+2.47 (-3.04 to 7.98)	+0.77 (-2.86 to 4.40)	+0.036 (-0.056 to 0.127)	-0.10 (-0.23 to 0.02)	+0.02 (-0.25 to 0.29)

All analyses are adjusted for age, ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e. stroke and/or coronary heart disease), other comorbidities (i.e. cancer, osteoarthritis, COPD, and/or asthma), diabetes mellitus, use of potentially weight-inducing psychotropics, use of lipid-modifying drugs, use of antihypertensives, hormonal replacement therapy (only female sex hormones (in women), and other sex hormones (in both sexes)), and menstrual status (in women). Non-corticosteroid users were taken as reference group for all analyses. Values can be converted to conventional units (i.e. mg/dL) by dividing by the following conversion factors: 0.0113 for triglycerides, 0.0259 for HDL-cholesterol, and 0.0555 for glucose. Abbreviations: *BMI*, body mass index; *DBP*, diastolic blood pressure; *GLU*, fasting plasma glucose; *HDL*, HDL-cholesterol; *SBP*, systolic blood pressure; *TG*, triglycerides; *WC*, waist circumference. *P<0.050, **P<0.010, ***P<0.001. [a]Adjusted mean differences (95% C.I.) are shown for log10-transformed triglycerides values.

Supplemental Table 3: Sensitivity analysis for the association between corticosteroid use and metabolic syndrome (in pre- and postmenopausal women) based on age.

Women (N=82 443)	<50 years (N=56 658)				≥50 years (N=25 785)			
			Metabolic syndrome				*Metabolic syndrome*	
	N	MetS, No. (%)[a]	Model 1	Model 2	N	MetS, No. (%)[a]	Model 1	Model 2
Total corticosteroid use	6241	759 (12.2)	**1.46 (1.34 to 1.58)*** **	**1.28 (1.17 to 1.41)*** **	3370	1115 (33.1)	**1.32 (1.22 to 1.43)*** **	**1.19 (1.09 to 1.30)*** **
• Local only use	6025	718 (14.1)	**1.43 (1.31 to 1.56)*** **	**1.26 (1.15 to 1.39)*** **	3145	1013 (32.2)	**1.28 (1.18 to 1.39)*** **	**1.16 (1.06 to 1.27)*** **
• Systemic use	216	41 (19.0)	**2.25 (1.59 to 3.19)*** **	**1.79 (1.25 to 2.57)** **	225	102 (45.3)	**1.84 (1.40 to 2.41)*** **	**1.62 (1.22 to 2.16)*** **
Multiple type use	1150	144 (12.5)	**1.49 (1.24 to 1.78)*** **	**1.32 (1.09 to 1.60)** **	585	197 (33.7)	**1.33 (1.11 to 1.59)** **	1.19 (0.98 to 1.43)
Single type use	5091	615 (12.1)	**1.45 (1.33 to 1.59)*** **	**1.28 (1.16 to 1.41)*** **	2785	918 (33.0)	**1.32 (1.21 to 1.43)*** **	**1.19 (1.09 to 1.31)*** **
• Systemic corticosteroid(s)	148	26 (17.6)	**2.03 (1.32 to 3.12)** **	**1.66 (1.07 to 2.59)***	163	75 (46.0)	**1.93 (1.40 to 2.65)*** **	**1.79 (1.28 to 2.49)*** **
• Topical corticosteroid(s)	1081	97 (9.0)	1.13 (0.91 to 1.39)	1.16 (0.93 to 1.44)	485	121 (24.9)	0.87 (0.71 to 1.08)	0.85 (0.68 to 1.06)
• Nasal corticosteroid(s)	1590	164 (10.3)	**1.22 (1.03 to 1.44)***	**1.24 (1.04 to 1.47)***	611	167 (27.3)	1.13 (0.94 to 1.35)	1.14 (0.95 to 1.38)
• Inhaled corticosteroid(s)	2112	314 (14.9)	**1.79 (1.58 to 2.03)*** **	**1.37 (1.19 to 1.58)*** **	1417	526 (37.1)	**1.55 (1.38 to 1.74)*** **	**1.32 (1.17 to 1.49)*** **
• Otological corticosteroid(s)	41	5 (12.2)	1.28 (0.50 to 3.29)	1.18 (0.45 to 3.13)	20	6 (30.0)	1.14 (0.43 to 3.05)	1.14 (0.42 to 3.12)
• Ocular corticosteroid(s)	52	4 (7.7)	0.82 (0.29 to 2.30)	0.76 (0.27 to 2.14)	50	14 (28.0)	1.02 (0.54 to 1.92)	1.01 (0.53 to 1.94)
• Intestinal corticosteroid(s)	42	4 (9.5)	1.14 (0.40 to 3.21)	0.84 (0.28 to 2.48)	24	7 (29.2)	1.14 (0.47 to 2.79)	0.92 (0.35 to 2.41)
• Other corticosteroid(s)	25	1 (4.0)	0.40 (0.05 to 3.00)	0.43 (0.06 to 3.24)	15	2 (13.3)	0.36 (0.08 to 1.61)	0.28 (0.06 to 1.37)
Men (N=58 436)	**<50 years (N=38 585)**				**≥50 years (N=19 851)**			
Total corticosteroid use	3495	582 (16.7)	**1.12 (1.02 to 1.23)***	1.08 (0.97 to 1.20)	2222	766 (34.5)	1.00 (0.91 to 1.09)	0.92 (0.83 to 1.02)
• Local only use	3378	557 (16.5)	**1.10 (1.00 to 1.22)***	1.07 (0.97 to 1.19)	2073	708 (34.2)	0.99 (0.90 to 1.09)	0.92 (0.83 to 1.03)
• Systemic use	117	25 (21.4)	1.47 (0.94 to 2.32)	1.22 (0.76 to 1.96)	149	58 (38.9)	1.06 (0.76 to 1.49)	0.90 (0.63 to 1.30)
Multiple type use	496	85 (17.1)	1.13 (0.89 to 1.44)	1.04 (0.81 to 1.34)	371	124 (33.4)	0.96 (0.77 to 1.20)	0.81 (0.64 to 1.03)
Single type use	2999	497 (16.6)	**1.11 (1.01 to 1.23)***	1.09 (0.97 to 1.21)	1851	642 (34.7)	1.00 (0.91 to 1.11)	0.94 (0.84 to 1.05)
• Systemic corticosteroid(s)	93	16 (17.2)	1.14 (0.66 to 1.97)	0.95 (0.53 to 1.68)	99	36 (36.4)	0.92 (0.61 to 1.40)	0.91 (0.58 to 1.42)
• Topical corticosteroid(s)	738	99 (13.4)	0.91 (0.73 to 1.13)	0.98 (0.78 to 1.22)	386	115 (29.8)	0.82 (0.65 to 1.02)	0.85 (0.67 to 1.07)
• Nasal corticosteroid(s)	917	133 (14.5)	0.95 (0.78 to 1.14)	1.00 (0.82 to 1.21)	404	129 (31.9)	0.97 (0.79 to 1.21)	0.97 (0.77 to 1.21)
• Inhaled corticosteroid(s)	1159	227 (19.6)	**1.33 (1.14 to 1.55)*** **	1.17 (0.995 to 1.39)	873	332 (38.0)	1.12 (0.97 to 1.29)	0.99 (0.84 to 1.16)
• Otological corticosteroid(s)	18	5 (27.8)	**3.04 (1.05 to 8.84)***	**3.66 (1.25 to 10.76)***	19	8 (42.1)	1.33 (0.53 to 3.33)	0.95 (0.35 to 2.58)
• Ocular corticosteroid(s)	42	11 (26.2)	1.99 (0.98 to 4.02)	**2.05 (1.00 to 4.19)***	47	14 (29.8)	0.79 (0.42 to 1.50)	0.92 (0.47 to 1.78)
• Intestinal corticosteroid(s)	17	3 (17.6)	1.30 (0.37 to 4.64)	1.52 (0.43 to 5.44)	15	6 (40.0)	1.21 (0.42 to 3.48)	0.98 (0.32 to 3.06)
• Other corticosteroid(s)	15	3 (20.0)	1.14 (0.31 to 4.15)	1.21 (0.32 to 4.55)	8	2 (25.0)	0.78 (0.16 to 3.94)	0.86 (0.17 to 4.38)

In model 1, associations were adjusted for age. In model 2, additional adjustments were performed for ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e. stroke and/or coronary heart disease), other comorbidities (i.e. cancer, osteoarthritis, COPD, and/or asthma), use of weight-inducing psychotropics, hormonal replacement therapy (only female sex hormones (only in women), and other sex hormones (in both sexes)), and menstrual status (only in women). Non-corticosteroid users were taken as reference group for all analyses. Values are shown as odds ratios with 95% C.I. *P<0.050, **P<0.010, ***P<0.001. [a]Numbers and percentages of subjects with metabolic syndrome diagnosis are given for the corresponding group of corticosteroid users.

Supplemental Table 4: Sensitivity analysis for the association between corticosteroid use and metabolic syndrome based on inflammatory status.

Women (N=82 443)	No inflammatory diseases (N=66 624)				Inflammatory disease(s) present (N=15 819)			
			Metabolic syndrome				*Metabolic syndrome*	
	N	MetS, No. (%)[a]	Model 1	Model 2	N	MetS, No. (%)[a]	Model 1	Model 2
Total corticosteroid use	4314	677 (15.7)	**1.22 (1.11 to 1.33)*** **	**1.19 (1.09 to 1.31)*** **	5297	1197 (22.6)	**1.24 (1.14 to 1.35)*** **	**1.21 (1.11 to 1.32)*** **
• Local only use	4058	611 (15.1)	**1.18 (1.08 to 1.30)*** **	**1.17 (1.06 to 1.29)** **	5112	1120 (21.9)	**1.21 (1.11 to 1.32)*** **	**1.18 (1.08 to 1.29)*** **
• Systemic use	256	66 (25.8)	**1.70 (1.26 to 2.30)*** **	**1.57 (1.15 to 2.14)** **	185	77 (41.6)	**2.07 (1.51 to 2.84)*** **	**1.81 (1.30 to 2.52)*** **
Multiple type use	389	79 (20.3)	**1.64 (1.25 to 2.14)*** **	**1.65 (1.25 to 2.18)*** **	1346	262 (19.5)	**1.08 (0.93 to 1.25)**	1.10 (0.94 to 1.29)
Single type use	3925	598 (15.2)	**1.18 (1.07 to 1.29)*** **	**1.15 (1.04 to 1.27)** **	3951	935 (23.7)	**1.30 (1.19 to 1.42)*** **	**1.24 (1.13 to 1.37)*** **
• Systemic corticosteroid(s)	222	59 (26.6)	**1.71 (1.24 to 2.36)** **	**1.58 (1.13 to 2.20)** **	89	42 (47.2)	**2.35 (1.51 to 3.67)*** **	**2.11 (1.33 to 3.34)** **
• Topical corticosteroid(s)	1253	153 (12.2)	0.96 (0.80 to 1.15)	0.96 (0.80 to 1.16)	313	65 (20.8)	1.00 (0.75 to 1.34)	1.02 (0.75 to 1.38)
• Nasal corticosteroid(s)	1710	232 (13.6)	**1.17 (1.01 to 1.35)***	**1.19 (1.03 to 1.38)***	491	99 (20.2)	1.10 (0.87 to 1.39)	1.17 (0.92 to 1.49)
• Inhaled corticosteroid(s)	526	125 (23.8)	**1.59 (1.28 to 1.98)*** **	**1.42 (1.13 to 1.78)** **	3003	715 (23.8)	**1.34 (1.21 to 1.48)*** **	**1.25 (1.13 to 1.39)*** **
• Otological corticosteroid(s)	52	8 (15.4)	1.17 (0.79 to 1.75)	1.04 (0.46 to 2.34)	9	3 (33.3)	1.41 (0.67 to 2.97)	1.60 (0.76 to 3.36)
• Ocular corticosteroid(s)	84	14 (16.7)	0.94 (0.69 to 1.28)	0.91 (0.49 to 1.70)	18	4 (22.2)	1.01 (0.56 to 1.80)	0.90 (0.28 to 2.92)
• Intestinal corticosteroid(s)	46	5 (10.9)	0.70 (0.27 to 1.83)	0.61 (0.22 to 1.69)	20	6 (30.0)	2.11 (0.76 to 5.81)	1.54 (0.50 to 4.71)
• Other corticosteroid(s)	32	2 (6.3)	0.35 (0.17 to 0.74)	0.28 (0.06 to 1.30)	8	1 (12.5)	0.41 (0.14 to 1.22)	0.41 (0.05 to 3.44)
Men (N=58 436)	**No inflammatory diseases (N=48 974)**				**Inflammatory disease(s) present (N=9462)**			
Total corticosteroid use	2868	590 (20.6)	0.94 (0.86 to 1.04)	0.96 (0.87 to 1.06)	2849	758 (26.6)	0.97 (0.88 to 1.08)	0.98 (0.88 to 1.10)
• Local only use	2697	542 (20.1)	0.93 (0.84 to 1.03)	0.95 (0.86 to 1.06)	2754	723 (26.3)	0.97 (0.87 to 1.08)	0.99 (0.89 to 1.11)
• Systemic use	171	48 (28.1)	1.11 (0.78 to 1.58)	1.05 (0.73 to 1.52)	95	35 (36.8)	1.08 (0.69 to 1.70)	0.87 (0.53 to 1.41)
Multiple type use	218	50 (22.9)	0.91 (0.65 to 1.27)	0.88 (0.61 to 1.27)	649	159 (24.5)	0.89 (0.73 to 1.08)	0.89 (0.72 to 1.09)
Single type use	2650	540 (20.4)	0.95 (0.86 to 1.05)	0.97 (0.87 to 1.08)	2200	599 (27.2)	1.00 (0.89 to 1.12)	1.01 (0.90 to 1.14)
• Systemic corticosteroid(s)	152	38 (25.0)	0.93 (0.64 to 1.37)	0.89 (0.60 to 1.34)	40	14 (35.0)	1.01 (0.50 to 2.04)	0.86 (0.40 to 1.84)
• Topical corticosteroid(s)	957	170 (17.8)	0.84 (0.71 to 1.00)	0.89 (0.75 to 1.07)	167	44 (26.3)	0.91 (0.63 to 1.31)	0.95 (0.65 to 1.39)
• Nasal corticosteroid(s)	1055	189 (17.9)	0.91 (0.77 to 1.07)	0.95 (0.80 to 1.13)	266	73 (27.4)	1.08 (0.81 to 1.44)	1.10 (0.82 to 1.48)
• Inhaled corticosteroid(s)	349	106 (30.4)	1.23 (0.96 to 1.57)	1.13 (0.87 to 1.47)	1683	453 (26.9)	0.99 (0.87 to 1.12)	1.00 (0.88 to 1.14)
• Otological corticosteroid(s)	30	8 (26.7)	1.27 (0.82 to 1.96)	1.16 (0.46 to 2.90)	7	5 (71.4)	**5.82 (2.39 to 14.16)***	4.87 (0.74 to 32.16)
• Ocular corticosteroid(s)	71	21 (29.6)	1.29 (0.98 to 1.69)	1.42 (0.82 to 2.45)	18	4 (22.2)	0.63 (0.35 to 1.14)	0.78 (0.25 to 2.41)
• Intestinal corticosteroid(s)	18	4 (22.2)	0.98 (0.54 to 1.79)	0.76 (0.20 to 2.85)	14	5 (35.7)	1.31 (0.73 to 2.35)	1.60 (0.49 to 5.28)
• Other corticosteroid(s)	18	4 (22.2)	1.15 (0.64 to 2.07)	1.15 (0.37 to 3.62)	5	1 (20.0)	0.64 (0.20 to 2.06)	0.79 (0.08 to 7.81)

Presence of inflammatory disease was defined as having osteoarthritis, asthma, and/or COPD. Subjects who had none of these three were classified as having no inflammatory diseases. In model 1, associations were adjusted for age. In model 2, additional adjustments were performed for ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e. stroke and/or coronary heart disease), other comorbidities (i.e. cancer, osteoarthritis, COPD, and/or asthma), use of weight-inducing psychotropics, hormonal replacement therapy (only female sex hormones (only in women), and other sex hormones (in both sexes)), and menstrual status (only in women). Non-corticosteroid users were taken as reference group for all analyses. Values are shown as odds ratios with 95% C.I. *P<0.050, **P<0.010, ***P<0.001.[a]Numbers and percentages of subjects with metabolic syndrome diagnosis are given for the corresponding group of corticosteroid users.

Supplemental Table 5: Sensitivity analysis for the association between corticosteroid use and metabolic syndrome stratified for obesity.

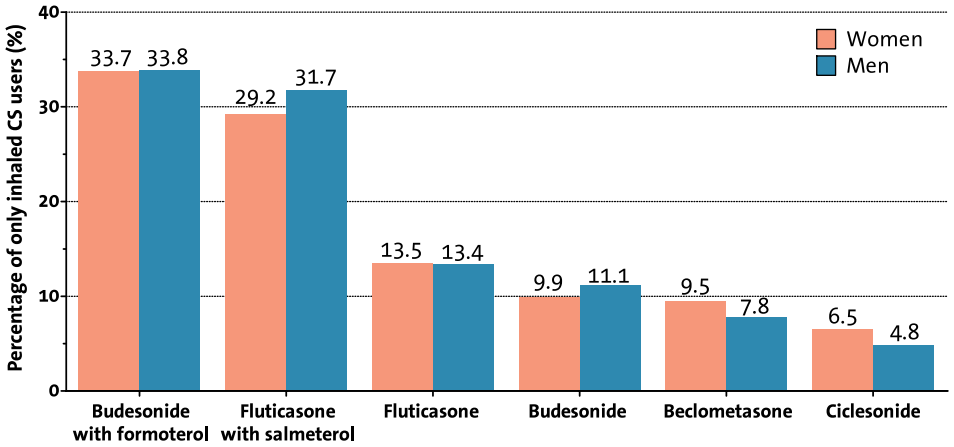
Women (N=82 443)	Non-obese (N=68 760)				Obese (N=13 683)			
			Metabolic syndrome				*Metabolic syndrome*	
	N	MetS, No. (%)[a]	Model 1	Model 2	N	MetS, No. (%)[a]	Model 1	Model 2
Total corticosteroid use	7445	896 (12.0)	**1.25 (1.15 to 1.35)*** **	**1.16 (1.07 to 1.27)*** **	2166	978 (45.2)	**1.15 (1.05 to 1.27)** **	1.09 (0.99 to 1.21)
• Local only use	7114	826 (11.6)	**1.23 (1.13 to 1.33)*** **	**1.15 (1.05 to 1.26)** **	2056	905 (44.0)	**1.11 (1.01 to 1.23)***	1.05 (0.95 to 1.17)
• Systemic use	331	70 (21.1)	**1.60 (1.19 to 2.13)** **	**1.38 (1.02 to 1.86)***	110	73 (66.4)	**2.31 (1.52 to 3.49)*** **	**2.16 (1.42 to 3.29)*** **
Multiple type use	1300	160 (12.3)	**1.32 (1.10 to 1.57)** **	**1.25 (1.03 to 1.50)***	435	181 (41.6)	0.97 (0.79 to 1.19)	0.94 (0.76 to 1.17)
Single type use	6145	736 (12.0)	**1.23 (1.13 to 1.34)*** **	**1.15 (1.05 to 1.26)** **	1731	797 (46.0)	**1.20 (1.08 to 1.34)*** **	**1.13 (1.01 to 1.27)***
• Systemic corticosteroid(s)	239	50 (20.9)	**1.54 (1.10 to 2.17)***	1.37 (0.97 to 1.96)	72	51 (70.8)	**2.91 (1.71 to 4.94)*** **	**2.76 (1.62 to 4.72)*** **
• Topical corticosteroid(s)	1306	113 (8.7)	0.91 (0.74 to 1.12)	0.90 (0.73 to 1.12)	260	105 (40.4)	1.03 (0.80 to 1.34)	1.00 (0.77 to 1.31)
• Nasal corticosteroid(s)	1835	175 (9.5)	1.15 (0.98 to 1.36)	1.18 (0.99 to 1.39)	366	156 (42.6)	1.22 (0.99 to 1.52)	**1.27 (1.02 to 1.58)***
• Inhaled corticosteroid(s)	2546	373 (14.7)	**1.43 (1.26 to 1.61)*** **	**1.24 (1.09 to 1.41)** **	983	467 (47.5)	**1.20 (1.05 to 1.37)** **	1.07 (0.92 to 1.24)
• Otological corticosteroid(s)	49	5 (10.2)	0.98 (0.37 to 2.61)	0.97 (0.36 to 2.59)	12	6 (50.0)	1.38 (0.43 to 4.42)	1.26 (0.39 to 4.12)
• Ocular corticosteroid(s)	85	13 (15.3)	1.18 (0.63 to 2.22)	1.15 (0.60 to 2.19)	17	5 (29.4)	0.59 (0.20 to 1.71)	0.56 (0.19 to 1.61)
• Intestinal corticosteroid(s)	54	6 (11.1)	1.20 (0.50 to 2.92)	0.97 (0.39 to 2.43)	12	5 (41.7)	0.93 (0.28 to 3.06)	0.85 (0.24 to 2.99)
• Other corticosteroid(s)	31	1 (3.2)	0.23 (0.03 to 1.72)	0.18 (0.02 to 1.48)	9	2 (22.2)	0.38 (0.08 to 1.94)	0.35 (0.07 to 1.89)
Men (N=58 436)	**Non-obese (N=49 938)**				**Obese (N=8498)**			
Total corticosteroid use	4739	785 (16.6)	1.01 (0.93 to 1.10)	1.00 (0.91 to 1.10)	978	563 (57.6)	0.92 (0.80 to 1.06)	0.91 (0.78 to 1.05)
• Local only use	4521	734 (16.2)	1.01 (0.92 to 1.10)	1.01 (0.92 to 1.11)	930	531 (57.1)	0.91 (0.79 to 1.05)	0.90 (0.78 to 1.05)
• Systemic use	218	51 (23.4)	1.06 (0.76 to 1.49)	0.96 (0.67 to 1.36)	48	32 (66.7)	1.21 (0.66 to 2.24)	1.11 (0.58 to 2.11)
Multiple type use	710	124 (17.5)	1.03 (0.84 to 1.27)	0.99 (0.79 to 1.23)	157	85 (54.1)	0.78 (0.56 to 1.08)	0.74 (0.52 to 1.04)
Single type use	4029	661 (16.4)	1.01 (0.92 to 1.10)	1.01 (0.91 to 1.11)	821	478 (58.2)	0.95 (0.82 to 1.10)	0.94 (0.81 to 1.11)
• Systemic corticosteroid(s)	162	34 (21.0)	0.95 (0.63 to 1.42)	0.89 (0.58 to 1.36)	30	18 (60.0)	0.95 (0.45 to 2.00)	0.97 (0.44 to 2.10)
• Topical corticosteroid(s)	978	137 (14.0)	0.90 (0.74 to 1.09)	0.97 (0.80 to 1.18)	146	77 (52.7)	0.78 (0.56 to 1.09)	0.76 (0.54 to 1.08)
• Nasal corticosteroid(s)	1129	154 (13.6)	0.94 (0.79 to 1.13)	0.99 (0.83 to 1.19)	192	108 (56.3)	0.99 (0.74 to 1.33)	0.96 (0.71 to 1.30)
• Inhaled corticosteroid(s)	1616	308 (19.1)	1.10 (0.97 to 1.26)	1.04 (0.89 to 1.20)	416	251 (60.3)	0.99 (0.80 to 1.22)	1.00 (0.80 to 1.25)
• Otological corticosteroid(s)	25	5 (20.0)	1.24 (0.44 to 3.53)	1.17 (0.40 to 3.49)	12	8 (66.7)	1.23 (0.36 to 4.23)	1.05 (0.28 to 3.89)
• Ocular corticosteroid(s)	70	12 (17.1)	0.91 (0.47 to 1.74)	0.95 (0.48 to 1.88)	19	13 (68.4)	1.23 (0.46 to 3.30)	1.40 (0.52 to 3.77)
• Intestinal corticosteroid(s)	28	7 (25.0)	1.45 (0.58 to 3.63)	1.47 (0.56 to 3.84)	4	2 (50.0)	0.85 (0.12 to 6.20)	0.53 (0.07 to 4.15)
• Other corticosteroid(s)	21	4 (19.0)	1.37 (0.44 to 4.21)	1.50 (0.49 to 4.66)	2	1 (50.0)	0.69 (0.04 to 10.95)	0.87 (0.05 to 14.93)

Non-obese is defined as having a BMI <30.0 kg/m^2 and obese as BMI ≥30.0 kg/m^2. In model 1, associations were adjusted for age. In model 2, additional adjustments were performed for ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e. stroke and/or coronary heart disease), other comorbidities (i.e. cancer, osteoarthritis, COPD, and/or asthma), use of weight-inducing psychotropics, hormonal replacement therapy (only female sex hormones (only in women), and other sex hormones (in both sexes)), and menstrual status (only in women). Non-corticosteroid users were taken as reference group for all analyses. Values are shown as odds ratios with 95% C.I. *P<0.050, **P<0.010, ***P<0.001. [a]Numbers and percentages of subjects with metabolic syndrome diagnosis are given for the corresponding group of corticosteroid users.

Supplemental Table 6: Sensitivity analysis for the association between corticosteroid use and metabolic syndrome in single type inhaled corticosteroid(s) users.

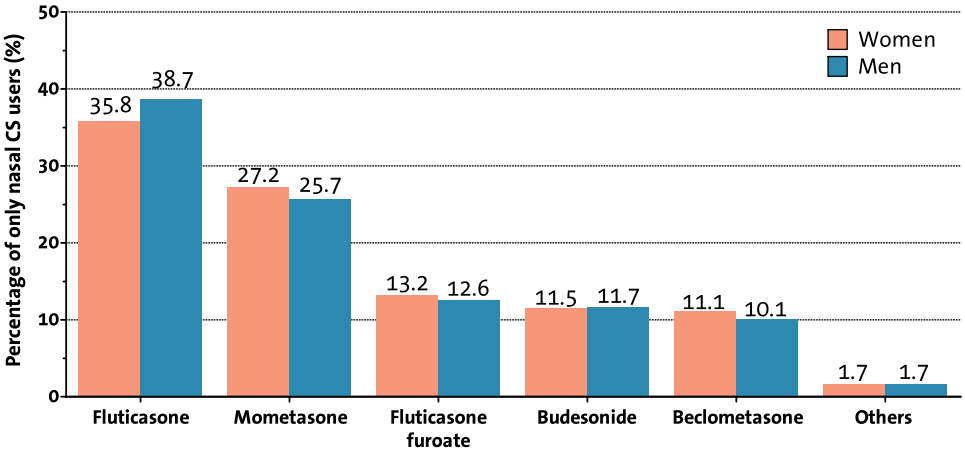
	Women				Men			
			Metabolic syndrome				*Metabolic syndrome*	
	N	MetS, No. (%)[a]	Model 1	Model 2	N	MetS, No. (%)[a]	Model 1	Model 2
Single type inhaled corticosteroid(s) use	3529	840 (23.8)			2032	559 (27.5)		
• Without beta-agonists	1318	290 (22.0)	**1.59 (1.38 to 1.82)*** **	**1.35 (1.17 to 1.57)*** **	705	166 (23.5)	1.04 (0.86 to 1.25)	1.01 (0.83 to 1.23)
• With beta-agonists	2211	550 (24.9)	**1.70 (1.53 to 1.88)*** **	**1.35 (1.20 to 1.51)*** **	1327	393 (29.6)	**1.30 (1.15 to 1.48)*** **	1.12 (0.98 to 1.29)

In model 1, associations were adjusted for age. In model 2, additional adjustments were performed for ethnicity, smoking, education level, alcohol use, physical activity, cardiovascular diseases (i.e. stroke and/or coronary heart disease), other comorbidities (i.e. cancer, osteoarthritis, COPD, and/or asthma), use of weight-inducing psychotropics, hormonal replacement therapy (only female sex hormones (only in women), and other sex hormones (in both sexes)), and menstrual status (only in women). Non-corticosteroid users were taken as reference group for all analyses. Values are shown as odds ratios with 95% C.I. ***P<0.001. [a]Numbers and percentages of subjects with metabolic syndrome diagnosis are given for the corresponding group of corticosteroid users.



Supplemental Figure 1: Distribution of use of inhaled corticosteroid agents in single type corticosteroid users.

Abbreviation: CS, corticosteroids.



Supplemental Figure 2: Distribution of use of nasal corticosteroid agents in single type corticosteroid users.

Abbreviation: CS, corticosteroids.

Supplemental References

1. Sheehan AH. Weight gain. In: Tisdale JE, Miller DA, eds. Drug-induced Diseases: Prevention, Detection, and Management. Bethesda, MD: American Society of Health-System Pharmacists; 2010: 629-642
2. Apovian CM, Aronne LJ, Bessesen DH, McDonnell ME, Murad MH, Pagotto U, Ryan DH, Still CD, Endocrine S. Pharmacological management of obesity: an endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2015; 100:342-362

Chapter 5

Anthropometric Measurements and Metabolic Syndrome in Relation to Glucocorticoid Receptor Polymorphisms in Corticosteroid Users

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Abstract

Introduction: *Corticosteroids are widely prescribed and their use has been linked to adverse cardiometabolic outcomes. A pivotal role in the action of corticosteroids is reserved for the glucocorticoid receptor (GR). Here, we assessed the relationship of glucocorticoid sensitivity altering GR polymorphisms with anthropometrics and metabolic syndrome (MetS) in corticosteroid users.*

Methods: *In this population-based cohort study (Lifelines), we genotyped 10,621 adult participants for GR hypersensitive (1/2 copies BclI and/or N363S) and GR resistant (1/2 copies ER22/23EK and/or 9β) variants. We assessed the relationship between functional GR polymorphisms with body mass index (BMI), waist circumference (WC), and MetS in users of corticosteroids.*

Results: *Overall corticosteroid use was associated with a significantly higher BMI and WC in GR wild-type users (BMI: +0.63 kg/m^2 [0.09-1.16], P=.022; WC: +2.03 cm [0.61-3.44], P=.005) and GR hypersensitive (BMI: +0.66 kg/m^2 [95% CI, 0.31-1.01); WC: +2.06 cm (1.13-2.98), both P<.001), but not in GR resistant users. Significantly higher WC in GR resistant carriers was observed only for inhaled corticosteroid users. With respect to MetS, again only GR wild-type users (odds ratio [OR] 1.44 [1.07-1.94], P=.017) and GR hypersensitives (OR 1.23 [95% CI, 1.00-1.50], P=.046) were more likely to have MetS; even more pronounced in only inhaled corticosteroid users (GR wild-type users, OR 1.64 [1.06-2.55], P=.027; GR hypersensitive users, OR 1.43 [1.08-1.91], P=.013).*

Conclusions: *Polymorphisms associated with increased GR sensitivity and wild-type GR are related to increased BMI, WC, and an increased MetS presence in corticosteroid users, especially of the inhaled types, when compared to nonusers. The adverse effects of corticosteroid use are less pronounced in users harboring GR resistant polymorphisms.*

Introduction

Corticosteroids are among the most commonly used drugs. We previously reported that more than 10% of the Dutch general population was using any type of corticosteroids [1], which could be explained by the high effectiveness and applicability of corticosteroids in extensive number of illnesses. Unfortunately, use of corticosteroids is also accompanied with widespread adverse effects. These adverse effects are especially observed with use of systemic corticosteroids due to their high systemic availability. Corticosteroid users have reported to suffer mainly from weight gain and neuropsychiatric changes [2], which are also frequently observed in patients with Cushing's syndrome. There is however increasing evidence that local corticosteroids can also induce systemic adverse effects. A large meta-analysis including users of corticosteroids showed that use of local forms, particularly of the inhaled formulations, also have increased risk of developing adrenal insufficiency [3].

The mode of action of corticosteroids does, however, not only depends on the amount of exposure but also on conditions at cellular level. An essential role in the pathway of glucocorticoid (GC) action is reserved for the glucocorticoid receptor (GR). Various GR polymorphisms have been reported of which some functional variants have extensively been investigated and shown to be associated with altered GC sensitivity [4]. In vivo as well as clinical studies assessing these have suggested that the intronic *BclI* variant and the N363S variant, the latter leading to a missense mutation in exon 2, are associated with increased GC sensitivity [5, 6]. On the other hand, the ER22/23EK and the 9β polymorphisms have been linked to GC resistance with carriers having for instance less cortisol suppression after dexamethasone administration [7] and smaller waist circumference (WC) in comparison to noncarriers [8].

These GR polymorphisms have been shown to be associated with alterations in body composition and several cardiometabolic parameters [9]. It remains unclear whether the GC sensitivity-altering polymorphisms could affect the vulnerability for developing adverse effects in corticosteroid users. This would especially be interesting for local types since the vast majority of the corticosteroid users are prescribed one of these forms, and therefore has a higher prevalence of users [1]. Moreover, our previous findings hint at systemic availability of the local corticosteroids, particularly of the inhaled forms, giving the association between these types with unfavorable cardiometabolic profile and metabolic syndrome (MetS) [1] as well as neuropsychiatric conditions and reduced executive functioning [10]. Hence, we investigated the relationship between four functional

GR polymorphisms with anthropometric measurements and MetS in corticosteroid users in the general population.

Subjects and Methods

Study Population

Data of adult individuals participating in the Lifelines cohort study were evaluated for the current study. Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviours of 167,729 persons living in the North of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics [11]. GWAS data were available for 13,378 participants of whom we included 10,621 after exclusion for subjects harboring both hypersensitive and resistant variants, non-reliable drug use data, non-fasting lab, and missing data on anthropometrics and/or MetS components. Written informed consent was provided by participants and study approval was obtained from the medical ethical committee of the University Medical Center Groningen, Groningen, The Netherlands.

Genetic Analysis

Participants were genotyped using the Illumina HumanCytoSNP GWAS platform. GWAS data was enriched using imputation with 1,000 genomes as a reference. Using PLINK verion 1.08p (Shaun Purcell, Harvard University), we extracted genotypes of functional GR SNPs: *Bcl*I, rs41423247; N363S, rs56149945; ER22/23EK, rs 6189 and 6190; and GR9β, rs6198. Users of corticosteroids were based on their genotype classified as either GR resistants (1/2 copies of ER22/23EK and/or 9β polymorphisms), GR wild types (WT; in case of two wild-type alleles), or GR hypersensitives (1/2 copies of *Bcl*I and/or N363S polymorphisms).

Use of Corticosteroids

The currently used drugs were after on-site container inspection classified conform the WHO Anatomical Therapeutical Chemical (ATC) code. We listed the ATC codes belonging to the various systemic corticosteroids (i.e. oral and parenteral) and local corticosteroids (i.e. dermal, nasal, inhaled, otological, ocular, intestinal, local oral, hemorrhoidal, gynecological). Participants using any type of corticosteroids were classified as “corticosteroid users” and further specified as “systemic users” (i.e. systemic with or without local types) or “local users” (i.e. users of only local type[s]). To assess the specific associations with the different local administration

forms, we additionally subclassified local users according to single-type use of three most prevalent forms: “inhaled types”, “nasal types”, and “dermal types”.

Anthropometrics and Metabolic Syndrome

Trained technicians performed all measurements according to standardized operating protocols as described previously [1]. Weight (kg) and height (m) were used to compute body mass index (kg/m^2). WC was measured at halfway the distance between front edge of lower ribs and the iliac crest. MetS was deemed present in case of at least three of the following five criteria as defined by the Joint Interim Statement [12]: [1] WC ≥88 cm (women) or ≥102 cm (men); [2] systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg, and/or use of antihypertensive agents given a previous diagnosis of hypertension; [3] triglycerides ≥1.7 mmol/L and/or use of lipid-modifying drugs; [4] HDL-cholesterol <1.3 mmol/L (women) or <1.0 mmol/L (men) and/or use of lipid-modifying drugs; [5] fasting serum glucose ≥5.6 mmol/L and/or use of blood-glucose lowering drugs.

Covariates

We considered data on age, sex, educational attainment, smoking and physical activity in order to control for confounding in the analyses. All covariates were self-reported and explained in detail elsewhere [1]. Educational attainment is related to the highest completed educational level and was categorized as: low (i.e. no education, primary, lower or preparatory vocational education, and lower general secondary education), middle (i.e. intermediate vocational education or apprenticeship, and higher general secondary education or pre-university secondary education), high (i.e. higher vocational education, and university) and other. With regard to smoking, participants were classified as nonsmoker, former smoker, or current smoker. Physical activity was based on the average number of days per week in which participants did at least half an hour of odd jobs, gardening, bicycling, or exercises combined. The percentage of missing data was 0.7% (educational attainment), 15.6% (smoking), and 9.0% (physical activity).

Statistical Analysis

Analyses were carried out with IBM SPSS Statistics version 22.0.0.2 (IBM Corp., Armonk, NY, USA). Student t test, Mann-Whitney U test or Chi Square test was performed to assess the crude differences in descriptive characteristics between nonusers and users of corticosteroids. Categorical variables were computed for separate analyses regarding specific corticosteroid user groups. For this, all nonusers were taken as reference group with each of the following user groups labelled separately based on GR genotypes (i.e. GR resistant, WT, or GR hypersensitive): “overall users”, “systemic (with or without local) type users”,

"local-types-only users", "inhaled-types-only users", "nasal-types-only users", and "dermal-types-only users". With respect to BMI and WC, we performed analyses of covariance to analyze the differences between nonusers and corticosteroid user groups. Logistic regression analyses were carried out for the association between MetS and corticosteroid use. For both type of analyses, we report the main models in which adjustments were made for age, sex, educational attainment, smoking, and physical activity. Interaction with sex was additionally assessed in all main models. Multiple imputation was carried out to handle missing data on covariates. P-values <.050 were considered statistically significant.

Results

Baseline Characteristics

Descriptive characteristics are shown in Table 1. Corticosteroids were used by 9.8% of the study population. A large proportion of the study population was harboring at least one GR hypersensitive variant (58.1%) with no significant differences in the distribution of the different genotypes between corticosteroid users and nonusers (P=.185). Distribution of the GR variants in nonusers and user groups are depicted in Table 2. The group of users consisted of more women (61.1% vs 57.9%, P=.049) and was on average older (50.2 [±11.7] vs 48.3 [±11.4] years, P<.001) in comparison to nonusers. Majority was using only local corticosteroids (94.7%) with highest number of users for inhaled (n=575), nasal (n=312) and dermal (n=226) types whereas 55 subjects were using systemic corticosteroids. After excluding 155 multiple type users, the number and percentage of single-type local corticosteroid use was as follows: 444 (77%) inhaled corticosteroids, 201 (64%) nasal corticosteroids, and 178 (79%) dermal corticosteroids.

Body Mass Index and Waist Circumference by GR Genotypes in Corticosteroid Users

Differences in BMI and WC between nonusers and users are shown in Figure 1. In the complete group of users, overall corticosteroid use was associated with increased BMI (+0.69 kg/m^2 [95% CI, 0.41 to 0.96]) and WC (+2.11 cm [95% CI, 1.34 to 2.88], both P<.001). All three genotypes had on average higher BMI and WC when compared to nonusers, however differences reached only statistical significance in GR WT users (BMI, +0.63 kg/m^2 [95% CI, 0.09 to 1.16], P=.022; WC, +2.03 cm [95% CI, 0.61 to 3.44], P=.005) and GR hypersensitive users (BMI, +0.66 kg/m^2 [95% CI, 0.31 to 1.01], P<.001; WC, +2.06 cm [95% CI, 1.13 to 2.98], P<.001).

No significant differences were found in systemic type users regarding BMI, however GR WT users had an increased WC (+6.55 cm [95% CI, 1.16 to 11.94], P=.017) in comparison to nonusers. In the combined group of users of locally

applied corticosteroids, only participants with GC hypersensitive variants had higher BMI (+0.72 kg/m^2 [95% CI, 0.36 to 1.08], P<.001), whereas all three genotypes had increased WC as compared to nonusers (GR resistant, +1.67 cm [95% CI, 0.16 to 3.17], P=.030; WT, +1.71 cm [95% CI, 0.24 to 3.17], P=.022; GR hypersensitive, +2.18 cm [95% CI, 1.24 to 3.13], P<.001).

Table 1: Descriptive characteristics of nonusers and corticosteroid users.

	All (N=10.621)	Nonusers (N=9,577)	Corticosteroid users (N=1,044)	P
Age (years)	48.5 (±11.5)	48.3 (±11.4)	50.2 (±11.7)	<.001
Sex (female)	6,187 (58.3)	5,549 (57.9)	638 (61.1)	.049
Educational attainment				
• Low	3,883 (36.6)	3,463 (36.2)	420 (40.2)	
• Middle	3,823 (36.0)	3,465 (36.2)	358 (34.3)	.076
• High	2,693 (25.4)	2,448 (25.6)	245 (23.5)	
• Other	222 (2.1)	201 (2.1)	21 (2.0)	
Smoking				
• Nonsmoker	4,081 (38.4)	3,638 (38.0)	443 (42.4)	.001
• Former smoker	3,728 (35.1)	3,358 (35.1)	370 (35.4)	
• Current smoker	2,812 (26.5)	2,581 (26.9)	231 (22.1)	
Physical activity				
• 0 days	421 (4.0)	381 (4.0)	40 (3.8)	.927
• 1-4 days	4,477 (42.2)	4,041 (42.2)	436 (41.8)	
• ≥5 days	5,723 (53.9)	5,155 (53.8)	568 (54.4)	
BMI (kg/m^2)	26.4 (±4.3)	26.3 (±4.2)	27.0 (±4.8)	<.001
Waist circumference (cm)	92.1 (±12.1)	91.8 (±12.1)	94.0 (±12.7)	<.001
Metabolic syndrome	2,446 (23.0)	2,153 (22.5)	293 (28.1)	<.001
Genotype*				
• GR resistant	2,162 (20.4)	1,937 (20.2)	225 (21.6)	.185
• GR wild type	2,286 (21.5)	2,046 (21.4)	240 (23.0)	
• GR hypersensitive	6,173 (58.1)	5,594 (58.4)	579 (55.5)	

Data are shown as numbers (percentage) and mean (±standard deviation). Abbreviation: *GR*, glucocorticoid receptor.
* GR resistant group includes participants with 1/2 copies of the ER22/23EK and/or 9β polymorphisms.
GR hypersensitive group includes participants with 1/2 copies of the *Bcl*I and/or N363S polymorphisms.

Stratification for single-type users showed that differences were mainly due to inhaled corticosteroid use. BMI was only significantly increased in inhaled type users with GR WT (+1.17 kg/m^2 [95% CI, 0.34 to 1.99], P=.005) and GR hypersensitive genotypes (+1.69 kg/m^2 [95% CI, 1.16 to 1.21], P<.001) and not in GR resistant users (+0.60 kg/m^2 [95% CI, -0.24 to 1.45], P=.163). WC was however higher in users of each GR genotype group when compared to nonusers (GR resistant, +2.34 cm [95% CI, 0.10 to 4.59], P=.040; WT, +3.80 cm [95% CI, 1.62 to 5.97], P<.001; GR hypersensitive genotype, +4.72 cm [95% CI, 3.34 to 6.11], P<.001). No interaction with sex was observed in any of the analyses.

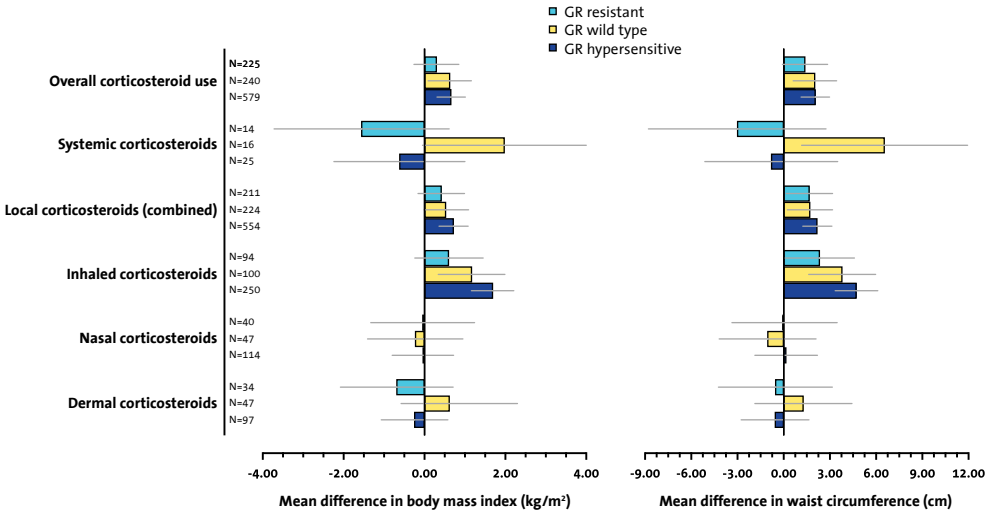

Figure 1: Differences in body mass index and waist circumference between nonusers and corticosteroid users by glucocorticoid receptor genotype.

Mean differences with the reference group of nonusers of corticosteroids (N=9,577) are shown in kg/m^2 (95% CI) for body mass index and cm (95% CI) for waist circumference. Analyses are adjusted for age, sex, educational attainment, smoking, and physical activity. Individuals with multiple type use are excluded for the analyses regarding inhaled, nasal, and dermal corticosteroid use.

Metabolic Syndrome and Corticosteroid Use by GR Genotype in Corticosteroid Users

Table 3 depicts the findings regarding differences in MetS presence between nonusers and users. MetS was in general more prevalent in corticosteroid users when compared to nonusers (28.1% vs 22.5%, P<.001). Within complete group of users, only those with a GR WT genotype (OR 1.44 [95% CI, 1.07 to 1.94], P=.017) or GR hypersensitive genotype (OR 1.23 [95% CI, 1.00 to 1.95], P=.046) were more likely to have MetS. Similar outcome was also observed in the combined local corticosteroid group, whereas no significant association was found in systemic type users. With respect to the former group, only inhaled corticosteroid use was linked to higher prevalence of MetS with GR WT and GR hypersensitive users being, respectively, 1.64 (95% CI, 1.06 to 2.55, P=.027) and 1.43 (95% CI, 1.08 to 1.91; P=.013) times more likely to have MetS in comparison to nonusers. There was no significant interaction with sex.

Table 2: Distribution of the glucocorticoid receptor variants in nonusers and corticosteroid user groups.

Genotype	First allele	Second allele	Nonusers (N=9,577)		Overall users (N=1,044)		Systemic users (N=55)		Local users (N=989)		Inhaled types only (N=444)		Nasal types only (N=201)		Dermal types only (N=178)	
			n	%	n	%	n	%	n	%	n	%	n	%	n	%
GR resistant	**WT**	**ER22/23EK**	200	2.1	38	3.6	0	0.0	38	3.8	20	4.5	4	2.0	7	3.9
	WT	**9β**	1,404	14.7	151	14.5	11	20.0	140	14.2	57	12.8	28	13.9	23	12.9
	ER22/23EK	**ER22/23EK**	9	0.1	5	0.5	1	1.8	4	0.4	1	0.2	0	0.0	1	0.6
	ER22/23EK	**9β**	92	1.0	13	1.2	1	1.8	12	1.2	6	1.4	4	2.0	1	0.6
	9β	**9β**	232	2.4	18	1.7	1	1.8	17	1.7	10	2.3	4	2.0	2	1.1
GR wild type	**WT**	**WT**	2,046	21.4	240	23.0	16	29.1	224	22.6	100	22.5	47	23.4	47	26.4
GR hypersensitive	**WT**	***Bcl*I**	3,490	36.4	348	33.3	16	29.1	332	33.6	142	32.0	67	33.3	63	35.4
	WT	**N363S**	341	3.6	32	3.1	1	1.8	31	3.1	16	3.6	7	3.5	4	2.2
	***Bcl*I**	***Bcl*I**	1,495	15.6	166	15.9	7	12.7	159	16.1	75	16.9	36	17.9	23	12.9
	***Bcl*I**	**N363S**	263	2.7	31	3.0	1	1.8	30	3.0	15	3.4	4	2.0	7	3.9
	N363S	**N363S**	5	0.1	2	0.2	0	0.0	2	0.2	2	0.5	0	0.0	0	0.0

Table 3: Association between metabolic syndrome and corticosteroid use by glucocorticoid receptor genotype.

	GR resistant users				GR wild type users				GR hypersensitive users			
	MetS n/N	MetS%	OR	95% CI	MetS n/N	MetS%	OR	95% CI	MetS n/N	MetS%	OR	95% CI
Overall users	61/225	27.1	1.15	0.84 to 1.58	73/240	30.4	**1.44**	**1.07 to 1.94***	159/579	27.5	**1.23**	**1.00 to 1.50***
Systemic users	5/14	35.7	1.17	0.36 to 3.81	8/16	50.0	2.45	0.84 to 7.21	8/25	32.0	0.99	0.40 to 2.43
Local users	56/211	26.5	1.14	0.82 to 1.59	65/224	29.0	**1.38**	**1.01 to 1.88***	151/554	27.3	**1.24**	**1.01 to 1.52***
• Inhaled types only	28/94	29.8	1.10	0.69 to 1.77	34/100	34.0	**1.64**	**1.06 to 2.55***	80/250	32.0	**1.43**	**1.08 to 1.91***
• Nasal types only	7/40	17.5	0.86	0.37 to 2.00	9/47	19.1	0.83	0.38 to 1.77	23/114	20.2	1.02	0.63 to 1.63
• Dermal types only	7/34	20.6	0.95	0.39 to 2.28	14/47	29.8	1.63	0.83 to 3.19	20/97	20.6	0.96	0.57 to 1.62

MetS prevalence in the reference group of nonusers (N=9,577) was 22.5%.
Analyses are adjusted for age, sex, educational attainment, smoking, and physical activity.
Abbreviations: *GR,* glucocorticoid receptor; *MetS*, metabolic syndrome; *OR*, odds ratio.
*P<.050.

Discussion

In this study, we investigated the relevance of functional polymorphisms of the GR in the association between systemic and local corticosteroid use with cardiometabolic outcomes in the general population. Corticosteroid users, in particular of the inhaled forms, have an increased BMI, WC and are more often burdened with MetS in comparison to nonusers. These differences are significantly evident in users harboring GR polymorphisms associated with GR hypersensitivity (*Bcl*I and/or N363S) and WT users, but less in users with GR resistant polymorphisms (i.e. ER22/23EK and/or 9β).

Genomic actions of activated GR are traditionally classified as transactivating or transrepressing. Adverse effects of supraphysiological GC exposure are considered to be mainly due to transactivation whereas the preferred anti-inflammatory response is induced by transrepression [13]. Earlier studies have performed functional assays to assess these GR-dependent effects in leukocytes of individuals with functional GR variants. Transactivational activity was increased with the N363S variant and decreased in case of ER22/23EK polymorphism [14], while the *in vitro* transrepressional effects were decreased in 9β carriers [15]. These in vitro observations are in line with the differences as observed in the current study with more adverse cardiometabolic effects in GR hypersensitive users of corticosteroids and vice versa in GR resistant users. Interestingly, Eipel and colleagues previously observed that pediatric patients harboring the N363S polymorphism more often developed GC-related hepatotoxicity and glucose abnormalities in the course of acute lymphoblastic leukemia (ALL) treatment [16]. Moreover, previous studies also with pediatric ALL patients showed that *Bcl*I carriers were also more likely to develop Cushingoid-like symptoms (e.g. adiposity, hypertension, diabetes) and depression during treatment with systemic corticosteroids [17], as well as a longer period of adrenal insufficiency after high-dose corticosteroid therapy in homozygous *Bcl*I carriers [18].

Supraphysiological exposure to GCs is known to induce lipogenesis and accumulation of central adipocytes conceivably due to the higher presence of GR in visceral area [19]. GCs can additionally increase (high-caloric) food intake [20, 21] and promote redistribution of fat tissue to central regions [22] which could further fuel these changes on adipocyte level. In the current study we found that overall use of corticosteroids was indeed associated with higher WC but mainly in users with GR WT and GR hypersensitive genotype. This could hint on protective effects of the GR resistant variants on changes in abdominal obesity with corticosteroid use, especially given the previous findings of (tendency to) lower WC in unselected carriers [8, 23]. Our findings in the current study were mainly evident for inhaled

corticosteroids and showed nevertheless that also users with GR resistant genotype had significantly higher WC albeit the difference was less pronounced in comparison to other users. It is conceivable that frequent and chronic use of these powerful agents, as would be anticipated in many inhaled type users, and often in combination with systemic corticosteroids would somehow outweigh the potential beneficial effects of GR resistant variants.

Similar differences were also evident with regard to BMI with greatest contrast between nonusers and inhaled corticosteroid users harboring GR hypersensitive variants. In contrast to WC, no significant differences were found for individuals with GR resistant variants. Despite the fact that both anthropometric measures are strongly linked to cardiovascular events [24], WC is, as an estimate for abdominal fat mass, the most important predictor and is in particular of interest in the context of GC effects. Excess of GCs stimulate redistribution of peripheral fat and accumulation of visceral fat by increasing synthesis and storage of lipids and adipose tissue hyperplasia by increasing differentiation of preadipocytes to mature adipocytes [25]. GCs also promote proteolysis in skeletal muscles while inhibiting protein synthesis which together could eventually lead to muscle atrophy [26]. Moreover, GCs also affect bone mineral density through various pathways leading to increased osteoclast activity and diminished osteoblast function which ultimately lead to bone loss [27]. This highlights the differential effects of GCs on body composition which can ultimately lead to varying effects regarding BMI. Furthermore, we previously showed that male carriers of the ER22/23EK variant had on average more lean mass and muscle strength [23] which could also contribute to the current observation.

The increased prevalence of MetS in corticosteroid users is in line with our previous observations in the complete cohort population [1]. As shown here, it seems however that this association is only significantly present in users carrying variants linked to GR hypersensitivity or those with a GR WT genotype. We have previously conducted the only study, as far as we know, on the link between GR polymorphisms and MetS in the general population and found increased risk in specific subgroups with the N363S (GR hypersensitive) variant [28]. Other smaller studies have investigated the association between GR variants and cardiometabolic features and demonstrated differences corresponding to altered GR sensitivity [29-31], however findings have not been consistent [5, 32, 33]. Since these studies did not take corticosteroid use into account, and given the relatively high percentage of users, it remains unclear to what extent the differences can be attributed to GR variations.

Contrary to our expectations, the differences in all outcomes between nonusers and users of systemic corticosteroids were highly variable and less consistent. This could largely be due to the small number of systemic corticosteroid users in the current study population (0.5%) and within the total group of users (5.3% vs 94.7% users of local types). In the group of locally applied corticosteroids, adverse outcomes were only consistently present in users of inhaled types. The majority of the inhaled type only group was using inhalers containing fluticasone (propionate) or budesonide, which are pharmacologically active upon use and are known to have relatively high GR binding affinity and lower protein-binding capacity [34] and thus to be more likely to induce systemic GC-related adverse events when entering circulation. Unfortunately, we have no data regarding the therapeutic effect of corticosteroids in user groups according to GR variants. In studies with selected study populations, however, it was shown that having two *BclI* variants is associated with a better treatment response to inhaled corticosteroids in asthmatic children [35]. Carriers of GR hypersensitive variants were also found to have a better therapeutic response to systemic corticosteroids in ALL [16, 36], inflammatory bowel disease [37], and nephrotic syndrome [38]. The opposite was observed in patients harboring GR resistant ER22/23EK or 9β variants and being treated with systemic corticosteroids [38, 39].

This is the first population-based study to assess cardiometabolic profile in users of systemic as well as local corticosteroid types in relation to GR variations. Among the strengths are the reliable and large-scale data collection on drug use and integrity of data regarding genotyping, BMI, and the MetS components. Since it concerns an observational study design, we cannot exclude residual confounding, despite adjustments for relevant confounders, and are not able to prove causality. Moreover, it remains unknown whether the findings can be extrapolated to other ethnicities given the fact that the study population involves mainly individuals from Caucasian race. Finally, larger longitudinal studies are needed to perform analyses for the separate polymorphisms and to confirm whether in systemic and local corticosteroid use the development of cardiometabolic features but also other clinically relevant GC-related adverse events (e.g., neuropsychiatric conditions) depend on GR variations and/or other factors in the GC pathway.

Conclusion

Corticosteroid users, in particular of inhaled corticosteroids, have an increased BMI, WC, and more often MetS in comparison to nonusers. These relationships are significantly evident in carriers of common GR genotypes associated with GR hypersensitivity or the WT genotype, but little to none present in users harboring GR resistant polymorphisms.

References

1. Savas M, Muka T, Wester VL, van den Akker ELT, Visser JA, Braunstahl GJ, et al. Associations Between Systemic and Local Corticosteroid Use With Metabolic Syndrome and Body Mass Index. J Clin Endocrinol Metab. 2017 Oct 01;102(10):3765-74.
2. Curtis JR, Westfall AO, Allison J, Bijlsma JW, Freeman A, George V, et al. Population-based assessment of adverse events associated with long-term glucocorticoid use. Arthritis Rheum. 2006 Jun 15;55(3):420-6.
3. Broersen LH, Pereira AM, Jorgensen JO, Dekkers OM. Adrenal Insufficiency in Corticosteroids Use: Systematic Review and Meta-Analysis. J Clin Endocrinol Metab. 2015 Jun;100(6):2171-80.
4. Savas M, van Rossum EFC. Impact of Glucocorticoid Receptor Polymorphisms on Glucocorticoid Action. In: Ilpo Huhtaniemi and Luciano Martini, (Eds.), Encyclopedia of Endocrine Diseases, Second Edition, vol. 3, pp. 147–156. Oxford: Academic Press; 2019.
5. Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, et al. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids in vivo. J Clin Endocrinol Metab. 1998 Jan;83(1):144-51.
6. van Rossum EF, Koper JW, van den Beld AW, Uitterlinden AG, Arp P, Ester W, et al. Identification of the BclI polymorphism in the glucocorticoid receptor gene: association with sensitivity to glucocorticoids in vivo and body mass index. Clinical Endocrinology. 2003 Nov;59(5):585-92.
7. van Rossum EF, Koper JW, Huizenga NA, Uitterlinden AG, Janssen JA, Brinkmann AO, et al. A polymorphism in the glucocorticoid receptor gene, which decreases sensitivity to glucocorticoids in vivo, is associated with low insulin and cholesterol levels. Diabetes. 2002 Oct;51(10):3128-34.
8. Syed AA, Irving JA, Redfern CP, Hall AG, Unwin NC, White M, et al. Association of glucocorticoid receptor polymorphism A3669G in exon 9beta with reduced central adiposity in women. Obesity. 2006 May;14(5):759-64.
9. van Rossum EF, Lamberts SW. Polymorphisms in the glucocorticoid receptor gene and their associations with metabolic parameters and body composition. Recent Prog Horm Res. 2004;59:333-57.
10. Savas M, Vinkers C, Rosmalen J, Hartman C, Wester VL, van den Akker ELT, et al. Systemic and local corticosteroid use is associated with reduced executive cognition, and mood and anxiety disorders. Neuroendocrinology. 2019 Jun 21.
11. Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, et al. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. Eur J Epidemiol. 2008;23(1):67-74.
12. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International

Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 2009 Oct 20;120(16):1640-5.

13. Stahn C, Lowenberg M, Hommes DW, Buttgereit F. Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. Mol Cell Endocrinol. 2007 Sep 15;275(1-2):71-8.
14. Russcher H, Smit P, van den Akker EL, van Rossum EF, Brinkmann AO, de Jong FH, et al. Two polymorphisms in the glucocorticoid receptor gene directly affect glucocorticoid-regulated gene expression. J Clin Endocrinol Metab. 2005 Oct;90(10):5804-10.
15. van den Akker EL, Russcher H, van Rossum EF, Brinkmann AO, de Jong FH, Hokken A, et al. Glucocorticoid receptor polymorphism affects transrepression but not transactivation. J Clin Endocrinol Metab. 2006 Jul;91(7):2800-3.
16. Eipel OT, Nemeth K, Torok D, Csordas K, Hegyi M, Ponyi A, et al. The glucocorticoid receptor gene polymorphism N363S predisposes to more severe toxic side effects during pediatric acute lymphoblastic leukemia (ALL) therapy. Int J Hematol. 2013 Feb;97(2):216-22.
17. Kaymak Cihan M, Karabulut HG, Yurur Kutlay N, Ilgin Ruhi H, Tukun A, Olcay L. Association Between N363S and BclI Polymorphisms of the Glucocorticoid Receptor Gene (NR3C1) and Glucocorticoid Side Effects During Childhood Acute Lymphoblastic Leukemia Treatment. Turk J Haematol. 2017 Jun 5;34(2):151-58.
18. de Ruiter RD, Gordijn MS, Gemke RJ, van den Bos C, Bierings MB, Rotteveel J, et al. Adrenal insufficiency during treatment for childhood acute lymphoblastic leukemia is associated with glucocorticoid receptor polymorphisms ER22/23EK and BclI. Haematologica. 2014 Aug;99(8):e136-7.
19. Marin P, Bjorntorp P. Endocrine-metabolic pattern and adipose tissue distribution. Horm Res. 1993;39 Suppl 3:81-5.
20. Epel E, Lapidus R, McEwen B, Brownell K. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. Psychoneuroendocrinology. 2001 Jan;26(1):37-49.
21. Udden J, Bjorntorp P, Arner P, Barkeling B, Meurling L, Rossner S. Effects of glucocorticoids on leptin levels and eating behaviour in women. J Intern Med. 2003 Feb;253(2):225-31.
22. Rebuffe-Scrive M, Krotkiewski M, Elfverson J, Bjorntorp P. Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. J Clin Endocrinol Metab. 1988 Dec;67(6):1122-8.
23. van Rossum EF, Voorhoeve PG, te Velde SJ, Koper JW, Delemarre-van de Waal HA, Kemper HC, et al. The ER22/23EK polymorphism in the glucocorticoid receptor gene is associated with a beneficial body composition and muscle strength in young adults. J Clin Endocrinol Metab. 2004 Aug;89(8):4004-9.

24. Flint AJ, Rexrode KM, Hu FB, Glynn RJ, Caspard H, Manson JE, et al. Body mass index, waist circumference, and risk of coronary heart disease: a prospective study among men and women. Obes Res Clin Pract. 2010 Jul;4(3):e171-e81.
25. Fardet L, Feve B. Systemic glucocorticoid therapy: a review of its metabolic and cardiovascular adverse events. Drugs. 2014 Oct;74(15):1731-45.
26. Salehian B, Kejriwal K. Glucocorticoid-induced muscle atrophy: mechanisms and therapeutic strategies. Endocr Pract. 1999 Sep-Oct;5(5):277-81.
27. Schacke H, Docke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther. 2002 Oct;96(1):23-43.
28. Wester VL, Koper JW, van den Akker EL, Franco OH, Stolk RP, van Rossum EF. Glucocorticoid receptor haplotype and metabolic syndrome: the Lifelines cohort study. Eur J Endocrinol. 2016 Dec;175(6):645-51.
29. Weaver JU, Hitman GA, Kopelman PG. An association between a Bc1I restriction fragment length polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women. J Mol Endocrinol. 1992 Dec;9(3):295-300.
30. Lin RC, Wang XL, Morris BJ. Association of coronary artery disease with glucocorticoid receptor N363S variant. Hypertension. 2003 Mar;41(3):404-7.
31. Srivastava N, Prakash J, Lakhan R, Agarwal CG, Pant DC, Mittal B. Influence of Bcl-1 Gene Polymorphism of Glucocorticoid Receptor Gene (NR3C1, rs41423247) on Blood Pressure, Glucose in Northern Indians. Indian J Clin Biochem. 2011 Apr;26(2):125-30.
32. Di Blasio AM, van Rossum EF, Maestrini S, Berselli ME, Tagliaferri M, Podesta F, et al. The relation between two polymorphisms in the glucocorticoid receptor gene and body mass index, blood pressure and cholesterol in obese patients. Clinical Endocrinology. 2003 Jul;59(1):68-74.
33. Mora M, Sanchez L, Serra-Prat M, Palomera E, Blanco J, Aranda G, et al. Hormonal determinants and effect of ER22/23EK glucocorticoid receptor gene polymorphism on health status deterioration in the participants of the Mataro Ageing Study. Age (Dordr). 2012 Jun;34(3):553-61.
34. Derendorf H, Nave R, Drollmann A, Cerasoli F, Wurst W. Relevance of pharmacokinetics and pharmacodynamics of inhaled corticosteroids to asthma. Eur Respir J. 2006 Nov;28(5): 1042–50.
35. Keskin O, Uluca U, Birben E, Coskun Y, Ozkars MY, Keskin M, et al. Genetic associations of the response to inhaled corticosteroids in children during an asthma exacerbation. Pediatr Allergy Immunol. 2016 Aug;27(5):507-13.
36. Xue L, Li C, Wang Y, Sun W, Ma C, He Y, et al. Single nucleotide polymorphisms in non-coding region of the glucocorticoid receptor gene and prednisone response in childhood acute lymphoblastic leukemia. Leuk Lymphoma. 2015 Jun;56(6):1704-9.
37. De Iudicibus S, Stocco G, Martelossi S, Drigo I, Norbedo S, Lionetti P, et al. Association of BclI polymorphism of the glucocorticoid receptor gene locus with response to glucocorticoids in inflammatory bowel disease. Gut. 2007 Sep;56(9):1319-20.

38. Teeninga N, Kist-van Holthe JE, van den Akker EL, Kersten MC, Boersma E, Krabbe HG, et al. Genetic and in vivo determinants of glucocorticoid sensitivity in relation to clinical outcome of childhood nephrotic syndrome. Kidney Int. 2014 Jun;85(6):1444-53.
39. Krupoves A, Mack D, Deslandres C, Seidman E, Amre DK. Variation in the glucocorticoid receptor gene (NR3C1) may be associated with corticosteroid dependency and resistance in children with Crohn's disease. Pharmacogenet Genomics. 2011 Aug;21(8):454-60.

Chapter 6

Systemic and Local Corticosteroid Use is Associated With Reduced Cognition and Mood and Anxiety Disorders

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Abstract

Background: *Use of local corticosteroids, especially the inhaled types, has increasingly been associated with systemic uptake and consequent adverse effects. In this study, we assessed the associations between the use of different corticosteroid types with cognitive and neuropsychiatric adverse effects related to high glucocorticoid exposure.*

Methods: *In 83,592 adults (mean age 44 years, 59% women) of the general population (Lifelines Cohort Study), we analyzed the relationship between corticosteroid use with executive cognitive functioning (Ruff Figural Fluency Test), and presence of mood and anxiety disorders (Mini-International Neuropsychiatric Interview survey). We performed additional exploration for effects of physical quality of life (QoL; RAND-36), and inflammation (high-sensitive C-reactive protein [CRP]).*

Results: *Cognitive scores were lower among corticosteroid users, in particular of systemic and inhaled types, when compared to nonusers. Users of inhaled types showed lower cognitive scores irrespective of physical QoL, psychiatric disorders, and high-sensitive CRP. Overall corticosteroid use was also associated with higher likelihood for mood and anxiety disorders. Users of inhaled corticosteroids were more likely to have mood disorders (OR 1.40 [95% CI 1.19–1.65], $p < 0.001$) and anxiety disorders (OR 1.19 [95% CI 1.06–1.33], $p = 0.002$). These findings were independent of physical QoL. A higher likelihood for mood disorders was also found for systemic users whereas nasal and dermal corticosteroid users were more likely to have anxiety disorders.*

Conclusions: *Commonly used local corticosteroids, in particular inhaled types, and systemic corticosteroids are associated with reduced executive cognitive functioning and a higher likelihood of mood and anxiety disorders in the general adult population.*

Introduction

Adverse effects of the glucocorticoid cortisol are usually observed in case of supraphysiological exposure as seen in patients with endogenous or exogenous Cushing's syndrome. Given the extent of cortisol action, high levels can lead to various physical as well as mental alterations. Cushing's syndrome patients, for example, develop obesity, hypertension, menstrual irregularities, and neuropsychiatric pathologies [1]. The incidence of endogenous Cushing's syndrome is extremely low with approximately 1–2 new cases per million persons annually [2]. However, the main cause of excessive glucocorticoid exposure is due to exogenous administration of drugs containing synthetic glucocorticoids [3]. These corticosteroids are one of the most prescribed drugs given their high anti-inflammatory and immunomodulatory potential, and many indications. Besides, the availability of corticosteroids in many systemic and local administration formulations makes them readily feasible and convenient for clinical use. We previously showed that nearly 11% of the adults of the general population of the Netherlands was using any type of corticosteroid, which is comparable with prescription numbers in ambulatory care in the United States [4].

The common assumption is that only systemic corticosteroid variants can induce systemic adverse events, and that the effects of the local forms are generally limited to the application site. However, mounting evidence is questioning this notion. A large meta-analysis, for example, demonstrated an increased risk of adrenal insufficiency also with local corticosteroid forms [5]. Moreover, we previously demonstrated that users of systemic corticosteroids as well as the local forms, in particular of the inhaled types, were more likely to have metabolic syndrome, increased waist circumference, higher body mass index (BMI), and other adverse cardiometabolic derangements in comparison to nonusers [6]. Both increased adrenal insufficiency risk and higher likelihood of frequent corticosteroid-related metabolic effects in local corticosteroid users support the idea of systemic absorption and subsequent systemic adverse effects of local corticosteroids. In this regard, it would be reasonable to also expect effects on the brain in the case of systemic availability of these types. Despite previous studies observing cognitive impairments [7, 8] and psychiatric disorders [9] in endogenous Cushing's syndrome patients and systemic corticosteroid users [10], there are, to our knowledge, no large population-based studies that have investigated the associations between cognitive and psychiatric indices and use of the various local corticosteroid types. In this study, we therefore assessed the relationship of systemic and local corticosteroid use with cognition and neuropsychiatric health in the general adult population.

Subjects and Methods

Study Population

We included data of adult participants of the Lifelines research program, which is a multi-disciplinary prospective population-based cohort study examining in a unique 3-generation design the health and health-related behaviors of 167,729 persons living in the north of The Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioral, physical and psychological factors that contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics [11]. Participants were included in case of complete data regarding outcomes of the assessment for cognitive functioning, neuropsychiatric health, and physical quality of life (QoL). After exclusion of participants with inconclusive information on drug use, there were in total 83,592 subjects eligible for the current study.

Corticosteroid Use

Current drug use was assessed by questionnaire and on-site inspection of drug containers. Drugs were subsequently coded ac- cording to their corresponding WHO Anatomical Therapeutic Chemical code. We filtered users of any type of corticosteroid and grouped them as being "corticosteroid users." Drugs with only mineralocorticoid action were not included. Subclassification was made for users of only local administration forms or users of systemic corticosteroids (i.e., oral and/or parenteral) with or without any of the other types. To elaborate the associations with specific administration forms, we further classified single-type users ac- cording to their use of only systemic, inhaled, nasal, dermal or other (i.e., otological, ocular, intestinal, local-oral, hemorrhoidal, or gynaecological) types.

Cognitive Functioning

The Ruff Figural Fluency Test (RFFT) is a measure of nonverbal fluency as part of executive cognitive functioning. The test contains 5 sheets each consisting of 35 identical frames of 5-dot patterns with or without distracting elements. For each part, participants are instructed to connect 2 or more dots per frame with straight lines and to make as many as possible unique designs without falling into repetition in 1 min [12]. The primary outcome is the total number of unique designs (i.e., unique design score), which can range from 0 to 175. The test was not performed in subjects who were consistently unable to properly hold a pen, had impaired vision, a score below 26 on the Mini-Mental State Examination, or had performed the test previously in another cohort study.

Neuropsychiatric Assessment

The Mini-International Neuropsychiatric Interview is a structured interview intended for diagnosing psychiatric disorders ac- cording to DSM-IV and ICD-10 diagnostic criteria [13]. Trained professionals administered the modules regarding the current diagnosis of major depressive episode, dysthymia, social phobia generalized anxiety disorder, agoraphobia without a history of panic disorder, and lifetime presence of panic disorder with or without agoraphobia. Patients were categorized as having a mood disorder if they met the diagnostic criteria for major depressive episode or dysthymia. Anxiety disorder was deemed present in case of any of the other assessed diagnoses.

Physical QoL

Due to potential confounding by disease burden, we planned to perform subgroup analyses with stratification for health-related QoL. For this purpose, we used the outcomes of the RAND-36 questionnaire, which is a commonly used survey consisting of 36 questions related to own health status. A weighted scoring and summation of selected items results in a score between 0 and 100 for 8 different domains [14]. By calculating the Z-score for each domain using Dutch reference population [15], we computed the aggregated physical component summary score [16] as a proxy for physical QoL. A higher score corresponds to a better QoL.

Covariates

To minimize confounding, we included potential covariates based on literature, biological plausibility, and statistical significance. Besides age and sex, we assessed self-reported educational attainment classified as low (i.e., no education, primary, lower or preparatory vocational education, and lower general secondary education), middle (i.e., intermediate vocational education or apprenticeship, and higher general secondary education or pre-university secondary education), high (i.e., higher vocational education, and university), and other. The use of psychotropic drugs was evaluated by screening current drug use for antiepileptics (ATC group N03), psycholeptics (N05; e.g., antipsychotics, anxiolytics), and/or psychoanaleptics (N06; e.g., antidepressants, psychostimulants). Lifestyle factors including physical activity, smoking, and alcohol use were assessed as previously described [6]. With regard to cardiometabolic factors, we included data on BMI, and presence of cardiovascular diseases. Trained technicians measured body weight (kg), and height (cm). Weight and height were used to compute BMI (kg/m^2). For cardiovascular diseases, subjects were asked to report if they had a past event of stroke, myocardial infarction, balloon angioplasty, and/or coronary artery bypass grafting. Any of the latter 3 conditions are also presented as coronary heart diseases in the results. Data of some covariates were missing for <5% of the

subjects, however, this was higher for physical activity (6.8%), smoking (7.0%), and alcohol use (9.1%).

Statistical Analysis

Differences in descriptive characteristics between users and nonusers were analyzed with Student t test or Mann-Whitney U test for continuous variables, and chi-square test for categorical data. Concerning the cognitive outcome, we performed analyses of covariance to assess differences in unique design score (RFFT analysis) between different routes of administration and for single-type users versus nonusers. We first analyzed crude differences, followed by adjustments for age, and sex. In the main model, we additionally adjusted for educational attainment, BMI, smoking, alcohol use, physical activity, cardiovascular diseases, and use of psychotropic drugs. The differences between users and nonusers in the binary outcomes for presence or absence of psychiatric disorders were assessed with logistic regression analyses. We performed similar adjustments for these analyses, and reported the crude and fully adjusted results. Interaction effects with sex and age were assessed in main analyses with complete group. With respect to disease burden, we additionally performed stratified analysis by either low (≤ median) or high (> median) physical component summary score. Moreover, given the recent finding that the unique design score is associated with anxiety and depression [17] we additionally repeated the analyses for cognitive functioning separately in participants with and without any of the assessed psychiatric disorders. IBM SPSS Statistics version 22.0.0.2 (IBM Corp., Armonk, NY, USA) was used to carry out multiple imputations for covariates with missing data, and to perform all analyses (2-sided). p values below 0.050 were considered statistically significant.

Sensitivity Analyses

Since corticosteroids are generally used in the presence of inflammatory processes and given the potential effect of inflammation on mental health and functioning, we performed sensitivity analyses to evaluate the relationship between the inflammatory marker C-reactive protein (CRP) with our outcomes and whether this contributed to the differences between users and nonusers. High-sensitive CRP (hsCRP) was measured with an immuno-nephelometric assay (CardioPhase hsCRP, Siemens Healthcare Diagnostics, Marburg, Germany). Data were available of 45,395 subjects of whom 89.6% were nonusers and 10.4% users. Coefficients were assessed in the main models for both cognition and psychiatric disorders.

Results

Subject Characteristics

Characteristics of the study population as a whole and stratified for corticosteroid use are shown in Table 1. Subjects were on average 44.2 years old and 58.8% were women. Corticosteroids were being used by 10.8% of the study population, which largely consisted of users of inhaled corticosteroids. The majority of users of the different administration forms were single-type users (Fig. 1). Physical QoL was higher in nonusers in comparison to users (53.3 [±7.2] vs. 50.3 [±9.0], $p < 0.001$).

Corticosteroid Use and Executive Cognitive Functioning

The total unique design score was 81.3 (±23.3) in the total study population. Overall corticosteroid use was associated with a 1.6 (95% CI 1.1–2.1, $p < 0.001$) lower cognitive score, which remained statistically significant after full adjustments of all specified covariates. Users of only local types and users of systemic (with or without local corticosteroids) had a lower score in comparison to nonusers (–0.9 [–1.4 to –0.4], $p < 0.001$; –3.0 [–5.1 to –1.0], $p = 0.004$ respectively). Within single-type users, only systemic corticosteroid users (–2.9 [–5.3 to –0.4], $p = 0.024$) and in- haled corticosteroid users (–2.1 [–2.9 to –1.4], $p < 0.001$) scored lower in unique design score when compared to non-corticosteroid users. Subgroup analyses stratified for either low or high physical QoL and for presence or absence of mood and/or anxiety disorders revealed consistently lower scores in inhaled corticosteroid users compared to nonusers (Fig. 2). With respect to other forms, only the use of systemic corticosteroids was associated with a significant lower score in subjects without mood and anxiety disorders (–3.1 [–5.8 to –0.5], $p = 0.021$), whereas no significant differences were found for the remaining types. No interaction with sex or age was observed. Corticosteroid Use and Mood and Anxiety Disorders Current mood and/or anxiety disorders were present in 11.1% of the total population, and were both more prevalent in corticosteroid users in comparison to nonusers (both $p < 0.001$; Table 1). Stratification for the main route of corticosteroid administration revealed associations for local types with both mood disorders (OR 1.24 [1.11–1.40]) and anxiety disorders (OR 1.18 [1.10–1.27], both $p < 0.001$; Table 2). Systemic corticosteroid use was only associated with mood disorders, which was especially evident in the single-type users (OR 1.75 [1.05–2.91], $p = 0.031$). Among users of local corticosteroids, users of only inhaled corticosteroids were more likely to have mood disorders (OR 1.40 [1.19–1.65], $p < 0.001$) and anxiety disorders (OR 1.19 [1.06–1.33], $p = 0.002$) in comparison to nonusers. For anxiety disorders, similar associations were present in nasal corticosteroid users (OR 1.21 [1.05–1.39], $p = 0.007$) and dermal corticosteroid users (OR 1.18 [1.01–1.40], $p = 0.043$). Interaction analyses for sex and age showed no significant differences. With subgroup analyses, inhaled corticosteroid use was found to be associated

Table 1: Characteristics of study sample.

		Corticosteroid use	
	All (N=83,592)	Nonusers (N=74,591)	Users (N=9,001)
Demographics			
Age (years)[a]	44.2 (±12.3)	44.1 (±12.3)	45.2 (±12.6)
Sex (female)[a]	49,174 (58.8)	43,512 (58.3)	5,662 (62.9)
Educational attainment[a]			
• Low	24,752 (29.6)	21,998 (29.5)	2,754 (30.6)
• Middle	32,879 (39.3)	29,491 (39.5)	3,388 (37.6)
• High	24,424 (29.2)	21,747 (29.2)	2,677 (29.7)
• Other	1,537 (1.8)	1,355 (1.8)	182 (2.0)
Use of psychotropic drugs[a]	7,441 (8.9)	6,331 (8.5)	1,110 (12.3)
High-sensitive CRP (mg/L)[a,b]	1.20 (2.20)	1.20 (2.10)	1.50 (2.90)
Lifestyle			
Physical activity			
• 0 days per week	4,071 (4.9)	3,628 (4.9)	443 (4.9)
• 1-4 days per week	38,632 (46.2)	34,568 (46.3)	4,064 (45.2)
• ≥5 days per week	40,889 (48.9)	36,395 (48.8)	4,494 (49.9)
Smoking[a]			
• Nonsmoker	38,790 (46.4)	34,428 (46.2)	4,362 (48.5)
• Former smoker	26,098 (31.2)	23,095 (31.0)	3,003 (33.4)
• Current smoker	18,704 (22.4)	17,068 (22.9)	1,636 (18.2)
Alcohol use[a]			
• None	18,490 (22.1)	16,310 (21.9)	2,180 (24.2)
• ≤1 drink/day	41,161 (49.2)	36,701 (49.2)	4,460 (49.6)
• 1-2 drinks/day	17,049 (20.4)	15,356 (20.6)	1,693 (18.8)
• >2 drinks/day	6,892 (8.3)	6,224 (8.3)	668 (7.4)
Cardiometabolic features			
BMI (kg/m^2)[a]	26.1 (±4.3)	26.0 (±4.3)	26.7 (±4.8)
Cardiovascular diseases[a]	1,762 (2.1)	1,526 (2.0)	236 (2.6)
• Stroke[a]	555 (0.7)	481 (0.6)	74 (0.8)
• Coronary heart disease[a]	1,253 (1.5)	1,084 (1.5)	169 (1.9)
Ruff Figural Fluency Test (executive cognitive functioning)			
Unique designs[a]	81.3 (±23.3)	81.5 (±23.3)	79.9 (±23.4)
MINI (psychiatric disorders)			
Mood and/or anxiety disorders[a]	9,301 (11.1)	8,064 (10.8)	1,237 (13.7)
• Mood disorders[a]	2,802 (3.4)	2,393 (3.2)	409 (4.5)
• Anxiety disorders[a]	8,212 (9.8)	7,138 (9.6)	1,074 (11.9)
RAND36 (health-related quality of life)			
Physical component summary score[a]	52.9 (±7.5)	53.3 (±7.2)	50.3 (±9.0)

All values are depicted as median (interquartile range), mean (±SD), or numbers (percentage).
Abbreviations: BMI, body mass index; CRP, C-reactive protein.
[a] Significant crude differences between nonusers and users;
[b] Data on high-sensitive CRP were available in 45,395 participants including 40,695 nonusers and 4,700 users.

Table 2: Association between corticosteroid use and psychiatric disorders.

		Mood Disorders				
			Crude model		Adjusted model[a]	
	N	Present (%)	OR	95% CI	OR	95% CI
Nonusers	74,591	2,393 (3.2)	Ref.		Ref.	
Overall users	9,001	409 (4.5)	**1.44**	**1.29, 1.60*****	**1.26**	**1.13, 1.41*****
Route						
Local	8,608	385 (4.5)	**1.41**	**1.27, 1.58*****	**1.24**	**1.11, 1.40*****
Systemic	393	24 (6.1)	**1.96**	**1.30, 2.97****	**1.58**	**1.02, 2.44***
Single type						
Systemic	280	18 (6.4)	**2.07**	**1.28, 3.35****	**1.75**	**1.05, 2.91***
Inhaled	3,248	182 (5.6)	**1.79**	**1.53, 2.09*****	**1.40**	**1.19, 1.65*****
Nasal	2,142	75 (3.5)	1.10	0.87, 1.38	1.06	0.83, 1.35
Dermal	1,620	46 (2.8)	0.88	0.66, 1.19	0.90	0.66, 1.22
Others	263	14 (5.3)	1.70	0.99, 2.91	1.63	0.93, 2.86

		Anxiety Disorders				
			Crude model		Adjusted model[a]	
	N	Present (%)	OR	95% CI	OR	95% CI
Nonusers	74,591	7,138 (9.6)	Ref.		Ref.	
Overall users	9,001	1,074 (11.9)	**1.28**	**1.20, 1.37*****	**1.17**	**1.09, 1.26*****
Route						
Local	8,608	1,035 (12.0)	**1.29**	**1.21, 1.38*****	**1.18**	**1.10, 1.27*****
Systemic	393	39 (9.9)	1.04	0.75, 1.45	0.86	0.61, 1.21
Single type						
Systemic	280	28 (10.0)	1.05	0.71, 1.55	0.89	0.59, 1.34
Inhaled	3,248	421 (13.0)	**1.41**	**1.27, 1.56*****	**1.19**	**1.06, 1.33****
Nasal	2,142	249 (11.6)	**1.24**	**1.09, 1.42****	**1.21**	**1.05, 1.39****
Dermal	1,620	177 (10.9)	1.16	0.99, 1.36	**1.18**	**1.01, 1.40***
Others	263	28 (10.6)	1.13	0.76, 1.67	1.04	0.69, 1.57

The group of non-corticosteroid users is taken as reference. [a]The analyses are adjusted for age, sex, educational attainment, body mass index, smoking, alcohol use, physical activity, cardiovascular diseases, and use of psychotropic drugs. *P<.050, **P<.010, ***P<.001.

with increased likelihood for mood and/ or anxiety disorders in both subjects with low physical QoL (OR 1.15 [1.01–1.29], p = 0.030) as well as high physical QoL (OR 1.32 [1.08–1.62], p = 0.008; Table 3). Among users with low physical QoL, relatively high effect sizes were found for users of nasal corticosteroids and users of the group of other corticosteroids, although the difference was significant only in the former group.

Table 3: Corticosteroid use and presence of mood and/or anxiety disorders by physical quality of life.

		Low Physical Quality of Life (N=41,796)				
			Crude model		Adjusted model[a]	
	N	Present (%)	OR	95% CI	OR	95% CI
Nonusers	36,011	4,740 (13.2)	Ref.		Ref.	
Overall users	5,785	906 (15.7)	**1.23**	**1.13, 1.32*** **	**1.15**	**1.06, 1.24****
Route						
Local	5,444	858 (15.8)	**1.23**	**1.14, 1.34*** **	**1.16**	**1.06, 1.26*** **
Systemic	341	48 (14.1)	1.08	0.80, 1.47	0.98	0.71, 1.35
Single type						
Systemic	238	35 (14.7)	1.14	0.79, 1.63	1.07	0.73, 1.56
Inhaled	2,248	372 (16.5)	**1.31**	**1.17, 1.47*** **	**1.15**	**1.01, 1.29***
Nasal	1,212	191 (15.8)	**1.23**	**1.05, 1.45****	**1.23**	**1.04, 1.45***
Dermal	882	126 (14.3)	1.10	0.91, 1.33	1.11	0.91, 1.36
Others	170	26 (15.3)	1.19	0.78, 1.81	1.18	0.76, 1.83

		High Physical Quality of Life (N=41,796)				
			Crude model		Adjusted model[a]	
	N	Present (%)	OR	95% CI	OR	95% CI
Nonusers	38,580	3,324 (8.6)	Ref.		Ref.	
Overall users	3,216	331 (10.3)	**1.22**	**1.08, 1.37****	**1.20**	**1.06, 1.36****
Route						
Local	3,164	329 (10.4)	**1.23**	**1.09, 1.39*** **	**1.21**	**1.07, 1.37****
Systemic	52	2 (3.8)	0.42	0.10, 1.74	0.47	0.11, 1.94
Single type						
Systemic	42	2 (4.8)	0.53	0.13, 2.20	0.55	0.13, 2.30
Inhaled	1,000	118 (11.8)	**1.42**	**1.17, 1.73*** **	**1.32**	**1.08, 1.62****
Nasal	930	90 (9.7)	1.14	0.91, 1.42	1.12	0.89, 1.41
Dermal	738	68 (9.2)	1.08	0.84, 1.39	1.17	0.91, 1.52
Others	93	8 (8.6)	1.00	0.48, 2.06	0.92	0.43, 1.96

The group of non-corticosteroid users is taken as reference. Mood and anxiety disorders are combined together due to otherwise small number of cases in users. [a]The analyses are adjusted for age, sex, educational attainment, body mass index, smoking, alcohol use, physical activity, cardiovascular diseases, and use of psychotropic drugs. *P<.050, **P<.010, ***P<.001.

Sensitivity Analyses

Corticosteroid users had in general a higher hsCRP than nonusers (median [IQR] 1.50 [2.90] vs. 1.20 [2.10] mg/L, $p < 0.001$; Table 1). With regard to cognition, there was a negative association between hsCRP and unique design score (B = –0.087, SE = 0.022, $p < 0.001$). Nevertheless, the use of systemic as well as inhaled corticosteroids was persistently associated with significantly lower cognitive scores when adjusted for hsCRP (Table 4). Additional adjustment for mood and anxiety disorders did not affect these results (data not shown). There was no association between hsCRP and the presence of mood and anxiety disorders.

Table 4: Differences in cognition between users and nonusers with available hsCRP data (N=45,395).

		Unique Design Score	
	N	Model 1	Model 2
Nonusers	40,695	Ref.	Ref.
Overall users	4,700	**-1.12 [-1.76, -0.48]*** **	**-1.07 [-1.71, -0.43]** **
Route			
Local	4,497	**-0.94 [-1.59, -0.28]** **	**-0.89 [-1.55, -0.24]** **
Systemic	203	**-5.18 [-8.10, -2.25]*** **	**-5.04 [-7.97, -2.12]*** **
Single type			
Systemic	153	**-5.27 [-8.64, -1.90]** **	**-5.13 [-8.50, -1.76]** **
Inhaled	1,783	**-2.33 [-3.34, -1.32]*** **	**-2.28 [-3.29, -1.27]*** **
Nasal	1,078	-0.37 [-1.65, 0.92]	-0.33 [-1.61, 0.96]
Dermal	856	0.99 [-0.45, 2.42]	1.00 [-0.44, 2.44]
Others	120	2.37 [-1.43, 6.17]	2.39 [-1.41, 6.19]

Adjusted mean differences (95% C.I.) in total unique design score between corticosteroids users and nonusers (reference). Model 1 is adjusted for age, sex, educational attainment, body mass index, smoking, alcohol use, physical activity, cardiovascular diseases, and use of psychotropic drugs. Model 2 is additionally adjusted for hsCRP. **P<.010, ***P<.001.

Discussion

In the current study, we show that use of both systemic and local corticosteroids, particularly the inhaled types, is associated with a reduced executive cognitive functioning and a higher likelihood of mood and anxiety disorders. With regard to the inhaled forms, these findings were persistent in both individuals with low and high physical QoL suggesting potential drug effects regardless of physical condition. Despite an inverse association between hsCRP levels and cognition, the use of systemic and inhaled types was independently associated with lower cognitive performance.

A reduction in executive cognitive functioning in local and systemic corticosteroid users, as found in this study, could hint on corticosteroid effects on the brain. Although multiple studies have shown an association between corticosteroid use and central nervous system disorders, the pathophysiology of exogenous corticosteroid action on the brain is still not well understood [18]. Corticosteroids have been described to affect various aspects of brain physiology, including selective hippocampal atrophy [19], neuronal plasticity [20, 21], neurotoxicity [22], and neurogenesis [23]. The hippocampus in particular is an important target of corticosteroids and strongly expresses both mineralocorticoid as well as glucocorticoid receptors [24–26]. Excess corticosteroids could lead to reversible and irreversible damage to hippocampal structure and thus contribute to cognitive

impairment [26]. Another brain region that seems susceptible to corticosteroid effects is the medial prefrontal cortex. Chronic corticosterone administration [27] and behavioral stress [28] have been demonstrated to result in a reorganization of apical dendrites in pyramidal neurons of the medial prefrontal cortex in rodent models. This reorganization may have functional consequences as reflected in glucocorticoid-induced changes in cognition, working memory and stress-related behavioral disorders [27–29]. Interestingly, the RFFT used as a measure of non-verbal fluency in the present study is especially sensitive to function [30] and dysfunction [31] of the right frontal lobe. In line with this, a previous placebo-controlled, crossover, randomized trial with healthy subjects receiving supraphysiological oral hydrocortisone found that corticosteroids indeed induced cognitive impairments which were especially related to frontal lobe dysfunction [32]. It is therefore conceivable that in case of systemic availability of local corticosteroids, in particular of the inhaled forms, these regions would also be exposed to supraphysiological glucocorticoid levels and subsequently impaired in their functioning.

We found that users of systemic and inhaled corticosteroids scored nearly 2–3 points lower on the RFFT when compared to nonusers. It is noteworthy to mention that these differences are relatively modest. Nevertheless, it is especially interesting that the outcomes point repeatedly in the same direction in all analyses for the systemic and the inhaled types. Among important contributors to RFFT score, age has consistently found to be negatively associated [12, 33] as was also observed in our cohort. In terms of clinical relevancy, the worse scores with inhaled and systemic corticosteroid use would on average correspond to lower scores as found with an age increase of 4.3 and 5.8 years, respectively, in our group of nonusers while controlling for other covariates.

Neuropsychiatric disorders are known to be one of the most prevalent and distressing adverse effects in users of systemic corticosteroids. Fardet and colleagues observed that approximately half of these users reported to suffer from neuropsychiatric complaints, including anxiety and depression, after they had started with corticosteroid treatment [34]. In addition, a small study with physically and mentally healthy subjects also showed behavioral changes in 75% of the participants after high-dose oral prednisone administration for 5 days [35]. Moreover, endogenous Cushing's syndrome has also frequently been linked with various psychopathologies [9, 36] among which mood and anxiety disorders as observed in the current study. In that sense, it would be conceivable that the inhaled types could also lead to these disorders in case of systemic absorption. This would even be more expected, given the high glucocorticoid receptor binding affinity of the frequently administered inhaled forms which is comparable to

nearly 10–20 times that of dexamethasone [37]. Interestingly, the unfavorable findings regarding cognition, mood and anxiety were especially evident in users of these types. However, it should be noted that the effect sizes are relatively small. Nevertheless, these and our previous findings of higher likelihood for metabolic syndrome, a higher BMI, and other cardiometabolic alterations in users of inhaled corticosteroids [6] are in line with frequently observed features in systemic corticosteroids users and Cushing's syndrome patients. These findings are consistent with our hypothesis of systemic availability and effects of inhaled corticosteroids in which case both somatic and brain effects would be observed when exposed to supraphysiological dosages.

An important consideration in understanding the effect of corticosteroids on the brain is their penetration of the blood-brain barrier. While corticosteroids, in general, pass through cell membranes to enter the brain on account of their lipophilicity, cells comprising the blood-brain barrier express proteins of the multidrug transporter system, which limit the access of exogenous molecules to the brain [24, 38]. Further functional studies are essential to demonstrate the access of the various exogenous corticosteroids to different brain areas and the implications for cognitive and psychiatric functioning.

The strength of this work lies in the in-depth phenotyping, extensive assessment, and completeness for the outcome observations in large number of subjects from the general adult population. An important limitation to mention is the observational nature which hinders drawing conclusions on causality and is prone to residual confounding. Moreover, we assessed only one aspect of the various cognitive functioning domains and merely in persons with no significant impairments with the Mini- Mental State Examination. Finally, there were no data available on the cumulative dose exposure. However, our main findings are particularly driven by the inhaled forms that are conceivably used in a chronic fashion giving the underlying often chronic indications such as asthma.

Conclusions

Commonly used local corticosteroids, in particular the inhaled types, and systemic corticosteroids are associated with reduced executive cognitive functioning and a higher likelihood of mood and anxiety disorders in the general adult population. Future confirmatory studies are needed to ratify our findings and to prove temporality, while further research should also assess the associations with other cognitive processes and psychiatric disorders.

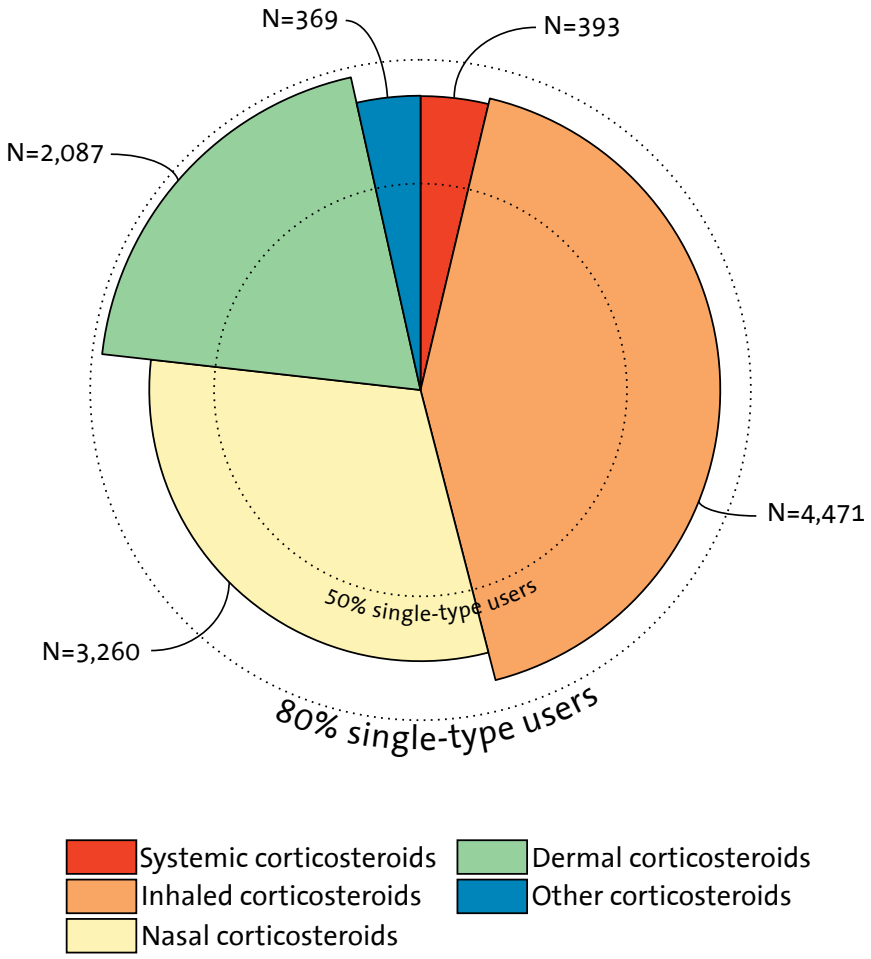

Figure 1: Distribution of corticosteroid use in the study sample.

Superimposed pie chart illustrating for each corticosteroid type the total number of users and the proportion of single-type use. The former is presented as the size of each slice and is also written in text beside. The radial length marks the percentage of users within each type who were not using any other corticosteroid types. These groups of single-type users were used to assess the associations for the specific administration forms. Single-type use was most prevalent in dermal corticosteroid users (77.6%) while nasal corticosteroids were relatively most often combined with other types of corticosteroids.

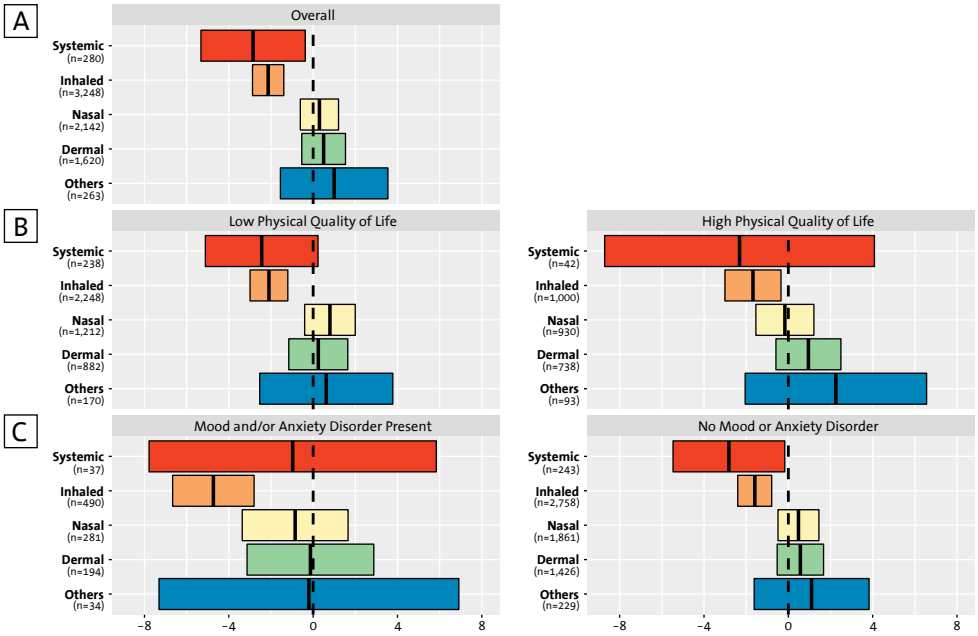

Figure 2: Executive cognitive functioning in corticosteroid users in comparison to nonusers.

Adjusted mean differences (95% CI) in unique design score between the single-type corticosteroid users, and nonusers as reference (a). The same analyses stratified for subjects with either low or high physical component summary score as proxy for physical QoL (b), and for presence or absence of mood and/or anxiety disorders (c). Analyses are adjusted for age, sex, educational attainment, BMI, smoking, alcohol use, physical activity, cardiovascular diseases, and use of psychotropic drugs.

References

1 Nieman LK. Cushing's syndrome: update on signs, symptoms and biochemical screening. Eur J Endocrinol. 2015 Oct;173(4):M33–8.

2 Lindholm J, Juul S, Jørgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, et al. Incidence and late prognosis of cushing's syndrome: a population-based study. J Clin Endocrinol Metab. 2001 Jan;86(1):117–23.

3 Nieman LK, Biller BM, Findling JW, Newell- Price J, Savage MO, Stewart PM, et al. The di- agnosis of cushing's syndrome: an endocrine society clinical practice guideline. J Clin En- docrinol Metab. 2008 May;93(5):1526–40.

4 Raofi S, Schappert SM. Medication therapy in ambulatory medical care: United States, 2003-04. Vital Health Stat 13. 2006 Dec;(163):1–40.

5 Broersen LH, Pereira AM, Jørgensen JO, Dekkers OM. Adrenal insufficiency in corticosteroids use: systematic review and meta- analysis. J Clin Endocrinol Metab. 2015 Jun; 100(6):2171–80.

6 Savas M, Muka T, Wester VL, van den Akker EL, Visser JA, Braunstahl GJ, et al. Associations between systemic and local corticosteroid use with metabolic syndrome and body mass index. J Clin Endocrinol Metab. 2017 Oct;102(10):3765–74.

7 Forget H, Lacroix A, Somma M, Cohen H. Cognitive decline in patients with Cushing's syndrome. J Int Neuropsychol Soc. 2000 Jan; 6(1):20–9.

8 Bourdeau I, Bard C, Forget H, Boulanger Y, Cohen H, Lacroix A. Cognitive function and cerebral assessment in patients who have Cushing's syndrome [ix.]. Endocrinol Metab Clin North Am. 2005 Jun;34(2):357–69.

9 Sonino N, Fava GA. Psychiatric disorders associated with Cushing's syndrome. Epidemiology, pathophysiology and treatment. CNS Drugs. 2001;15(5):361–73.

10 Judd LL, Schettler PJ, Brown ES, Wolkowitz OM, Sternberg EM, Bender BG, et al. Adverse consequences of glucocorticoid medication: psychological, cognitive, and behavioral effects. Am J Psychiatry. 2014 Oct;171(10):1045–51.

11 Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, et al. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. Eur J Epidemiol. 2008;23(1):67–74.

12 Ruff RM, Light RH, Evans RW. The ruff figural fluency test - a normative study with adults. Dev Neuropsychol. 1987;3(1):37–51.

13 Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Inter- view (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59 Suppl 20:22–33.

14 VanderZee KI, Sanderman R, Heyink JW, de Haes H. Psychometric qualities of the RAND 36-Item Health Survey 1.0: a multidimensional measure of general health status. Int J Behav Med. 1996;3(2):104–22.

15 Aaronson NK, Muller M, Cohen PD, Essink- Bot ML, Fekkes M, Sanderman R, et al. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. J Clin Epidemiol. 1998 Nov;51(11): 1055–68.
16 Ware JE, Kosinski M, Keller SD. Sf-36 physical and mental component summary measures: A user's manual. Boston (MA): The Health Institute, New England Medical Center; 1994.
17 Gulpers B, Lugtenburg A, Zuidersma M, Verhey FR, Voshaar RC. Anxiety disorders and figural fluency: A measure of executive function. J Affect Disord. 2018 Jul;234:38–44.
18 Ciriaco M, Ventrice P, Russo G, Scicchitano M, Mazzitello G, Scicchitano F, et al. Corticosteroid-related central nervous system side effects. J Pharmacol Pharmacother. 2013 Dec; 4(5 Suppl 1):S94–8.
19 McEwen BS. Possible mechanisms for atrophy of the human hippocampus. Mol Psychiatry. 1997 May;2(3):255–62.
20 Woolley CS, Gould E, McEwen BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res. 1990 Oct;531(1-2):225–31.
21 Watanabe Y, Gould E, McEwen BS. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res. 1992 Aug;588(2):341–5.
22 Sapolsky RM. The possibility of neurotoxicity in the hippocampus in major depression: a primer on neuron death. Biol Psychiatry. 2000 Oct;48(8):755–65.
23 Odaka H, Adachi N, Numakawa T. Impact of glucocorticoid on neurogenesis. Neural Regen Res. 2017 Jul;12(7):1028–35.
24 De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. Endocr Rev. 1998 Jun; 19(3):269–301.
25 de Kloet ER, Oitzl MS, Joëls M. Functional implications of brain corticosteroid receptor diversity. Cell Mol Neurobiol. 1993 Aug;13(4): 433–55.
26 Brown ES, Rush AJ, McEwen BS. Hippocampal remodeling and damage by corticosteroids: implications for mood disorders. Neuropsychopharmacology. 1999 Oct;21(4):474–84.
27 Wellman CL. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. J Neurobiol. 2001 Nov;49(3):245–53.
28 Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, et al. Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cor- tex. Neuroscience. 2004;125(1):1–6.
29 McKlveen JM, Myers B, Herman JP. The me- dial prefrontal cortex: coordinator of autonomic, neuroendocrine and behavioural responses to stress. J Neuroendocrinol. 2015 Jun;27(6):446–56.
30 Foster PS, Williamson JB, Harrison DW. The Ruff Figural Fluency Test: heightened right frontal lobe delta activity as a function of performance. Arch Clin Neuropsychol. 2005 Jun; 20(4):427–34.

31 Ruff RM, Allen CC, Farrow CE, Niemann H, Wylie T. Figural fluency: differential impairment in patients with left versus right frontal lobe lesions. Arch Clin Neuropsychol. 1994 Jan;9(1):41–55.

32 Young AH, Sahakian BJ, Robbins TW, Cowen PJ. The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers. Psychopharmacology (Berl). 1999 Aug;145(3):260–6.

33 Izaks GJ, Joosten H, Koerts J, Gansevoort RT, Slaets JP. Reference data for the Ruff Figural Fluency Test stratified by age and educational level. PLoS One. 2011 Feb;6(2):e17045.

34 Fardet L, Flahault A, Kettaneh A, Tiev KP, Généreau T, Tolédano C, et al. Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion. Br J Dermatol. 2007 Jul;157(1):142–8.

35 Wolkowitz OM, Rubinow D, Doran AR, Brei- er A, Berrettini WH, Kling MA, et al. Prednisone effects on neurochemistry and behavior. Preliminary findings. Arch Gen Psychiatry. 1990 Oct;47(10):963–8.

36 Pereira AM, Tiemensma J, Romijn JA. Neuropsychiatric disorders in Cushing's syndrome. Neuroendocrinology. 2010;92 Suppl 1:65–70.

37 Daley-Yates PT. Inhaled corticosteroids: potency, dose equivalence and therapeutic index. Br J Clin Pharmacol. 2015 Sep;80(3): 372–80.

38 Meijer OC, de Lange EC, Breimer DD, de Boer AG, Workel JO, de Kloet ER. Penetration of dexamethasone into brain glucocorticoid targets is enhanced in mdr1A P-glycoprotein knockout mice. Endocrinology. 1998 Apr;139(4):1789–93.

Chapter 7

Hair Glucocorticoids as Biomarker for Endogenous Cushing's Syndrome: Validation in Two Independent Cohorts

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Abstract

Background/Aims: *The current diagnostic workup of Cushing's syndrome (CS) requires various tests which only capture short-term cortisol exposure, whereas patients with endogenous CS generally have elevated cortisol levels over longer periods of time. Scalp hair assessment has emerged as a convenient test in capturing glucocorticoid concentrations over long periods of time. The aim of this multicenter, multinational, prospective, case-control study was to evaluate the diagnostic efficacy of scalp hair glucocorticoids in screening of endogenous CS.*

Methods: *We assessed the diagnostic performances of hair cortisol (HairF), hair cortisone (HairE), and the sum of both (sumHairF+E), as measured by a state-of-the-art LC-MS/MS technique, in untreated patients with confirmed endogenous CS (n = 89) as well as in community controls (n = 295) from the population-based Lifelines cohort study.*

Results: *Both glucocorticoids were significantly elevated in CS patients when compared to controls. A high diagnostic efficacy was found for HairF (area un- der the curve 0.87 [95% CI: 0.83–0.92]), HairE (0.93 [0.89–0.96]), and sumHairF+E (0.92 [0.88–0.96]) (all p < 0.001). The participants were accurately classified at the optimal cutoff threshold in 86% of the cases (81% sensitivity, 88% specificity, and 94% negative predictive value [NPV]) by HairF, in 90% of the cases (87% sensitivity, 90% specificity, and 96% NPV) by HairE, and in 87% of the cases (86% sensitivity, 88% specificity, and 95% NPV) by the sumHairF+E. HairE was shown to be the most accurate in differentiating CS patients from controls.*

Conclusion: *Scalp hair glucocorticoids, especially hair cortisone, can be seen as a promising biomarker in screening for CS. Its convenience in collection and workup additionally makes it feasible for first-line screening.*

Introduction

Cushing's syndrome (CS) results from excessive exposure to glucocorticoid hormones and is associated with significant morbidity and mortality [1]. After exclusion of exogenous CS caused by glucocorticoid-containing drugs, a variety of endogenous diseases can give rise to increased secretion of cortisol. Approximately 70% of the cases of endogenous CS are caused by a pituitary adenoma producing excessive ACTH, stimulating the adrenal to produce cortisol (i.e., Cushing's disease). The remainder of endogenous CS cases mostly consist of adrenal causes and ectopic ACTH production [1].

Endogenous CS is rare but often presents with common and therefore nonspecific signs and symptoms such as weight gain, fatigue, metabolic syndrome features, and depression [2]. Features more specific for CS include easy bruising, facial plethora, and proximal myopathy, but these do not occur in all patients [3]. This clinical dilemma can cause a significant delay in diagnosis, which is often made when the condition has been existing for an extended period of time and patients display multiple signs and symptoms of CS. Current guidelines recommend three different first-line screening tests: 24-h urinary free cortisol (UFC), late-night salivary cortisol (LNSC), and the 1-mg dexamethasone suppression test [4]. All three tests rely on patient compliance for the collection of samples or drug intake, and their limitations often necessitate repeated testing. Furthermore, they may be influenced by several factors such as kidney function (for UFC), gingival microtrauma (for LNSC), and drug use (for the dexamethasone suppression test).

Recently, we reported on the largest study thus far using measurements of scalp hair cortisol in patients with CS [5]. Scalp hair offers information about integrated cortisol exposure over months of time [6]. This may be particularly valuable in CS, where cortisol production may often vary across days. In our study, hair cortisol provided a 93% sensitivity and 91% specificity for CS, comparing well to first-line tests [5]. Furthermore, hair analysis can be used to create retrospective timelines of cortisol exposure, which can be helpful in cases of cyclic CS [7, 8].

All studies measuring hair cortisol in CS thus far relied on immunoassays to quantify cortisol. A recent advance in the development of hair steroid analysis is hair steroid profiling using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Recently, we have validated a method which measures hair values of cortisol, cortisone, testosterone, androstenedione, dehydroepiandrosterone sulfate, and 17α-hydroxyprogesterone [9]. In contrast to immunoassays, LC-MS/MS is less prone to interference, offers higher sensitivity, and can be used to measure multiple steroids simultaneously. The aim of this study was to assess the diagnostic

efficacy of hair cortisol (HairF) and cortisone (HairE) measured by LC-MS/ MS in two independently collected cohorts of patients with endogenous CS.

Subjects and Methods

Study Participants

Our study population consisted of 295 controls from the general Dutch population, which had also been included in our previous study [10], and 89 patients with proven endogenous CS. All controls were recruited from Lifelines, which is a multidisciplinary, prospective, population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the north of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, physical, and psychological factors which contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics [11]. Patients were recruited from two clinic sites, one in the Netherlands (Erasmus MC, Rotterdam; n = 19) and one in Germany (Klinikum der Ludwig-Maximilians-Universität München, Munich; n = 70). Diagnostic workup was performed ac- cording to the guideline [4] and the diagnosis of CS, de novo or recurrent, was biochemically established by experienced endocrinologists and proven by surgery and/or additional investigations (e.g., bilateral inferior petrosal sinus sampling).

Scalp Hair Measurements

In all participants, a scalp hair sample of approximately 100– 150 hairs was collected from the posterior vertex. The hair was cut as close to the scalp as possible and after sample collection stored in an envelope in the dark at room temperature. The protocol for hair processing and analysis was adapted from the previous method described in detail elsewhere [9]. In short, approximately 20 mg of the proximal 3 cm (or the entire length of the hair sample, if the hair was shorter than 3 cm) was weighed and cut into 1-cm-long pieces. The hair was washed in 2 mL of LCMS-grade isopropanol for 2 min and allowed to fully dry. Steroids were extracted overnight in 1.4 mL of LCMS-grade methanol, and 100 µL of cortisol-d3 and cortisone-d8 as internal standards for 18 h at 25 °C while the samples were being gently shaken. After extraction, hair samples were centrifuged at 4,369 g (4,500 rpm) for 5 min, and 900 µL of the extract was transferred to a clean tube. We then added 750 µL of methanol to the hair samples, which were spun down again, after which another 900 µL of extract was transferred to the tubes with the extract. The extracts were evaporated under a continuous nitrogen stream at 37 °C, reconstituted in 1 mL of purified water and 20 µL of methanol, and purified using solid-phase extraction.

Cortisol and cortisone concentrations were subsequently quantified by LC-MS/MS using a Xevo TQ-S system (Waters, Milford, MA, USA). HairF and HairE were successfully determined in 91 and 97% of the study participants. Data on both hair glucocorticoids were available for 89% of the study population. The interassay coefficient of variation for cortisol and cortisone was 14.8 and 15.3%, respectively. The intra-assay coefficient of variation for cortisol and cortisone was <11 and <8%, respectively. The lower limit of quantification of cortisol and cortisone was <1.3 and <9.3 pg/ mg, respectively. For research purposes, HairF and HairE measurements below the lower limit of quantification were included in the analyses as quantitative measures, since no recognized substitution method exists.

Statistical Analysis

We used SPSS version 24 (IBM Corp., Armonk, NY, USA) and RStudio version 1.0.136 (RStudio, Inc., Boston, MA, USA) with the pROC package [12] for the statistical analyses. The hair glucocorticoid values were logarithmically transformed to achieve a normal distribution and are reported as geometric means and 95% CI. The baseline characteristics were analyzed using ANCOVA if continuous, and using x2 tests if categorical. Associations between HairF and HairE were assessed by Pearson's correlation. The diagnostic efficacy of HairF, HairE, and the sum of HairF and HairE (sumHairF+E) for CS screening was assessed using receiver operating characteristic (ROC) curves.

Optimal cutoffs, defined as the curve points closest to the top-left corner, were initially determined for cohorts 1 and 2 separately. For the main analyses, both cohorts were combined and optimal cutoff values were determined for the complete population. De- Long's test was used to compare ROC curves between the two cohorts. Paired analyses were additionally performed to assess the discriminating ability of the different outcomes relative to each other. Moreover, we computed the diagnostic accuracy (i.e., the percentage of correctly classified subjects) and other diagnostic performance parameters (i.e., sensitivity, specificity, positive predictive value [PPV], negative predictive value [NPV], positive likelihood ratio [LR+], and negative likelihood ratio [LR–]). Given the intraindividual and interassay coefficients of variation, we additionally calculated diagnostic performance parameters at 15 and 30% higher and lower levels than the optimal cutoffs. Furthermore, we performed sensitivity analyses in order to account for potential effects of exogenous glucocorticoids on hair glucocorticoid concentrations [10]. We repeated the main ROC analyses with only nonusers in the control cohort. This resulted in exclusion of a total of 38 controls who had used any type of exogenous glucocorticoids in the previous 3 months. Among these participants, hair analyses

were successful in 36/38 for HairF and sumHairF+E, and in 37/38 for HairE. All outcomes were considered statistically significant in case of a p value <0.05.

Results

Descriptive Characteristics and Hair Glucocorticoid Concentrations

The subjects' characteristics and concentrations of hair glucocorticoids are shown in Table 1 and Figure 1. On average, the controls were younger (42.3 years) than the patients (50.2 years). The majority of the participants were women in both the control group (74.6%) and the CS group (74.2%). Hair glucocorticoids stratified by sex are shown in online supplementary Table S1. In general, men had higher levels on all measures; however, significant sex differences in the three in- dices were only present in the controls. Both male and female CS patients had higher values than the controls of same sex (all $p < 0.001$). Overall, there was a strong linear association between HairF and HairE ($r = 0.821$, $p < 0.001$). The geometric mean HairF was higher in the CS patients of cohort 1 (17.3 pg/mg [95% CI: 9.5–31.3]) and cohort 2 (11.7 pg/mg [95% CI: 8.5–16.2]) than in the controls (2.7 pg/mg [95% CI: 2.5–2.9]) (both $p < 0.001$). HairE was also significantly higher in the patients (cohort 1: 37.9 pg/mg [95% CI: 21.7–66.3]; cohort 2: 40.9 pg/mg [95% CI: 30.8–54.4]) than in the controls (8.2 pg/mg [95% CI: 7.8–8.7]) (both $p < 0.001$). The geometric mean of the sum of both hair glucocorticoids was also higher in the CS patients than in the controls. There were no statistically significant differences in hair glucocorticoids between the two patient cohorts.

Table 1: Descriptive characteristics and hair glucocorticoids in controls and Cushing's syndrome patients.

		Cushing's syndrome patients		
	Controls (N=295)	Cohort 1 (N=19)	Cohort 2 (N=70)	Combined (N=89)
Female (%)	220 (74.6%)	16 (84.2%)	50 (71.4%)	66 (74.2%)
Age (years)	42.3 (±11.5)	44.2 (±16.7)	51.8 (±15.4)	50.2 (±15.9)
Hair glucocorticoids (pg/mg)				
• Hair cortisol (HairF)	2.7 (2.5 to 2.9)	17.3 (9.5 to 31.3)	11.7 (8.5 to 16.2)	12.7 (9.6 to 16.9)
• Hair cortisone (HairE)	8.2 (7.8 to 8.7)	37.9 (21.7 to 66.3)	40.9 (30.8 to 54.4)	40.2 (31.4 to 51.5)
• Sum hair glucocorticoids (sumHairF+E)	11.2 (10.6 to 12.0)	63.7 (39.4 to 102.9)	49.7 (38.1 to 65.0)	52.6 (41.8 to 66.2)

Data are shown as numbers (percentage), median (±SD), and geometric mean (95% CI).

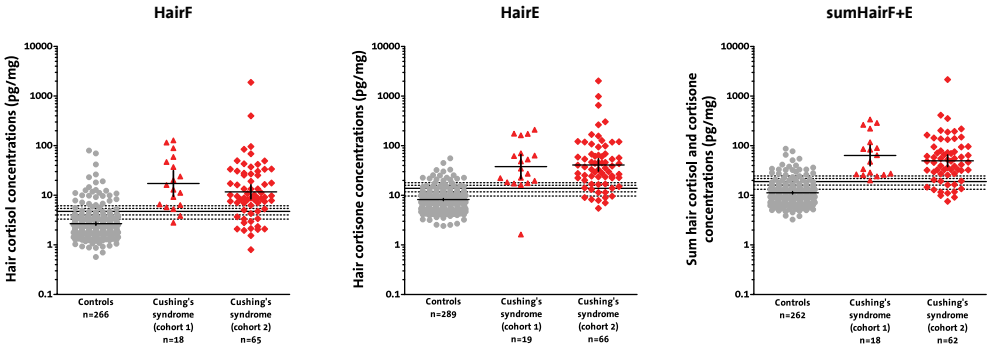


Figure 1: Distribution of hair glucocorticoid concentrations in controls and Cushing's syndrome patients.

Hair cortisol (HairF), hair cortisone (HairE), and the sum of both (sumHairF+E) are shown for community controls, as well as for Cushing's syndrome patients from two independent cohorts. The data for each group are summarized as the geometric mean with corresponding 95% CI. The solid black lines correspond to the optimal cutoff values, and the dashed lines above and below indicate levels corresponding to 15 and 30% above and below the optimal cutoff values, respectively.

Diagnostic Efficacy of Hair Glucocorticoids for Screening of CS

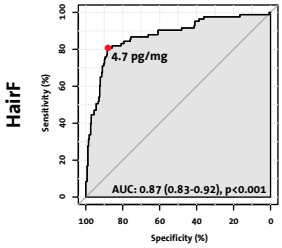
ROC curves with corresponding diagnostic performance parameters for HairF, HairE, and sumHairF+E are depicted in Figure 2. Analyses stratified by sex are shown in online supplementary Figure S1. All three indices showed a strongly significant differentiating efficacy among CS patients from both cohorts separately and combined ($p < 0.001$ for all areas under the curve [AUCs]).

For HairF, an optimal cutoff of 4.7 pg/mg (AUC 0.87 [95% CI: 0.83–0.92]) was observed, with an accuracy of 86%, a sensitivity of 81%, and a specificity of 88%. A positive test result confirmed CS with 68% probability, whereas the NPV was 94%. In regard to HairE, the ROC analysis yielded an optimal cutoff of 13.8 pg/mg (AUC 0.93 [0.89–0.96]). This allowed the correct identification of 74/85 CS patients and 261/289 controls, corresponding to 90% accuracy, 87% sensitivity, and 90% specificity. The PPV and NPV with HairE was 73 and 96%, respectively. The sum of both hair glucocorticoids also showed a high diagnostic efficacy with an AUC of 0.92 (95% CI: 0.88– 0.96). The optimal sumHairF+E cutoff was 18.9 pg/mg, with a corresponding sensitivity of 86% and a specificity of 88%. At this cutoff, 69/80 CS patients and 230/262 controls were identified correctly, yielding an accuracy of 87% with a PPV of 68% and an NPV of 95%.

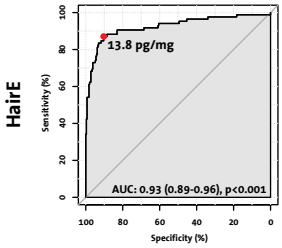
In the context of sensitivity analyses to take potential influencing effects of glucocorticoid-containing drugs into account, we found nearly identical AUCs when only nonusers were considered as controls ($p < 0.001$ for all three indices; data not

shown). Moreover, the optimal cutoff levels with corresponding sensitivity and specificity were also roughly the same for HairF (4.7 pg/mg; 81% sensitivity, 87% specificity), HairE (13.8 pg/mg; 87% sensitivity, 89% specificity), and sumHairF+E (16.2 pg/mg; 89% sensitivity, 85% specificity). Diagnostic accuracy at these levels was 86% for HairF, 89% for HairE, and 86% for sumHairF+E.

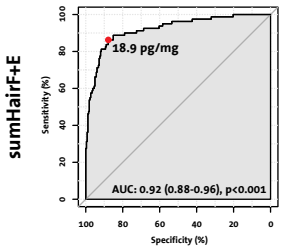
The optimal cutoff for all outcomes was lower in cohort 2 than in cohort 1; however, only the sum of hair glucocorticoids was statistically significantly different in diagnostic efficacy between the two cohorts (Fig. 3). Paired ROC analysis of the hair glucocorticoids showed that HairE and sumHairF+E were more accurate than HairF in the screening of CS in the complete study population (both $p < 0.010$; Fig. 4), whereas HairE was marginally more accurate than the sum value ($p = 0.041$).



	Value (pg/mg)	Accuracy (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR positive	LR negative
OC-30%	3.3	78	86.7	75.6	52.6	94.8	3.6	0.2
OC-15%	4.0	83	81.9	83.5	60.7	93.7	5.0	0.2
OC	**4.7**	**86**	**80.7**	**88.0**	**67.7**	**93.6**	**6.7**	**0.2**
OC+15%	5.4	86	75.9	88.7	67.7	92.2	6.7	0.3
OC+30%	6.1	86	74.7	89.8	69.7	91.9	7.4	0.3



	Value (pg/mg)	Accuracy (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR positive	LR negative
OC-30%	9.7	74	90.6	69.2	46.4	96.2	2.9	**0.1**
OC-15%	11.8	86	88.2	85.8	64.7	96.1	6.2	**0.1**
OC	**13.8**	**90**	**87.1**	**90.3**	**72.5**	**96.0**	**9.0**	**0.1**
OC+15%	15.9	91	83.5	93.1	78.0	95.1	12.1	**0.2**
OC+30%	18.0	90	76.5	94.5	80.2	93.2	13.8	**0.3**



	Value (pg/mg)	Accuracy (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)	LR positive	LR negative
OC-30%	13.3	75	91.3	70.6	48.7	96.4	3.1	**0.1**
OC-15%	16.1	85	88.8	84.4	63.4	96.1	5.7	**0.1**
OC	**18.9**	**87**	**86.3**	**87.8**	**68.3**	**95.4**	**7.1**	**0.2**
OC+15%	21.8	88	81.3	90.5	72.2	94.0	8.5	**0.2**
OC+30%	24.6	89	80.0	92.0	75.3	93.8	10.0	**0.2**

Figure 2: Receiver operating characteristic curve analyses of the diagnostic performance of hair glucocorticoids for Cushing's syndrome.

The red dots refer to the OC value for screening of Cushing's syndrome. The table summarizes the different diagnostic performance parameters at the OC level and other specified levels. AUC, area under the curve; HairE, hair cortisone concentrations; HairF, hair cortisol concentrations; sumHairF+E, sum of HairF and HairE; OC, optimal cutoff threshold; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

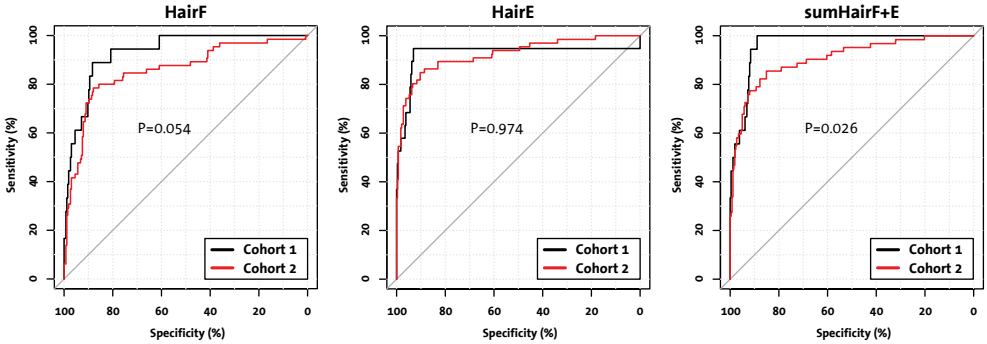


Figure 3: Comparison of receiver operating characteristic curves for screening of Cushing's syndrome by hair glucocorticoids between two independent patient cohorts.

HairE, hair cortisone concentrations; HairF, hair cortisol concentrations; sumHairF+E, sum of HairF and HairE.

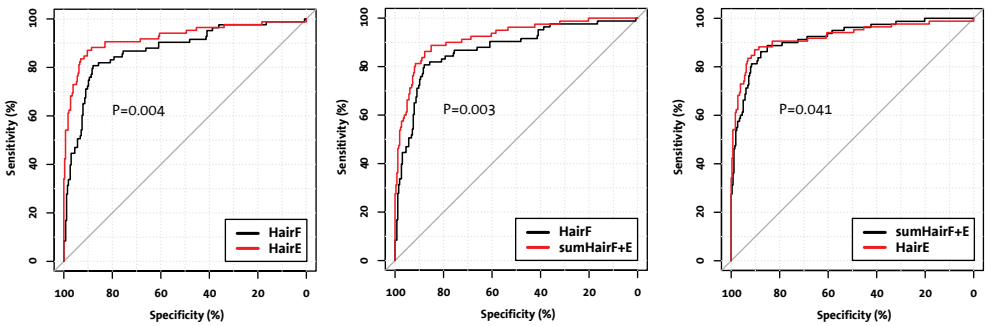


Figure 4: Paired analyses for differences in diagnostic efficacy between hair glucocorticoids for screening of Cushing's syndrome.

Hair cortisone (HairE) was more accurate than hair cortisol (HairF) and the sum of both glucocorticoids (sumHairF+E) in differentiating patients with Cushing's syndrome from controls. sumHairF+E was also statistically significantly better than HairF with respect to diagnostic efficacy.

Discussion

In this multicenter study, we evaluated, for the first time, the diagnostic efficacy of scalp hair cortisol and cortisone concentrations as measured by LC-MS/MS for the screening of CS in two independent patient cohorts. We showed that both glucocorticoids were significantly elevated in patients when compared to community controls, while there were no differences between the patient cohorts. With respect to diagnostic performance, we found a high differentiating capacity of HairF (accuracy 86%, sensitivity 81%, and specificity 88%), HairE (accuracy 90%, sensitivity 87%, and specificity 90%), and the sum of both (accuracy 87%, sensitivity 86%, and specificity 88%). Excluding users of exogenous glucocorticoids in the control cohort revealed no significant effects on these findings. Paired

analyses showed that HairE was more accurate than HairF or the sum of both in distinguishing patients from controls.

Assessment of cortisol concentrations in scalp hair has previously been performed by us and others to compare levels between CS patients and controls [5, 7, 8, 13, 14]. Published studies consistently showed clearly elevated levels in patients in the proximal 1- and 3-cm hair segments. Recently, we have also investigated the diagnostic efficacy of HairF in distinguishing CS patients from healthy controls, as well as patients suspected of CS but in whom the diagnosis was eventually excluded. High sensitivity and specificity were observed with similar optimal cutoffs for both analyses [5]. However, this and previous studies have only analyzed HairF and have performed analyses by immunoassay, which is, among others, prone to cross-reactivity and is inferior to LC-MS/MS with respect to selectivity and detection. Findings of local production of 11β-hydroxysteroid dehydrogenase (11β-HSD) types 1 and 2 – which are, respectively, responsible for the conversion of cortisone into cortisol and vice versa – in skin, hair follicles, and other cutaneous appendages [15-17] also complicate the interpretation of prior findings in CS patients. It therefore remains questionable whether the measured hair cortisol concentrations only reflect the actual past exposure to cortisol or whether these are altered due to local conversion by 11β-HSDs.

Here, we showed for the first time that HairF as well as HairE is elevated in CS patients and that both glucocorticoids possess a high diagnostic efficacy. Moreover, we showed a relatively better diagnostic performance of HairE in distinguishing patients from controls when compared to HairF. Another test that might also be prone to local conversion effects is the first-line screening test with salivary cortisol – this because of the 11β-HSD2 activity in parotid tissue [17]. A previous study by Perogamvros et al. [18] focused on both salivary glucocorticoids in non-cushingoid patients and found, similarly to the current work, higher concentrations of cortisone than of cortisol, whereas the opposite was true for the free fractions in serum. Interestingly, sampling after adrenal stimulation with ACTH injection showed salivary cortisone to reflect free serum cortisol more accurately than salivary cortisol. An evaluation of salivary cortisol and cortisone in another study with CS patients indeed revealed a high diagnostic accuracy of both measures [19]. Additionally, a recent study by Kapoor et al. [20] with radiolabeled cortisol experiments on primates confirmed that circulating cortisol is taken up in hair and can be measured. Importantly, the authors also showed that a substantial proportion of the administered cortisol was incorporated as cortisone. More research, however, is needed to understand the dynamics between cortisol and

cortisone at the local level and to investigate the additional value of cortisone measurements.

The diagnostic efficacy of screening tests depends on the chosen cutoff value for differentiating patients from subjects without the disease. This makes it challenging to place our results in the context of the recommended tests. Nevertheless, Elamin et al. [21] have systematically summarized and pooled the results of the traditional tests in the diagnostic workup of CS. Based on this, the diagnostic efficacy of hair glucocorticoids, especially of HairE, seems to be quite similar to that of midnight salivary cortisol (pooled LR+ 8.8 and LR– 0.1) and UFC (pooled LR+ 10.6 and LR– 0.2), even though most of the included studies had a small population with a fairly high prevalence of CS [21]. Since the diagnosis of CS could not rely on a single screening test, further research should especially address the diagnostic effectiveness of hair glucocorticoids in combination with other recommended tests. Besides, as mentioned in the guideline and also observed here, there is a substantial proportion of false positives with the screening tests, due among other things to the high prevalence of (mild) hypercortisolistic cushingoid-like conditions (e.g., psychiatric disorders, diabetes mellitus, and obesity) and the rare occurrence of CS [4]. Therefore, the recommendation to restrict testing to subjects with a high a priori probability of having CS could reasonably be ex- tended to hair glucocorticoid assessment.

The current screening tests are subject to several difficulties and limitations which are less severe or completely absent with scalp hair measurements. From the patient's perspective, hair sampling is noninvasive and does not require following specific instructions (e.g., collection of urine output for at least 24 consecutive hours for UFC) or impose restrictions (e.g., fasting or no teeth brushing before saliva collection for LNSC) as with the recommended tests; also, hair samples can be collected, stored, and posted by mail with ease, which is especially useful for patients who have to cover long distances to a clinic site. For care professionals, it is convenient that hair measurements are not dependent on the time of day or patient compliance and are not influenced by acute stressors. The unique feature

of these measurements of covering long-term glucocorticoid exposure makes them additionally useful in the screening for cyclical CS. The current guideline recommends UFC or salivary cortisol measurements in case of suspicion of cyclical CS [4]; however, these tests can yield normal results when patients are screened after the periodical increase in cortisol levels. We previously demonstrated the usefulness of hair measurements in such situations in multiple patients who had normal screening test results at the time of evaluation but had retrospectively elevated cortisol concentrations in hair segments corresponding to the period of cushingoid signs and symptoms [7].

The large number of patients and controls and the multicenter evaluation are among the major strengths of the current work. Moreover, all hair glucocorticoid concentrations were determined with high sensitivity and specificity using a state-of-the-art LC-MS/MS technique. This study is, however, limited in the way that controls from the community were not screened for CS. Nevertheless, given the rarity of this disorder, with less than 5 cases per million individuals [22], it is very unlikely that controls were misclassified. Moreover, the results were not adjusted for potential confounders such as UV exposure [23], hair washing, or diabetes mellitus [24]. It is, however, questionable whether these factors would have substantially influenced the outcomes, because of the large (5–6 fold) differences between controls and CS patients in hair glucocorticoid levels.

In conclusion, scalp hair assessment for hair glucocorticoids, in particular for cortisone concentrations, shows a high diagnostic efficacy in differentiating CS patients from controls. Because of its simplicity and noninvasive sampling, as well as its diagnostic performance, it may be seen as a promising biomarker and a potential addition to the armamentarium of CS screening tests. To allow the uniform use of fixed cutoff values, we recommend further efforts to standardize or harmonize results between international centers.

References

1 Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. Lancet. 2015 Aug; 386(9996):913–27.

2 Newell-Price J, Trainer P, Besser M, Grossman A. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr Rev. 1998 Oct;19(5):647–72.

3 Ross EJ, Linch DC. Cushing's syndrome – killing disease: discriminatory value of signs and symptoms aiding early diagnosis. Lancet. 1982 Sep;2(8299):646–9.

4 Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, et al. The diagnosis of Cushing's syndrome: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2008 May;93(5):1526–40.

5 Wester VL, Reincke M, Koper JW, van den Akker EL, Manenschijn L, Berr CM, et al. Scalp hair cortisol for diagnosis of Cushing's syndrome. Eur J Endocrinol. 2017 Jun;176(6): 695–703.

6 Wester VL, van Rossum EF. Clinical applications of cortisol measurements in hair. Eur J Endocrinol. 2015 Oct;173(4):M1–10.

7 Manenschijn L, Koper JW, van den Akker EL, de Heide LJ, Geerdink EA, de Jong FH, et al. A novel tool in the diagnosis and follow-up of (cyclic) Cushing's syndrome: measurement of long-term cortisol in scalp hair. J Clin Endocrinol Metab. 2012 Oct;97(10):E1836–43.

8 Thomson S, Koren G, Fraser LA, Rieder M, Friedman TC, Van Uum SH. Hair analysis provides a historical record of cortisol levels in Cushing's syndrome. Exp Clin Endocrinol Diabetes. 2010 Feb;118(2):133–8.

9 Noppe G, de Rijke YB, Dorst K, van den Akker EL, van Rossum EF. LC-MS/MS-based method for long-term steroid profiling in human scalp hair. Clin Endocrinol (Oxf). 2015 Aug;83(2):162–6.

10 Wester VL, Noppe G, Savas M, van den Akker EL, de Rijke YB, van Rossum EF. Hair analysis reveals subtle HPA axis suppression associated with use of local corticosteroids: the Lifelines cohort study. Psychoneuroendocrinology. 2017 Jun;80:1–6.

11 Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, et al. Universal risk factors for multifactorial diseases: LifeLines: a three-generation population-based study. Eur J Epidemiol. 2008;23(1):67–74.

12 Robin X, Turck N, Hainard A, Tiberti N, Li- sacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011 Mar;12(1):77.

13 Hodes A, Lodish MB, Tirosh A, Meyer J, Belyavskaya E, Lyssikatos C, et al. Hair cortisol in the evaluation of Cushing syndrome. Endocrine. 2017 Apr;56(1):164–74.

14 Manenschijn L, Koper JW, Lamberts SW, van Rossum EF. Evaluation of a method to measure long term cortisol levels. Steroids. 2011 Sep-Oct;76(10-11):1032–6.

15 Tiganescu A, Walker EA, Hardy RS, Mayes AE, Stewart PM. Localization, age- and site-dependent expression, and regulation of 11β-hydroxysteroid dehydrogenase type 1 in skin. J Invest Dermatol. 2011 Jan;131(1):30–6.

16 Hennebert O, Chalbot S, Alran S, Morfin R. Dehydroepiandrosterone 7alpha-hydroxylation in human tissues: possible interference with type 1 11beta-hydroxysteroid dehydrogenase-mediated processes. J Steroid Biochem Mol Biol. 2007 May;104(3-5):326–33.

17 Smith RE, Maguire JA, Stein-Oakley AN, Sasano H, Takahashi K, Fukushima K, et al. Localization of 11beta-hydroxysteroid dehydrogenase type II in human epithelial tissues. J Clin Endocrinol Metab. 1996 Sep;81(9):3244–8.

18 Perogamvros I, Keevil BG, Ray DW, Trainer PJ. Salivary cortisone is a potential biomarker for serum free cortisol. J Clin Endocrinol Metab. 2010 Nov;95(11):4951–8.

19 Antonelli G, Ceccato F, Artusi C, Marinova M, Plebani M. Salivary cortisol and cortisone by LC-MS/MS: validation, reference intervals and diagnostic accuracy in Cushing's syndrome. Clin Chim Acta. 2015 Dec;451 Pt B: 247–51.

20 Kapoor A, Schultz-Darken N, Ziegler TE. Radiolabel validation of cortisol in the hair of rhesus monkeys. Psychoneuroendocrinology. 2018 Nov;97:190–5.

21 Elamin MB, Murad MH, Mullan R, Erickson D, Harris K, Nadeem S, et al. Accuracy of diagnostic tests for Cushing's syndrome: a systematic review and metaanalyses. J Clin Endocrinol Metab. 2008 May;93(5):1553–62.

22 Lindholm J, Juul S, Jørgensen JO, Astrup J, Bjerre P, Feldt-Rasmussen U, et al. Incidence and late prognosis of Cushing's syndrome: a population-based study. J Clin Endocrinol Metab. 2001 Jan;86(1):117–23.

23 Wester VL, van der Wulp NR, Koper JW, de Rijke YB, van Rossum EF. Hair cortisol and cortisone are decreased by natural sunlight. Psychoneuroendocrinology. 2016 Oct;72:94–6.

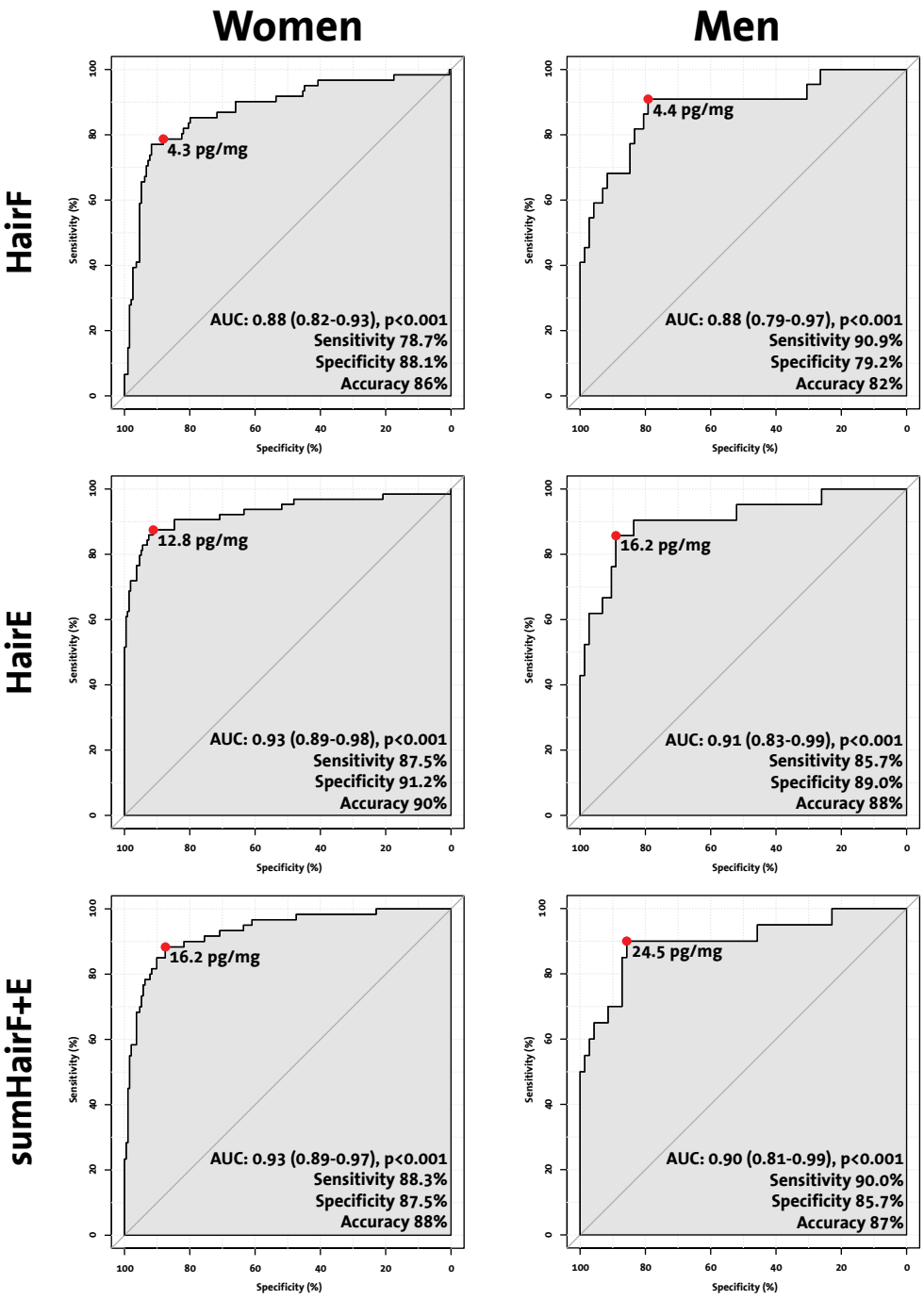
24 Staufenbiel SM, Penninx BW, de Rijke YB, van den Akker EL, van Rossum EF. Determinants of hair cortisol and hair cortisone con- centrations in adults. Psychoneuroendocrinology. 2015 Oct;60:182–94.

Supplementary Data

Supplementary Table S1: Hair glucocorticoids in controls and Cushing's syndrome patients by sex.

				Cushing's syndrome patients					
		n	Controls	n	Cohort 1	n	Cohort 2	n	Combined
Hair glucocorticoids (pg/mg)									
Hair cortisol (HairF)	Female	194	2.5 (2.3 to 2.8)	15	14.9 (7.7 to 29.0)	46	10.1 (6.9 to 15.0)	61	11.1 (8.0 to 15.5)
	Male	72	3.2 (2.7 to 3.8)	3	36.0 (2.6 to 502.9)	19	16.6 (9.0 to 30.7)	22	18.4 (10.6 to 32.1)
Hair cortisone (HairE)	Female	216	7.8 (7.4 to 8.3)	16	34.8 (18.6 to 65.1)	48	40.2 (28.9 to 56.0)	64	38.8 (29.2 to 51.6)
	Male	73	9.4 (8.3 to 10.7)	3	60.1 (3.4 to 1063.9)	18	42.6 (22.9 to 79.3)	21	44.8 (25.8 to 77.8)
Sum hair glucocorticoids (sumHairF+E)	Female	192	10.7 (10.0 to 11.5)	15	58.6 (34.6 to 99.3)	45	47.8 (34.4 to 66.3)	60	50.3 (38.3 to 66.0)
	Male	70	12.7 (11.1 to 14.6)	3	96.2 (5.9 to 1559.6)	17	55.4 (33.6 to 91.2)	20	60.2 (38.0 to 95.3)

Data are shown as geometric mean (95% CI).



Supplementary Figure S1: ROC analyses for diagnostic performance of hair glucocorticoids for Cushing's syndrome stratified for sex.

Chapter 8

Anthropometrics in Relation to Endogenous and Exogenous Glucocorticoids During Combined Lifestyle Intervention With Cognitive Behavioral Therapy

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Manuscript submitted

Abstract

Introduction: *Obesity shows striking similarities with a hypercortisolistic state and is associated with elevated cortisol levels. Here, we evaluated changes in and associations between anthropometric measurements, body composition, and long-term cortisol during lifestyle intervention in obesity. We additionally assessed the role of systemic corticosteroid use on the effect on anthropometric changes.*

Methods: *In this prospective longitudinal study, 118 subjects with obesity (mean age 41.8 years, BMI 40.2 kg/m2) initially participated in a combined lifestyle intervention with cognitive behavioral therapy. Anthropometrics, body composition (measured by DXA), and scalp hair cortisol (HairF) and cortisone (HairF) concentrations (as a measure of chronic glucocorticoid exposure) were evaluated at baseline, after the intensive phase (week 10), and at end of intervention (week 75).*

Results: *Anthropometrics improved significantly and were sustained till end of intervention (weight, -5.6 kg [SE, ±0.9]); waist circumference, -7.4 cm [±0.8]); BMI, -1.9 kg/m2 [±0.3]). Weight change was mainly due to loss of fat mass. No significant weight loss was observed in systemic corticosteroid users. HairF significantly decreased after 75 weeks (mean log change: -0.23 log pg/mg [SE, ±0.06], P=.002; mean absolute change: -1.69 pg/mg [SE, ±0.74]). However, changes in hair glucocorticoids and anthropometric measurements were not interrelated.*

Conclusions: *Combined lifestyle intervention with cognitive behavioral therapy decreases long-term cortisol concentrations and improves anthropometric measurements and body composition in obesity. Associations between these changes cannot fully explain these outcomes, suggesting mediation by other factors. Use of systemic corticosteroids might be considered as an important hindering factor in weight-loss attempts.*

Introduction

Obesity has reached pandemic proportions with worldwide nearly 40% of the adult population being overweight or obese [1]. The health consequences of obesity are widespread, including elevated risks on cardiovascular diseases, cancer, and psychiatric diseases as well as premature mortality [2, 3]. The underlying causes of obesity can be quite diverse and multifactorial and necessitate different approaches [4]. Additional specialized care might be required in case of presence of for example monogenetic forms of obesity, endocrinopathies, sleep disturbances, or use of certain drugs that could potentially contribute to weight gain or hinder weight loss. However, a healthy diet combined with an adequate amount of physical activity remains the cornerstone for each intervention.

Intriguing similarities exist between common obesity and Cushing's syndrome, which is caused by exaggerated production of glucocorticoids. Both entities are for example characterized by increased waist circumference, metabolic disorders and psychological distress. This has led to the hypothesis that cortisol institutes an important role in the development and/or maintenance of obesity [5]. Previous studies have investigated the relationship between cortisol and obesity and indeed found hypothalamus-pituitary-adrenal (HPA) axis disturbances like increased cortisol production [6], disruptions in diurnal rhythm of cortisol secretion [7], and increased activity of the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD) type 1 which is responsible for conversion of the inactive glucocorticoid cortisone into the active hormone cortisol [8]. However, many of these investigations have measured cortisol in traditional matrices like blood, saliva, and urine which only informs about short-term levels whereas the weight-inducing effect are usually observed after long-term overexposure to cortisol. Nowadays, we are able to measure cortisol in scalp hair which allows retrospective investigation of cortisol concentrations over a prolonged period of time [9]. Scalp hair grows with approximately one cm per month, which makes it a valid marker for cortisol exposure in the previous months to years. We are nowadays able to detect cortisol and cortisone in hair with high sensitivity and specificity by liquid-chromatography tandem mass spectrometry (LC-MS/MS) [10].

Interventions aimed at achieving a sustainable healthy lifestyle have been shown to be effective with respect to weight loss [11]. However, it remains currently unknown whether this will be accompanied by reduction in glucocorticoid levels. Since it is not clear whether elevated cortisol levels are the cause or consequence of obesity, it would be interesting to see whether weight loss alters long-term glucocorticoid levels. Hence, we performed a combined lifestyle intervention with cognitive behavioral therapy to assess the effects on and changes over time in

the interrelation between anthropometric measurements, body composition, and long-term cortisol and cortisone concentrations in individuals with obesity. We additionally investigated whether use of drugs containing synthetic glucocorticoids (i.e. corticosteroids) during the intervention influences changes in anthropometric measurements.

Methods

Study design and population

All participants were recruited at obesity center CGG (Centrum Gezond Gewicht), a Dutch multidisciplinary national referral center for patient-tailored diagnostics and treatment of obesity. Reasons for referral to our center are evaluation of underlying causes and personalized therapeutic advice with optional participation in our combined lifestyle program for patients with obesity. Patients are initially seen by a physician for diagnostics to identify underlying causes and contributing factors including drug use, (neuro-) endocrine, genetic, mental, and lifestyle-related factors [4]. A patient-tailored plan is subsequently set-up. Patients are eligible for inclusion in our in-house combined lifestyle intervention with cognitive behavioral therapy when it concerns a lifestyle-related obesity, no underlying causes that necessitate individual treatment are detected and if they meet the following inclusion criteria: body mass index (BMI) >30 kg/m^2, age ≥18 years, ability to speak Dutch, ≥1 obesity-related comorbidity (e.g., dyslipidemia, hypertension, non-alcoholic fatty liver disease, diabetes mellitus type 2, obstructive sleep apnea syndrome, polycystic ovary syndrome, or osteoarthritis), no intellectual disability, no current wish to become pregnant, and no (severe) behavioral problems obstructing functioning in a group. Between October 2011 and June 2018, we included 122 subjects with obesity who participated in our intervention.

Combined lifestyle intervention plus cognitive behavioral therapy

Participants for the combined lifestyle intervention with cognitive behavioral therapy are after medical approval screened by a dietician, physical therapist, and psychologist for factors that necessitate individual therapy, such as binge eating disorder, major depression or motor impairment hampering group activities. When inclusion criteria are met and no severe obstacles are foreseen by either the physician or the allied health professionals, patients are allowed to be included in the program. Twice a year a group of 10-12 patients starts for a 75-week trajectory. During this period group sessions take place once a week during week 1 to 10, biweekly from week 11 to 18, once a month between weeks 18 and 26, and once in 3 months from week 26 to 75. Group sessions include 1,5 hours of nutritional advices and psychotherapy offered by a dietician together with a psychologist, and subsequently 1,5 hours of exercise led by two physical therapists. The

nutrition education protocol focuses on a balanced normocaloric diet according to the Dutch food-based dietary guideline [12]. During all sessions, cognitive behavioral therapy techniques were used to create awareness and to restructure dysfunctional thoughts about lifestyle (food and exercise), weight (loss) and self-esteem. The exercise sessions contain a combination of aerobic endurance training and anaerobic resistance training, with the aim to stimulate exercise in the home-setting and to improve both cardiorespiratory and muscular fitness and strength. Between sessions, patients were offered homework assignments to promote their active participation in the program, as well as to explore how they can organize their lifestyle in a more healthy and personalized way. The long duration of the intervention together with the reduction over time in session frequency was specifically aimed to coach them through all stages of behavioral change while also gradually increasing their independence and the chance of sustained lifestyle adjustments.

Data collection and individual characteristics

Three individual evaluation moments were planned during the treatment: the first at start of the intervention (baseline), the second after the intensive phase (week 10), and the third at the end of the maintenance phase (week 75). Various measurements and collections were performed around these three moments. Socioeconomic status was based on neighborhood level status score as calculated by the Netherlands Institute for Social Research [13]. Scores were assigned according to zip code at time of start of the intervention and were, based on tertiles for scores in the Netherlands in the corresponding year, defined as low, middle, or high socioeconomic status. Anthropometric measurements were assessed by dedicated trained outpatient clinic assistants. A wall-mounted stadiometer was used to measure height. Weight was measured using a calibrated scale while the patient was clothed and standing without shoes. BMI is calculated as weight divided by height in meters squared (kg/m^2). Waist circumference was measured unclothed between the superior anterior iliac crest and below the lowest rib after normal expiration, with patients standing. All anthropometric parameters were rounded to the nearest decimal. In addition, from September 2014 onwards, Dual-Energy X-Ray Absorptiometry (DXA) scans were performed for body composition analysis. Baseline DXA scans on the Lunar Prodigy (GE Healthcare, Madison, WI, USA) were therefore available in a subgroup of 38 participants of whom 21 completed the intervention and had scans available at all three moments. Accordingly, the following body composition parameters were assessed in this subgroup: total mass (kg), fat mass (kg), lean mass (kg), fat and lean mass percentage of total body mass (%), android fat mass (% fat), gynoid fat mass (% fat), and android/gynoid ratio.

Corticosteroid use

We assessed the role of systemic corticosteroids, i.e. oral and parenteral (e.g. intra-articular injections) administration forms in the changes of anthropometrics measurements during intervention. Corticosteroid use was assessed by checking current drug use at the different evaluation moments. Patients were specifically asked whether they were using and/or had used any type of corticosteroid since previous clinic site visit. Moreover, we also cross-checked by evaluating the questionnaire with respect to the item comprising corticosteroid use in the previous three months before hair sample collection.

Scalp hair analysis

Hair samples were cut as close as possible to the scalp from the posterior vertex for assessment of long-term cortisol and cortisone concentrations. Participants were additionally requested to complete a questionnaire about relevant hair-related characteristics like hair color, treatment, and washing frequency as well as corticosteroid use in the past three months. We assessed concentrations in the proximal three cm or complete length if total sample length was shorter in samples taken at baseline and at the end of the intervention (week 75). As hair growths with approximately one cm per month, this would roughly resemble the exposure in the previous three months. For the second evaluation moment (week 10), we assessed only the proximal one cm to prevent overlap with the period before the start of the intervention. Analyses were performed as described in detail elsewhere [10]. In short, proximal segments were weighted, cut in smaller pieces, and subsequently washed in LCMS grade isopropanol. Cortisol and cortisone were extracted using methanol and extracts were cleaned up by solid-phase extraction. Detection and quantification was performed by LC-MS/MS on Xevo TQ-S system (Waters, Milford, MA, USA).

Statistical analysis

Differences in baseline characteristics were assessed with ANOVA or Mann-Whitney U test for continuous variables, and Chi-square tests or Fisher's exact test for categorical variables. Hair samples were available and analysed in 81 individuals at baseline, whereas samples were obtained at all three moments in 57 completers. In order to prevent potential influence of systemic corticosteroid use on glucocorticoid concentrations, we excluded individual data of two users at baseline [14]. Of the 79 available samples, cortisol and cortisone were successfully determined in 96% (n=76) and 99% (n=78) of cases. For the longitudinal analyses of changes in hair glucocorticoids, individual data of 8/57 participants were excluded because of systemic corticosteroid use in the periods matching with analysed hair segments. Hair cortisol and cortisone in the complete set of hair

samples of the remaining 49 participants could respectively be determined in 41/49 (91%) and 45/49 (92%). Hair glucocorticoid concentrations were 10log-transformed to approximate a normal distribution. To investigate changes over time, we performed complete-case analysis with Friedman test for non-normally distributed data and repeated-measures ANOVA for normally distributed data with Greenhouse-Geisser corrections in case of violation of sphericity. For statistical comparisons between evaluation moments, we performed post-hoc analyses with Bonferroni adjustments in case of overall significant change over time (P_{time} <.050). Group descriptives for changes for all outcomes are shown as means with standard errors. We conducted ANCOVAs to assess the relationship (crude and adjusted for age and sex) between baseline measures with each other and the associations in changes between baseline and end of intervention for hair glucocorticoids and anthropometric measurements. With respect to the latter, hair glucocorticoid data from one patient at 75 weeks was excluded due to high probability of matrix interference. Bootstrapping with 1,000 resamples was ran in case of doubt about normal distribution of the dependent variable. Mixed-design ANOVAs were performed to investigate interaction in changes of anthropometric measurements with age, sex, and systemic corticosteroid use during the intervention. Data were analyzed by using SPSS version 25 (IBM Corp., Armonk, NY, USA), graphs were made in RStudio version 1.0.136 (RStudio, Inc., Boston, MA, USA). Statistical significance was defined as a P-value below .050.

Results

Baseline characteristics

Descriptive baseline characteristics of the complete study population are shown in Table 1. A total of four participants were excluded from analyses due to medical circumstances (cancer, n=2; pregnancy, n=2) that required specific attention in the course of the intervention. One individual had undergone bariatric surgery during the intervention, hence only data before the operation were included in this case.

Overall, 31 subjects (26%) dropped out during the intervention whereas 87 were completers. At baseline, participants were mainly female (74%) with a mean age of 41.8 years (SD, ±13.3) and a mean BMI of 40.2 (±5.9) kg/m^2. There were no statistically significant differences in demographics, anthropometric measurements or other assessed characteristics between completers and dropouts (Table 1). Hair glucocorticoids were well correlated to one another (r=0.722, P<.001). Within this group of subjects with obesity, no significant baseline associations were found between hair cortisol concentrations and any of the other parameters, whereas hair cortisone concentrations were only positively associated with waist circumference (B=0.005, P=.029). However, this lost significance after adjustment for age and sex.

Table 1: Baseline characteristics.

	N	All participants (N=118)	N	Completers (N=87)	N	Dropouts (N=31)
Demographics						
Age (years)	118	41.8 (±13.3)	87	41.9 (±13.3)	31	41.4 (±13.5)
Sex (female, %)	118	87 (74)	87	66 (76)	31	21 (68)
Socioeconomic status (n, %)	118		87		31	
• Low		53 (45)		37 (43)		16 (52)
• Middle		27 (23)		18 (21)		9 (29)
• High		38 (32)		32 (37)		6 (19)
Anthropometrics						
Weight (kg)	118	118.1 (±19.8)	87	118.8 (±19.8)	31	116.3 (±20.1)
Waist circumference (cm)	118	113.9 (±15.1)	87	114.9 (±14.7)	31	111.0 (±16.0)
Body mass index (kg/m^2)	118	40.2 (±5.9)	87	40.2 (±5.8)	31	40.1 (±6.3)
Body composition [a]						
Total mass (kg)	38	115.7 (±17.9)	29	116.7 (±18.1)	9	112.7 (±18.0)
Fat mass (kg)	38	55.9 (±11.7)	29	56.6 (±12.4)	9	53.7 (±9.5)
Lean mass (kg)	38	56.8 (±9.8)	29	57.1 (±9.4)	9	56.1 (±11.3)
Fat mass percentage (%)	38	48.1 (±5.5)	29	48.3 (±5.6)	9	47.7 (±5.4)
Lean mass percentage (%)	38	49.3 (±5.2)	29	49.2 (±5.3)	9	49.6 (±5.2)
Android fat (%)	38	56.6 (±6.0)	29	56.7 (±6.5)	9	56.0 (±4.5)
Gynoid fat (%)	38	50.7 (±6.3)	29	50.9 (±6.2)	9	50.2 (±7.0)
Android/gynoid ratio	38	1.12 (±0.12)	29	1.12 (±0.12)	9	1.13 (±0.11)
Scalp hair analysis						
Hair color (n, %)	79		59		20	
• Black		6 (8)		4 (7)		2 (10)
• Brown		35 (44)		28 (48)		7 (35)
• Blond		28 (35)		23 (39)		5 (25)
• Red		2 (3)		1 (2)		1 (5)
• Grey		8 (10)		3 (5)		5 (25)
Hair treatment, yes (n, %)	79	39 (49)	59	31 (53)	20	8 (40)
Hair washing frequency (n, %)	79		59		20	
• 0-2 times/week		21 (27)		15 (25)		6 (30)
• 3-4 times/week		29 (37)		22 (37)		7 (35)
• >4 times/week		29 (37)		22 (37)		7 (35)
Hair cortisol concentrations (log pg/mg)	76	0.60 (±0.37)	57	0.60 (±0.37)	19	0.59 (±0.37)
Hair cortisone concentrations (log pg/mg)	78	1.12 (±0.30)	58	1.13 (±0.29)	20	1.09 (±0.33)

Values are shown as mean (±SD) and numbers (percentage).
[a] Measurements are performed on dual-energy X-ray absorptiometry scanner.

Effects of Combined Lifestyle Intervention on Anthropometric Measurements and Body Composition

Changes in anthropometrics and body composition measurements are shown in Table 2. The average weight loss at 75 weeks was 5.6 kg (SE, ±0.9) corresponding to a relative loss of 4.7% (±0.7) total body weight. Nearly half of the completers (39/87) lost ≥5% of their total weight at start, including 23.0% (20/87) of the participants who lost at least 10% body weight. The decrease in weight, waist circumference and BMI was already present at the first evaluation (10 weeks, all P<0.001). These changes were maintained and even declined slightly further, although statistically non-significant, to the end of the intervention. No interaction with age or sex was observed. Weight loss was in particular due to loss of fat mass in the subgroup with DXA measurements (Table 2). Although there was no significant change in absolute lean mass over time, the mean lean mass percentage relative to total body mass increased after 10 weeks (+1.5%, P=.004) and 75 weeks (+2.4%, P<.001) whereas the opposite was observed for fat mass (-1.6%, P=.002, and -2.5%, P<.001, respectively).

Table 2: Changes in anthropometrics and body composition.

	N	P_{time}	Baseline to 10 weeks	10 weeks to 75 weeks	Baseline to 75 weeks
Anthropometrics					
Weight (kg)	87	**<.001**	**-5.3 (±0.4)*** **	-0.3 (±0.8)	**-5.6 (±0.9)*** **
Waist circumference (cm)	84	**<.001**	**-6.7 (±0.6)*** **	-0.7 (±0.8)	**-7.4 (±0.8)*** **
Body mass index (kg/m^2)	87	**<.001**	**-1.8 (±0.1)*** **	-0.1 (±0.3)	**-1.9 (±0.3)*** **
Body composition[a]					
Total mass (kg)	21	**<.001**	**-5.1 (±0.9)** **	-2.4 (±1.5)	**-7.4 (±1.6)** **
Fat mass (kg)	21	**<.001**	**-4.3 (±0.7)*** **	-2.1 (±1.2)	**-6.4 (±1.3)*** **
Lean mass (kg)	21	.264	-0.7 (±0.3)	-0.3 (±0.5)	-1.0 (±0.5)
Fat mass percentage (%)	21	**<.001**	**-1.6 (±0.3)** **	-0.9 (±0.6)	**-2.5 (±0.6)*** **
Lean mass percentage (%)	21	**<.001**	**+1.5 (±0.3)** **	+0.9 (±0.6)	**+2.4 (±0.6)*** **
Android fat (%)	21	**.011**	-2.0 (±0.7)	-1.0 (±0.9)	**-3.0 (±1.0)***
Gynoid fat (%)	21	**<.001**	**-2.0 (±0.4)** **	-1.3 (±0.7)	**-3.3 (±0.6)*** **
Android/gynoid ratio	21	.675	+0.004 (±0.015)	+0.007 (±0.015)	+0.011 (0.020)

Changes are shown as mean (±SE).
[a] Measurements are performed on dual-energy X-ray absorptiometry scanner.
*P<.050, **P<.010, ***P<.001.

Influence of Systemic Corticosteroid Use on Anthropometric Measurements During Intervention

Systemic corticosteroids were used by 17/87 (19.5%) of the completers during the intervention: seven patients had used during the first 10 weeks, 13 patients between 10 and 75 weeks and three patients during both time periods. Weight change during the intervention was different according to use of systemic corticosteroids ($P_{interaction}$=.041; Figure 1). Stratified analyses showed significant

changes in both groups, however, systemic corticosteroid users only lost body weight at the beginning (-3.53 kg [SE, ±0.80], P=.018) and gained on average 2.02 kg [±2.01] between weeks 10 and 75 resulting in no significant changes between start and end of the intervention (-1.51 kg [±2.39], P=.510). The proportion of participants who lost ≥5% total body weight at end of intervention in users and nonusers was respectively 35.3% (6/17) and 47.1% (33/70). Weight loss of ≥10% total body weight was achieved by 11.8% (2/17) of the users and 25.7% (18/70) of the nonusers. There was no significant interaction effect between systemic corticosteroid use and changes in waist circumference (P=.170) or BMI (P=.058).

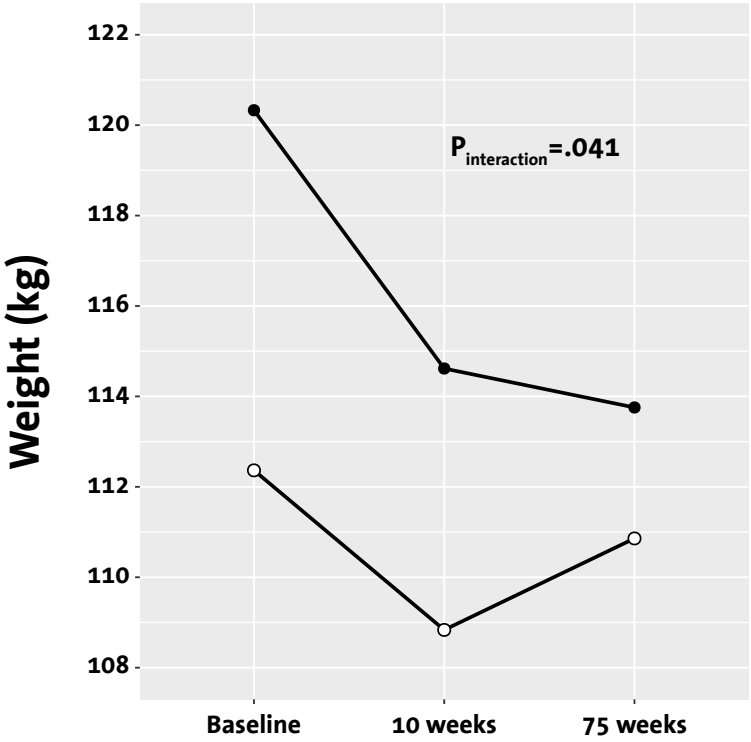

Figure 1: Differential effects of combined lifestyle intervention with cognitive behavioral therapy on weight by systemic corticosteroid use.

Average weight of nonusers (n=70; closed circles) and systemic corticosteroid users (n=17; open circles) at evaluation moments. Although the two groups did not significantly differ on average weight, there was a significant difference in change over time between corticosteroid users and nonusers ($P_{interaction}$=.041).

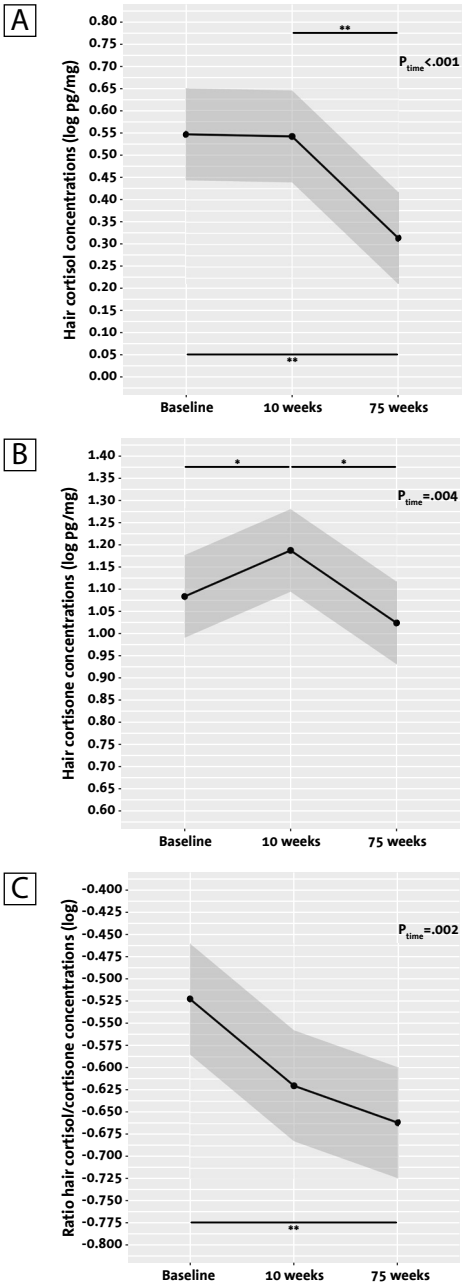

Figure 2: Hair glucocorticoid concentrations during combined lifestyle intervention with cognitive behavioral therapy in individuals with obesity.

Average (95% CI) log-transformed hair cortisol concentrations (panel A; N=41), hair cortisone concentrations (panel B; N=45), and ratio of both (panel C; N=39) at baseline, 10 weeks, and at 75 weeks. Asterisks denote significant changes between evaluation moments. *P<.050, **P<.010.

Effects of Combined Lifestyle Intervention on Long-term Glucocorticoid Concentrations

Hair cortisol and hair cortisone concentrations changed significantly over time (Figure 2). Hair cortisol concentrations were relatively comparable between start and week 10 but decreased at the end of the intervention (0 to 75 weeks, mean log change: -0.23 log pg/mg [SE, ±0.06], P=.002; mean absolute change: -1.69 pg/mg [SE, ±0.74]). In contrast, hair cortisone concentrations were initially increased (mean log change: +0.10 log pg/mg [±0.03], P=.008; mean absolute change: +5.00 pg/mg [±1.73]) followed by a significant decrease between 10 and 75 weeks (mean log change: -0.16 log pg/mg [±0.05], P=.013; mean absolute change: -4.72 pg/mg [±2.25]] to roughly baseline levels. We conducted additional analysis for the ratio of log-transformed hair cortisol/cortisone concentrations and observed a decreasing trend over time with a significant lower ratio at end of intervention in comparison to baseline (mean log change: -0.14 log [±0.04], P=.003; mean absolute change: -0.16 [±0.09]). Changes were similar between sexes and there was no interaction with age.

Associations between Changes in Long-term Glucocorticoid Concentrations and Anthropometric measurements

There were no significant associations in the changes between baseline and 75 weeks in hair cortisol concentrations and weight (B=0.013, P=.807), waist circumference (B=0.064, P=.167), and BMI (B=0.046, P=.789). No significant associations were either found between hair cortisone concentrations and weight (B=0.001, P=.997) waist circumference (B=0.043, P=.833), and BMI (B=-0.031, P=.945). Adjustments for age and sex did not influence the results.

Discussion

In the current study, we show that a 75-week combined lifestyle intervention consisting of healthy nutrition and exercise combined with cognitive behavioral therapy leads to a favorable change in anthropometric measurements and body composition, with loss of fat mass and a relative gain of lean mass, and also decreases long-term systemic cortisol concentrations as assessed in scalp hair. The changes in hair glucocorticoids were independent of anthropometric changes. Interestingly, use of commonly prescribed systemic corticosteroids seemed to hamper the weight loss during the intervention.

An effective combined lifestyle intervention includes three components: diet, exercise and behavioral therapy [15, 11]. Many weight management programs have previously been reported [16]. A study which was conducted in line with our study design related to a three-way approach from dieticians, physical therapists and

psychologists [17]. In that program, patients with diabetes (type I or II) and obesity were offered a short 12-week intervention including a person-specific dietitian-led hypocaloric diet with meal replacements, exercise physiologist-led exercise intervention, and psychologist-led cognitive behavioral support [17]. Participants achieved an impressive weight reduction of an average of −9.7% (±3.6) at the end of intervention [18], of which nearly 80% was loss of fat mass, and −6.4% (±7.7) of body weight after five-year follow-up [19], with first year weight regain mainly determining the wide inter-individual variability. This study differed from ours in exclusive recruitment of patients with diabetes and use of a hypocaloric meal-replacement, but was quite comparable with respect to the severity of obesity as well as weight loss on the long-term.

The underlying causes of elevated cortisol exposure in obesity are multiple and not completely elucidated. Whilst in the case of Cushing's syndrome the cause for the elevated cortisol exposure is due to exaggerated production from the adrenal glands, changes in obesity are more susceptible to interaction between increased central HPA axis activity and tissue-specific alterations in cortisol metabolism [20]. Johnstone *et al.* have thoroughly investigated the cortisol secretion as well as metabolism in a small group of men with obesity after weight loss due to starvation or short-term very low calorie diet [21]. Caloric restriction with dietary approach did not change net cortisol levels but resulted in lower cortisol production as well as reduced metabolism, whereas starvation prompted greater cortisol levels without relevant changes in metabolism hinting on stress-activated cortisol production. Similar findings with regard to cortisol concentrations were also observed in other small studies in which weight loss was achieved with short-term caloric restrictions [22, 23]. Importantly, the dietary approach in our intervention was aimed on a normocaloric and healthy diet which may be easier to implement in daily life in a sustainable way.

Our findings of unaltered cortisol levels on the short-term are in accordance with those previous studies, but the long-term changes could hint on alterations on distinct levels. A reduced HPA axis activity, increased cortisol metabolism and/or alterations in 11βHSD enzymes could explain the decrease in cortisol at end of the intervention. With regard to the latter, 11βHSD1 enzyme activity is particularly increased in adipose tissue in obesity [8] whereas the expression of 11βHSD2 (which converts cortisol into cortisone) is halved and inversely related with 11βHSD1 expression levels as well as central obesity and body fat mass [24]. Given the decrease in body fat mass and waist circumference, alterations in activities of these enzymes, especially of 11βHSD1 due to its abundant expression, would be expected. This could explain the observed changes in long-term glucocorticoids,

as measured in hair, and fit the decrease in hair cortisol/cortisone-ratio. A reduced 11βHSD1 expression in adipocytes [25, 26] and lower ratio of urinary cortisol/cortisone-metabolites [27] was also found in patients who had lost significant weight loss after bariatric surgery during long-term follow-up. Another study could not detect changes in expression levels after weight loss which could possibly be due to small sample size with lesser severity of obesity and shorter duration of follow-up [24].

In the present study we expected a decrease in weight and waist circumference to be related to decrease in long-term cortisol concentrations, however we did not observe such associations. This may be due to the influence of other changes that are simultaneously induced by the intervention that also affect HPA axis activity and glucocorticoid metabolism. It was previously shown that, for instance, the changes in cortisol production and metabolism after weight loss with very low calorie diet were quickly reversed, even in the absence of weight regain, when *ad libitum* feeding was allowed [21]. A more recent study also showed that changes in macronutrients intake in obesity altered cortisol metabolism independent of weight loss [28]. Further research should reveal the relevance of these and other important factors in the regulation of cortisol exposure in individuals with obesity undergoing weight loss attempts.

We recently found that drug use and particularly of corticosteroids was the most common self-reported cause for experienced periods of marked weight gain in patients with obesity [29]. Moreover, we additionally reported that corticosteroid use was more than twice as common in patients with obesity when compared to non-obese individuals [30]. Hence, before the start of the intervention, use of drugs with potential weight gaining side effects was optimized where possible. Despite explicit instructions to refrain from use of corticosteroids if not necessary, with also sending this information to the general practitioners (GPs) of the participants, about 20% of the completers had used some form of systemic corticosteroids during the intervention. This happened in particular after the intensive phase wherein the time between visits gradually became longer. More than half of these users had received a corticosteroid injection by their GPs or other medical specialists which, unfortunately, are often erroneously assumed to have little to no systemic effects. A meta-analysis on the occurrence of adrenal insufficiency in users of various types of corticosteroids, however, showed that intra-articular injections give an equally increased risk on adrenal insufficiency as for with use of oral administration forms [31]. Moreover, Guaraldi *et al.* showed that administration of a single intrabursal injection with corticosteroids suppressed the HPA axis in all participants already at the first day after administration and found that this

was still not completely recovered up to the final evaluation at 45 days [32]. These findings suggest systemic uptake and supraphysiological exposure which can lead to various (metabolic) effects associated with an hypercortisolistic state and potentially antagonize weight loss attempts. It would hence be recommended to explicitly monitor, and if possible stop, taper, or replace drugs with potential weight-gaining side effects in patients engaging in a weight-loss program.

Among the strengths of the current study are the long duration of the intervention, relatively low number of dropouts, and repeated assessments of detailed body composition and both long-term cortisol as well as cortisone concentrations. Moreover, measurements for the hair glucocorticoids were performed on LC-MS/MS enabling high sensitivity and specificity. Also DXA scans, used for assessment of fat mass and lean mass, is a high quality measurement of body composition. However, due to changing standardized examinations over time, DXA scans were not available for all participants. Other limitations are the lack of data on cumulative dose exposure of exogenous corticosteroids and the fact that scalp hair samples were not available in all patients.

Conclusions

Combined lifestyle intervention with cognitive behavioral therapy is beneficial for patients with obesity in terms of a sustained loss in weight and waist circumference while improving body composition, as well as lowering long-term cortisol concentrations. The lack of significant associations between improvements in long-term glucocorticoids (cortisol and cortisone) and anthropometric parameters suggest influences of other factors related to both. Further studies should assess the effects of other glucocorticoid and weight-related factors (e.g. sleeping patterns, inflammation markers, psychological state, dietary composition) in this sense. The use of systemic corticosteroids, including the often overlooked corticosteroid injections, might be considered as an important hindering factor in weight loss attempts.

References

1. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2014 Aug 30;384(9945):766-81.
2. Reilly JJ, Kelly J. Long-term impact of overweight and obesity in childhood and adolescence on morbidity and premature mortality in adulthood: systematic review. Int J Obes (Lond). 2011 Jul;35(7):891-8.
3. Abdelaal M, le Roux CW, Docherty NG. Morbidity and mortality associated with obesity. Ann Transl Med. 2017 Apr;5(7):161.
4. van der Valk ES, van den Akker ELT, Savas M, Kleinendorst L, Visser JA, Van Haelst MM, et al. A comprehensive diagnostic approach to detect underlying causes of obesity in adults. Obes Rev. 2019 Jun;20(6):795-804.
5. Bjorntorp P, Rosmond R. Obesity and cortisol. Nutrition. 2000 Oct;16(10):924-36.
6. Marin P, Darin N, Amemiya T, Andersson B, Jern S, Bjorntorp P. Cortisol secretion in relation to body fat distribution in obese premenopausal women. Metabolism. 1992 Aug;41(8):882-6.
7. Al-Safi ZA, Polotsky A, Chosich J, Roth L, Allshouse AA, Bradford AP, et al. Evidence for disruption of normal circadian cortisol rhythm in women with obesity. Gynecol Endocrinol. 2018 Apr;34(4):336-40.
8. Rask E. Tissue-Specific Changes in Peripheral Cortisol Metabolism in Obese Women: Increased Adipose 11 -Hydroxysteroid Dehydrogenase Type 1 Activity. J Clin Endocr Metab. 2002 Jul;87(7):3330-36.
9. Wester VL, van Rossum EF. Clinical applications of cortisol measurements in hair. Eur J Endocrinol. 2015 Oct;173(4):M1-10.
10. Noppe G, de Rijke YB, Dorst K, van den Akker EL, van Rossum EF. LC-MS/MS-based method for long-term steroid profiling in human scalp hair. Clin Endocrinol (Oxf). 2015 Aug;83(2):162-6.
11. Webb VL, Wadden TA. Intensive Lifestyle Intervention for Obesity: Principles, Practices, and Results. Gastroenterology. 2017 May;152(7):1752-64.
12. Stichting Voedingscentrum Nederland. Richtlijnen Schijf van Vijf. Available at: https://www.voedingscentrum.nl/Assets/Uploads/voedingscentrum/Documents/Professionals/Schijf%20van%20Vijf/Voedingscentrum%20Richtlijnen%20Schijf%20van%20Vijf%202016%204.pdf. Accessed 05 January, 2020.
13. Vliegenthart J, Noppe G, van Rossum EF, Koper JW, Raat H, van den Akker EL. Socioeconomic status in children is associated with hair cortisol levels as a biological measure of chronic stress. Psychoneuroendocrinology. 2016 Mar;65:9-14.
14. Wester VL, Noppe G, Savas M, van den Akker ELT, de Rijke YB, van Rossum EFC. Hair analysis reveals subtle HPA axis suppression associated with use of local corticosteroids: The Lifelines cohort study. Psychoneuroendocrinology. 2017 Jun;80:1-6.

15. Yumuk V, Tsigos C, Fried M, Schindler K, Busetto L, Micic D, et al. European Guidelines for Obesity Management in Adults. Obes Facts. 2015;8(6):402-24.
16. Avenell A, Robertson C, Skea Z, Jacobsen E, Boyers D, Cooper D, et al. Bariatric surgery, lifestyle interventions and orlistat for severe obesity: the REBALANCE mixed-methods systematic review and economic evaluation. Health Technol Assess. 2018 Nov;22(68):1-246.
17. Hamdy O, Carver C. The Why WAIT program: improving clinical outcomes through weight management in type 2 diabetes. Curr Diab Rep. 2008 Oct;8(5):413-20.
18. Mottalib A, Sakr M, Shehabeldin M, Hamdy O. Diabetes Remission after Nonsurgical Intensive Lifestyle Intervention in Obese Patients with Type 2 Diabetes. J Diabetes Res. 2015;2015:468704.
19. Hamdy O, Mottalib A, Morsi A, El-Sayed N, Goebel-Fabbri A, Arathuzik G, et al. Long-term effect of intensive lifestyle intervention on cardiovascular risk factors in patients with diabetes in real-world clinical practice: a 5-year longitudinal study. BMJ Open Diabetes Res Care. 2017;5(1):e000259.
20. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DE, Johnson O, et al. Tissue-specific dysregulation of cortisol metabolism in human obesity. J Clin Endocrinol Metab. 2001 Mar;86(3):1418-21.
21. Johnstone AM, Faber P, Andrew R, Gibney ER, Elia M, Lobley G, et al. Influence of short-term dietary weight loss on cortisol secretion and metabolism in obese men. Eur J Endocrinol. 2004 Feb;150(2):185-94.
22. Wabitsch M, Hauner H, Heinze E, Bockmann A, Benz R, Mayer H, et al. Body fat distribution and steroid hormone concentrations in obese adolescent girls before and after weight reduction. J Clin Endocrinol Metab. 1995 Dec;80(12):3469-75.
23. Tomiyama AJ, Mann T, Vinas D, Hunger JM, Dejager J, Taylor SE. Low calorie dieting increases cortisol. Psychosom Med. 2010 May;72(4):357-64.
24. Engeli S, Bohnke J, Feldpausch M, Gorzelniak K, Heintze U, Janke J, et al. Regulation of 11beta-HSD genes in human adipose tissue: influence of central obesity and weight loss. Obes Res. 2004 Jan;12(1):9-17.
25. Simonyte K, Olsson T, Naslund I, Angelhed JE, Lonn L, Mattsson C, et al. Weight loss after gastric bypass surgery in women is followed by a metabolically favorable decrease in 11beta-hydroxysteroid dehydrogenase 1 expression in subcutaneous adipose tissue. J Clin Endocrinol Metab. 2010 Jul;95(7):3527-31.
26. Woods CP, Corrigan M, Gathercole L, Taylor A, Hughes B, Gaoatswe G, et al. Tissue specific regulation of glucocorticoids in severe obesity and the response to significant weight loss following bariatric surgery (BARICORT). J Clin Endocrinol Metab. 2015 Apr;100(4):1434-44.
27. Rask E, Simonyte K, Lonn L, Axelson M. Cortisol metabolism after weight loss: associations with 11 beta-HSD type 1 and markers of obesity in women. Clin Endocrinol (Oxf). 2013 May;78(5):700-5.

28. Stimson RH, Johnstone AM, Homer NZ, Wake DJ, Morton NM, Andrew R, et al. Dietary macronutrient content alters cortisol metabolism independently of body weight changes in obese men. J Clin Endocrinol Metab. 2007 Nov;92(11):4480-4.
29. Savas M, Wester VL, Visser JA, Kleinendorst L, van der Zwaag B, van Haelst MM, et al. Extensive Phenotyping for Potential Weight-Inducing Factors in an Outpatient Population with Obesity. Obes Facts. 2019 Jun 19;12(4):369-84.
30. Savas M, Wester VL, Staufenbiel SM, Koper JW, van den Akker ELT, Visser JA, et al. Systematic Evaluation of Corticosteroid Use in Obese and Non-obese Individuals: A Multi-cohort Study. Int J Med Sci. 2017;14(7):615-21.
31. Broersen LH, Pereira AM, Jorgensen JO, Dekkers OM. Adrenal Insufficiency in Corticosteroids Use: Systematic Review and Meta-Analysis. J Clin Endocrinol Metab. 2015 Jun;100(6):2171-80.
32. Guaraldi F, Gori D, Calderoni P, Castiello E, Pratelli L, Leporati M, et al. Comparative assessment of hypothalamic-pituitary-adrenal axis suppression secondary to intrabursal injection of different glucocorticoids: a pilot study. J Endocrinol Invest. 2019 Sep;42(9):1117-24.

Chapter 9

Long-Term Cortisol Exposure and Associations With Height and Comorbidities in Turner Syndrome

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Abstract

Context: *Turner syndrome (TS) usually manifests in traits as short stature and premature ovarian failure. Many patients also have an increased risk of cardiometabolic disorders and psychological distress, which are features that overlap with those of a prolonged state of hypercortisolism.*

Objective: *To investigate whether TS is associated with increased long-term cortisol concentrations as measured in scalp hair and whether these are linked to cardiometabolic and psychological parameters.*

Design: *Prospective observational case-control study.*

Setting: *Academic outpatient TS expertise center.*

Participants: *Fifty-five patients with TS (53% 45,X karyotype), and 110 age-matched female community control subjects from the general population-based Lifelines cohort study.*

Main Outcome Measures: *Hair cortisol concentrations (HCC), anthropometrics, biochemical parameters, and psychological questionnaires for perceived stress (Perceived Stress Scale–14), fatigue (Checklist Individual Strength–20), and health-related quality of life (RAND-36).*

Results: *Compared with control subjects, patients with TS had higher HCC [geometric mean, 3.51 pg/mg (95% CI, 2.64 to 4.65) vs 2.39 pg/mg (2.13 to 2.68); P=0.003] and a worse cardiometabolic profile in terms of fasting glucose, and triglycerides. HCC was only associated with total cholesterol levels (standardized β=0.294; P=0.047) and was not associated with the psychological outcomes. A higher HCC was inversely associated with height only in patients with TS (standardized β=-0.307; P=0.023).*

Conclusion: *Patients with TS are chronically exposed to higher cortisol levels, which is associated with short stature and increased total cholesterol levels, and potentially contributes to the known elevated cardiovascular disease risk.*

Introduction

Turner syndrome (TS), one of the most prevalent genetic syndromes in women (1:2500 live-born females), derives from a (partial) loss or abnormality of one of the X chromosomes. Patients with TS are predisposed to a wide range of comorbidities, which require multidisciplinary care and thorough medical examination [1]. Besides characteristic abnormalities such as short stature, webbed neck, and premature ovarian failure, patients are particularly prone to osteoporotic fractures, congenital cardiac defects, ischemic heart disease, and metabolic syndrome traits [2]. With regard to the latter, patients with TS tend to be overweight or obese [3], and have hypertension [4], dyslipidemia [5], and glucose intolerance or diabetes mellitus type 2 [6]. Despite the genetic alterations, it remains largely unknown whether these commonly encountered derangements are mainly a direct consequence of the genetic anomaly [e.g., SHOX gene [7]] or are secondary to physiological and endocrine changes, as frequently observed in patients with TS.

Interestingly, increased exposure to the stress hormone cortisol is known to induce cardiometabolic abnormalities, prompt psychological distress and fatigue, hamper growth, and decrease bone mineral density [8], which all are frequently observed in women with TS [2, 9]. How- ever, relatively little attention has been paid to cortisol secretion in patients with TS. Only one study, to our knowledge, has investigated cortisol levels in TS in the past decade [10]. Onder et al. [10] assessed cortisol levels before and after ACTH injection in a small group of patients with TS and observed high baseline and peak levels. Nonetheless, this concerned a time-point measurement and the investigators did not assess the relationship between cortisol and metabolic outcomes or other cortisol-related comorbidities. To our knowledge, no information is available yet regarding long-term cortisol levels in patients with TS. This is especially of interest because previously mentioned comorbidities usually manifest after prolonged exposure to elevated cortisol levels.

The conventional methods for cortisol assessment in serum, saliva, and urine can only provide estimates of cortisol levels within a small time window and are highly variable due to the diurnal and pulsatile variability in cortisol secretion. Another method measures cortisol levels in scalp hair, which circumvents many limitations of the previous methods and enables retrospective assessment of cortisol secretion in the previous weeks to months, depending on the length of the collected sample [11]. Earlier studies demonstrated relatively high intraindividual stability in cortisol levels in hair taken from the posterior vertex, with simultaneous as well as repeated sampling over time [12, 13]. Moreover, the reliability of hair cortisol as a matrix for assessment of long-term cortisol exposure has been demonstrated by

Short et al. [14]. Since hair is assumed to grow with approximately 1 cm/mo; Short et al. [14] investigated the relationship between cortisol levels measured in a single 1-cm hair sample, with the average of salivary cortisol levels assessed with three daily measurements for 30 days. They found a strong association between both out- comes. We and others have shown that hair cortisol concentrations (HCCs) are associated with various outcomes related to chronic exposure to elevated cortisol levels, such as greater waist circumference [15], psychopathology [16], and Cushing syndrome [17, 18]. Timelines of HCC also were demonstrated to be highly valuable in screening of patients suspected of cyclic Cushing syndrome, given the normal cortisol levels with traditional (i.e., short-term) assessments but increased HCC in segments matching with the course of Cushingoid features [19]. Now, we are able to measure HCC with an advanced liquid chromatography-tandem mass spectrometry (LC-MS/MS) method [20] that enables more sensitive and specific measurements in comparison with the traditional immunoassays.

Because many patients with TS experience a multitude of comorbidities that also occur in a state of hypercortisolism, we hypothesized that TS is associated with increased cortisol exposure and this would be associated with known stress-related consequences commonly reported in TS. Hence, we assessed long-term cortisol levels in scalp hair of patients with TS and matched community control subjects. Among patients with TS, we also investigated HCC in relationship to cardiometabolic measures, perceived stress, fatigue, and health-related quality of life (QoL).

Subjects and Methods

Study population

Consecutive patients visiting our academic outpatient TS expertise center between August 2014 and May 2015 were asked to participate in this study. Sixty-seven patients with confirmed TS, stated in a genetic test report and/or documented by a treating physician in an electronic health record, and having scalp hair at the posterior vertex were initially included. As a control group, we included female participants in the Lifelines cohort study, from whom we previously had determined cortisol levels in scalp hair [21]. Lifelines is a multidisciplinary, prospective, population-based cohort study examining, in a unique three-generation design, the health and health-related behaviors of 167,729 persons living in the north of the Netherlands. It uses a broad range of investigative procedures in assessing the biomedical, sociodemographic, behavioral, physical, and psychological factors that contribute to the health and disease of the general population, with a special focus on multimorbidity and complex genetics [22]. Patients with TS and female control subjects were excluded in case of oral, intra-articular, or topical-on-scalp

corticosteroid use, which resulted in the exclusion of three patients and five control subjects. HCC were successfully determined in 55 patients, who were finally included in this study. All participants provided informed consent. The study protocol was approved by the local medical ethical committee and was conducted in accordance with the Declaration of Helsinki.

Anthropometrics and biochemical assessments

At site visits, weight and height were measured and used for computing body mass index (BMI; calculated as kg/m^2). GH therapy was previously received by 37 of 50 patients (74%) with available information regarding treatment. Blood samples were collected to determine values of the following cardiometabolic parameters: fasting glucose (mmol/L), total cholesterol (mmol/L), high-density lipoprotein and low-density lipoprotein cholesterol (mmol/L), and triglycerides (mmol/L). Analyses were separately performed for patients and control subjects on Roche Cobas c501 and Roche Modular P chemistry analyzers, respectively (Basel, Switzerland).

Psychological parameters

Among patients with TS, self-reported data by questionnaires in Dutch were collected for subjective measures of perceived stress, fatigue, and health-related QoL. Perceived stress was assessed by using the Perceived Stress Scale–14, which consists of 14 items related to nonspecific stress appraisal in the past month [23]. All answers are noted on a 5-point Likert scale (score range, 0 to 4) and are summed to a total score, which ranges between 0 and 56. A higher score represents a higher level of perceived stress. The Checklist Individual Strength–20 (CIS-20) was applied to assess perceived fatigue. This questionnaire consists of 20 statements assessing different aspects of fatigue in the past 2 weeks (e.g., "I feel tired") on a 7-point Likert scale with preset answer categories ranging from "yes, that is true," to "no, that is not true" [24, 25]. Scores are summed to a total fatigue score, with higher scores indicating higher levels of perceived fatigue. To measure health-related QoL, the RAND-36 was applied. The answers on this 36-item survey were used to calculate scores for eight different health-related domains [26, 27]. From these domains, the aggregated physical component summary (PCS) score and mental component summary (MCS) score can be obtained, which are, respectively, indicative of physical and mental QoL [28]. We initially calculated sex-specific Z-scores for all domains, using previously collected reference data from a population study among Dutch individuals [29]. Higher scores on PCS and MCS reflect more beneficial outcomes. The median time difference between collection of hair samples and questionnaires was 6 weeks.

Scalp HCC

Long-term cortisol exposure was assessed in scalp hair collected from the posterior vertex. We analyzed HCC in the proximal 3 cm, or complete length if the sample was shorter, which approximately corresponds to cortisol exposure in the preceding 3 months. At time of sample collection, participants were asked to complete a questionnaire about hair-related factors, such as hair washing frequency, which could potentially affect cortisol concentrations. From the collected sample, about 20 mg of hair was cut and subsequently processed as described elsewhere [18]. Cortisol concentrations were eventually quantified by LC-MS/MS (Xevo TQ-S System; Waters, Milford, MA).

Statistical analysis

All analyses were performed with SPSS Statistics, version 24 (IBM, Armonk, NY) and RStudio, version 1.0.136 (Boston, MA). To reduce residual confounding, we performed age-based optimal matching of patients with female control subjects from our historical cohort at 1:2 ratio by using the MatchIt package (30). Differences in baseline characteristics, anthropometrics, and cardiometabolic parameters between groups in continuous outcomes were analyzed with ANOVA or Mann-Whitney U test, and with x2 test or Fisher exact test in case of categorical parameters. HCCs were base 10–log transformed to achieve a normal distribution and are reported as geometric mean with corresponding 95% CIs. We used analysis of covariance to assess the crude difference in long-term cortisol exposure between patients with TS and matched control subjects. In a stepwise manner, we corrected for significant differences between the groups in hair characteristics and baseline variables. Within patients with TS, associations between transformed HCC and other measures were assessed with linear regression analyses (crude and adjusted for age) and noted as standardized coefficients (b) with corresponding P values. A two-tailed test with significance level of 0.05 was considered for all analyses.

Results

Baseline characteristics of patients and matched control subjects are shown in Table 1. Patients with TS were slightly younger than control subjects [median age, 31.0 years (interquartile range, 18.0) vs 35.0 years (10.3); P=0.016]. There was a significant difference in body weight and height between groups, with patients being especially shorter [mean (6SD), 1.56 m (60.07) vs 1.71 m (60.06); P<0.001]. Within the patient group, 52.7% had the classical monosomy karyotype (45,X), whereas the remaining had X-mosaicism (45,X/46,XX or 45,X/ 47,XXX; 18.2%), isochromosome Xq (18.2%), or other variants (10.9%). There were no significant differences between patients with classical monosomy and non-monosomy

karyotypes in any of the outcomes except for triglycerides (Table 2). With respect to GH treatment, patients who were previously treated were younger [median age, 26.0 years (interquartile range, 12.0) vs 44.0 years (26.0); P<0.001] and taller [1.59 m (0.09) vs 1.52 m (0.09); P=0.006] compared with untreated patients. There were no other significant differences in outcomes (data not shown).

Table 1: Baseline characteristics of study participants.

	Turner syndrome (N=55)	Matched controls (N=110)	P-value
Sex, female	55 (100%)	110 (100%)	-
Age, years	31.0 (18.0)	35.0 (10.3)	.016
Weight, kg	65.0 (18.2)	72.3 (17.5)	<.001
Height, m	1.56 (±0.07)	1.71 (±0.06)	<.001
Karyotype			
• Classical monosomy (45,x)	29 (52.7%)	N/A	-
• Non-monosomy	26 (47.3%)	N/A	
Hair characteristics			
Natural hair color			
• Brown	29 (52.7%)	44 (40.0%)	
• Blond	18 (32.7%)	55 (50.0%)	
• Black	3 (5.5%)	1 (0.9%)	.086
• Grey	3 (5.5%)	8 (7.3%)	
• Red	2 (3.6%)	2 (1.8%)	
Hair washing frequency, p/week[a]			
• 0-2 times	23 (41.8%)	23 (20.9%)	
• 3-4 times	20 (36.4%)	42 (38.2%)	.008
• > 4 times	12 (21.8%)	45 (40.9%)	
Hair treatment, yes[a,b]	15 (27.3%)	53 (48.2%)	.010

Values are shown as number (%), median (interquartile range), and median (±SD).
[a] Hair washing frequency and hair treatment concern the three month period prior to sampling;
[b] Hair treatment includes dyeing, bleaching, and perming.

Long-term cortisol exposure

Patients with TS had higher HCC compared with matched control subjects [geometric mean, 3.51 pg/mg (95% CI, 2.64 to 4.65) vs 2.39 pg/mg (2.13 to 2.68), P=0.003; Fig. 1]. Two overweight patients (with non- monosomy) with the shortest stature had particularly high HCC. With respect to hair characteristics, control subjects washed their hair more often and a greater proportion had undergone a hair treatment. Stepwise adjustments for these hair features, age, and weight did not change the results. However, the difference between the groups in HCC was no longer significant after additional adjustment for height. Simple linear regression analyses showed a strong inverse relationship between HCC and height for the complete study population; however, stratified analysis revealed only significant associations in TS (unadjusted, β=-0.307, P=0.023; adjusted for age, β=-0.353, P=0.014; Fig. 2A).

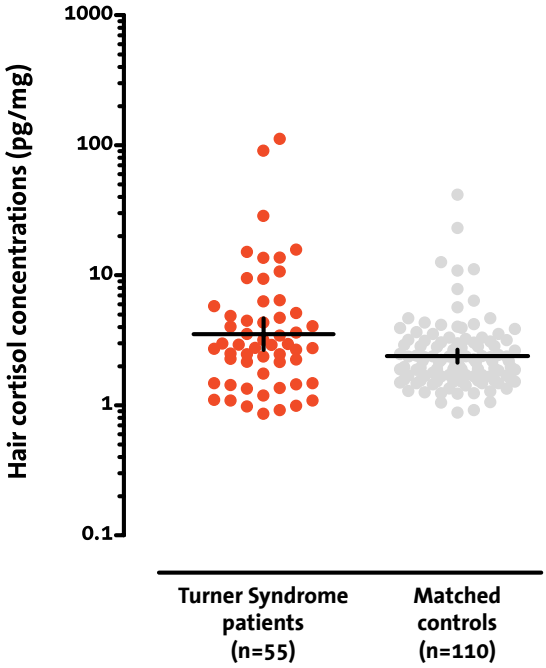

Figure 1: Long-term cortisol concentrations as measured in scalp hair of patients with TS and age-matched, female, community control subjects.

Table 2: Cardiometabolic traits and psychometrics by karyotype in Turner syndrome.

	n	45,X (n=29)	n	Non-monosomy (n=26)	P-value
Age, years	29	31.0 (17.0)	26	29.0 (17.8)	.755
Weight, kg	29	65.0 (17.5)	26	65.5. (19.7)	.607
Height, m	29	1.58 (0.14)	26	1.54 (0.10)	.188
Hair cortisol concentrations, pg/mg	29	3.10 (2.19 to 4.39)	26	4.02 (2.49 to 6.48)	.673
Cardiometabolic traits					
BMI, kg/m²	29	25.3 (7.2)	26	27.9 (7.1)	.117
Fasting glucose, mmol/L	23	4.9 (0.8)	18	5.3 (1.2)	.762
Total cholesterol, mmol/L	25	4.6 (0.9)	21	5.2 (1.2)	.181
HDL-cholesterol, mmol/L	25	1.8 (0.6)	21	1.5 (0.7)	.125
LDL-cholesterol, mmol/L	25	2.6 (0.9)	21	3.0 (1.1)	.076
Triglycerides, mmol/L	25	0.9 (0.6)	21	1.2 (0.5)	.015
Psychometrics					
PSS-14	16	22.0 (9.3)	13	19.0 (7.0)	.110
CIS-20	18	70.5 (27.0)	17	50.0 (36.5)	.405
RAND-36					
• Physical QoL (PCS)	15	53.9 (8.8)	12	56.0 (4.6)	.300
• Mental QoL (MCS)	15	50.3 (12.3)	12	55.2 (21.2)	.548

Data are shown as median (interquartile range) and geometric mean (95% CI).
Abbreviations: *BMI*, body mass index; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *MCS*, Mental Component Summary score; *PCS*, Physical Component Summary score; *QoL*, quality of life.

HCC and cardiometabolic traits

The cardiometabolic parameters for the patient and control groups are listed in Table 3. Patients with TS had a worse cardiometabolic profile in terms of higher fasting glucose (P=0.011) and triglyceride (P<0.001) levels, with also a near-significant higher BMI. Among patients with TS, HCC had a significant positive association with total cholesterol levels (unadjusted, β=0.294, P=0.047; adjusted for age, β=0.312, P=0.040; Fig. 2B). No associations were found with other cardiometabolic measures. There was a near-significant association with low-density lipoprotein–cholesterol level (unadjusted, β=0.273; P=0.066). Adjustment for age did not affect these results.

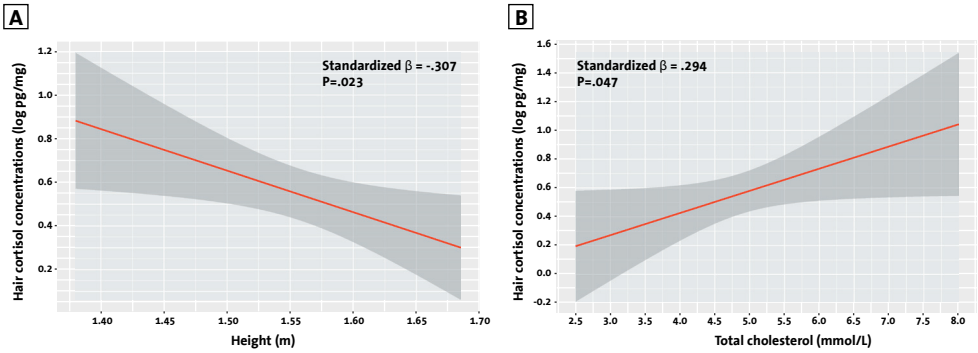

Figure 2: Associations between HCCs with height and total cholesterol levels in patients with TS.

HCCs were (A) inversely associated with height and (B) positively associated with total cholesterol levels.

HCC and psychological parameters

The scores on the questionnaires are shown in Table 3. The Perceived Stress Scale–14 score was positively, although not significantly, associated with HCC (β=0.257; P=0.178) in patients. There was neither a significant association between HCC and indices of fatigue (Checklist Individual Strength–20 β=-0.190; P=0.275), physical QoL (RAND-36 PCS β=-0.124; P=0.539), or mental QoL (RAND-36 MCS β=-0.249; P=0.210). Results were not affected by age. In exploratory analyses, we assessed the association between height and the psycho- metrics and did not find any significant relationships.

Table 3: Cardiometabolic traits and psychometrics in Turner syndrome and controls.

	n	Turner syndrome	n	Matched controls	P-value
Cardiometabolic traits					
BMI, kg/m²	55	25.8 (7.9)	110	25.1 (5.2)	.071
Fasting glucose, mmol/L	41	5.1 (1.0)	102	4.8 (0.4)	.011
Total cholesterol, mmol/L	46	4.9 (±0.9)	105	4.6 (±0.8)	.128
HDL-cholesterol, mmol/L	46	1.6 (±0.5)	105	1.6 (±0.3)	.505
LDL-cholesterol, mmol/L	46	3.0 (±0.9)	105	3.0 (±0.7)	.808
Triglycerides, mmol/L	46	1.1 (0.7)	105	0.8 (0.4)	<.001
Psychometrics					
PSS-14	29	22.6 (±8.7)		N/A	
CIS-20	35	65.8 (±22.4)		N/A	
RAND-36					
• Physical QoL (PCS)	27	55.5 (6.9)		N/A	
• Mental QoL (MCS)	27	52.9 (14.5)			

Values are shown as median (interquartile range) and median (±SD).
Abbreviations: *BMI*, body mass index; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *MCS*, Mental Component Summary score; *PCS*, Physical Component Summary score; *QoL*, quality of life.

Discussion

In this study, we found that long-term cortisol concentrations in scalp hair were elevated in patients with TS when compared with matched control subjects and that hair cortisol was positively associated with short stature and total cholesterol levels in patients with TS. Patients also had a worse cardiometabolic profile in terms of fasting glucose and triglyceride levels. No associations were found between HCC and perceived stress, fatigue, or health-related QoL.

The long-term consequences of elevated chronic cortisol exposure in patients with TS remain unknown. Our finding of ~50% higher cortisol levels, however, could be relevant because subtle hypercortisolism was previously found to be related to a higher risk of cardiovascular events and mortality [31]. This is particularly important because cardiovascular diseases are among the primary causes of premature mortality in patients with TS [2]. Previous research with scalp-hair assessments also hinted at a relation between long-term cortisol exposure and cardiac events. Retrospective assessment of cortisol exposure with scalp hair, for instance, revealed increasing levels in the months before an acute cardiac event and was found to be a primary predictor for such cases [32]. We also showed that high HCC was independently associated with an increased cardiovascular disease presence after adjusting for various potential other risk factors [33]. The worse cardiometabolic profile in combination with higher cortisol levels, which were associated with higher cholesterol levels, therefore may be detrimental in regard to cardiac outcomes. Because our patients were relatively young and cardiovascular events usually take a long time to develop, follow-up studies perhaps could reveal

if high HCCs contribute to elevated cardiovascular risk and reveal its potency to predict individual cardiometabolic diseases and eventually cardiac events among patients with TS.

Height was significantly negatively associated with HCC in patients, whereas no significant relation was found in control subjects (β=-0.122; P=0.205). The causal direction of this relationship remains uncertain; however, increased exposure to cortisol impairs growth rate, as observed in pediatric patients with endogenous and exogenous Cushing syndrome [34]. Accordingly, patients should have been exposed to higher cortisol levels much earlier (i.e., during the growth phase), because our population was, on average, older than 30 years. On the other hand, short stature is associated with less social competence [35] and is perceived as problematic by a large proportion of patients [36], which could conceivably induce psychological and biological stress. Concordantly, an earlier randomized controlled trial with GH therapy in TS showed that a higher growth rate, indeed, was associated with better psychological state, including greater self-perception and less teasing [37].

On the basis of the various mechanisms controlling cortisol exposure, several hypotheses can be proposed for the relatively higher cortisol levels in TS. High levels of psychological distress are related to elevated HCCs, as seen in different patient populations [16]. Many patients with TS eventually manage to become well integrated in society, but there are several issues that may affect the psychological burden over time. Regarding their motor, cognitive, and emotional development, young girls with TS tend to develop slightly slower than most of their age mates [35, 36, 38–40]. Combined with their slow development, their atypical appearance may make these girls vulnerable for exclusion or teasing [41]. With increasing age, more attention and consciousness might also be raised toward health and infertility, which, in turn, may contribute to the development of low self-concept, low self-esteem, social anxiety, or depression [42, 43]. This could subsequently further increase psychological stress and eventually lead to increased levels of cortisol secretion. With respect to the assessed psychological parameters here, we did not find significant relationships between any of the assessments and HCC. This perhaps could be explained by differences in dynamics between psychological and biological stress, and differences in time coverage between the scalp-hair analysis and the included questionnaires.

Another underlying cause for the increased exposure could be related to the body composition. Various studies assessing HCCs found a positive relationship with visceral fat mass and waist circumference in children [44] and adults [15]. Gravholt et al. [45] showed that patients with TS, with an average BMI comparable to that

of our patients, have relatively greater total fat mass and visceral fat mass in comparison with matched control subjects. Unfortunately, we have no data on body composition; however, based on the high rates of overweight and obesity in our patient population (i.e., 62%), a relatively high visceral fat mass would be expected. Furthermore, the liver is frequently involved in the spectrum of comorbidities associated with TS, and abnormalities range from elevated liver enzymes to nonalcoholic fatty liver disease and even fulminant liver cirrhosis [46]. With regard to the latter, patients with TS have a nearly sixfold greater risk of liver cirrhosis developing [2]. These changes are believed to be related to overweight and architectural hepatic changes with or due to changes in the vasculature [46]. The relationship between cortisol levels and liver disease has been described and linked to various mechanisms that might lead to subclinical hypercortisolism [47]. Targher et al. [48] showed, for example, that overweight patients with a biopsy-based diagnosis of nonalcoholic fatty liver disease had higher levels of urinary free cortisol and postdexamethasone suppression–test cortisol compared with control subjects matched for age, sex, and BMI. Interestingly, they also found a strong positive relation between the severity of liver damage and cortisol concentrations [48]. Hence, it could be proposed that potential liver abnormalities in patients with TS are associated with either overactivation of the hypothalamus-pituitary-adrenal axis and/or alterations in related hepatic processes (e.g., decreased breakdown by hepatic cortisol-metabolizing enzymes), which eventually lead to higher cortisol levels and vice versa.

In this study, we investigated long-term cortisol exposure in patients with TS. With the state-of-the-art LC-MS/MS technique, we measured cortisol levels with greater sensitivity and specificity than in previous assessments with immunoassays. Other strengths of this study include phenotyping for cardiometabolic and psychological parameters, and matching with a relatively large group of female control subjects from the general population. Our study, however, is limited by the retrospective assessment of hair characteristics, the absence of similar psychological assessments in control women, lack of data on other potential confounders (e.g., lifestyle, dietary intake), the different methods used to measure biochemical parameters, and because the observational study design does not omit residual confounding.
In conclusion, patients with TS had increased chronic exposure to cortisol, as measured in scalp hair, compared with matched control subjects. This was associated with short stature and increased total cholesterol levels, and could potentially contribute to the known elevated cardiovascular disease risk in TS.

References

1. Saenger P. Turner's syndrome. N Engl J Med. 1996;335(23): 1749–1754.
2. Gravholt CH, Juul S, Naeraa RW, Hansen J. Morbidity in Turner syndrome. J Clin Epidemiol. 1998;51(2):147–158.
3. Elsheikh M, Conway GS. The impact of obesity on cardiovascular risk factors in Turner's syndrome. Clin Endocrinol (Oxf). 1998; 49(4):447–450.
4. Freriks K, Timmermans J, Beerendonk CC, Verhaak CM, Netea-Maier RT, Otten BJ, Braat DD, Smeets DF, Kunst DH, Hermus AR, Timmers HJ. Standardized multidisciplinary evaluation yields significant pre- viously undiagnosed morbidity in adult women with Turner syndrome. J Clin Endocrinol Metab. 2011;96(9):E1517–E1526.
5. Ross JL, Feuillan P, Long LM, Kowal K, Kushner H, Cutler GB, Jr. Lipid abnormalities in Turner syndrome. J Pediatr. 1995;126(2): 242–245.
6. Bakalov VK, Cheng C, Zhou J, Bondy CA. X-chromosome gene dosage and the risk of diabetes in Turner syndrome. J Clin Endocrinol Metab. 2009;94(9):3289–3296.
7. Rao E, Weiss B, Fukami M, Rump A, Niesler B, Mertz A, Muroya K, Binder G, Kirsch S, Winkelmann M, Nordsiek G, Heinrich U, Breuning MH, Ranke MB, Rosenthal A, Ogata T, Rappold GA. Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syn- drome. Nat Genet. 1997;16(1):54–63.
8. Lacroix A, Feelders RA, Stratakis CA, Nieman LK. Cushing's syndrome. Lancet. 2015;386(9996):913–927.
9. Boman UW, Mo¨ ller A, Albertsson-Wikland K. Psychological as- pects of Turner syndrome. J Psychosom Obstet Gynaecol. 1998; 19(1):1–18.
10. Onder A, Aycan Z, Cetinkaya S, Kendirci HN, Bas VN, Agladioglu SY. Assessment of the 21-hydroxylase deficiency and the adrenal functions in young females with Turner syndrome. J Pediatr Endocrinol Metab. 2012;25(7-8):681–685.
11. Wester VL, van Rossum EF. Clinical applications of cortisol measurements in hair. Eur J Endocrinol. 2015;173(4):M1–M10.
12. Sauve´ B, Koren G, Walsh G, Tokmakejian S, Van Uum SH. Measurement of cortisol in human hair as a biomarker of systemic exposure. Clin Invest Med. 2007;30(5):E183–E191.
13. Stalder T, Steudte S, Miller R, Skoluda N, Dettenborn L, Kirschbaum C. Intraindividual stability of hair cortisol concen- trations. Psychoneuroendocrinology. 2012;37(5):602–610.
14. Short SJ, Stalder T, Marceau K, Entringer S, Moog NK, Shirtcliff EA, Wadhwa PD, Buss C. Correspondence between hair cortisol concentrations and 30-day integrated daily salivary and weekly urinary cortisol measures. Psychoneuroendocrinology. 2016;71: 12–18.

15. Jackson SE, Kirschbaum C, Steptoe A. Hair cortisol and adiposity in a population-based sample of 2,527 men and women aged 54 to 87 years. Obesity (Silver Spring). 2017;25(3):539–544.
16. Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, van Rossum EF. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology. 2013;38(8): 1220–1235.
17. Hodes A, Meyer J, Lodish MB, Stratakis CA, Zilbermint M. Mini- review of hair cortisol concentration for evaluation of Cushing syndrome. Expert Rev Endocrinol Metab. 2018;13(5):225–231.
18. Savas M, Wester VL, de Rijke YB, Rubinstein G, Zopp S, Dorst K, van den Berg SAA, Beuschlein F, Feelders RA, Reincke M, van Rossum EFC. Hair glucocorticoids as biomarker for endogenous Cushing's syndrome: validation in two independent cohorts [published online ahead of print 13 February 2019]. Neuroen- docrinology. doi: 10.1159/000498886.
19. Manenschijn L, Koper JW, van den Akker EL, de Heide LJ, Geerdink EA, de Jong FH, Feelders RA, van Rossum EF. A novel tool in the diagnosis and follow-up of (cyclic) Cushing's syndrome: measurement of long-term cortisol in scalp hair. J Clin Endocrinol Metab. 2012;97(10):E1836–E1843.
20. Noppe G, de Rijke YB, Dorst K, van den Akker EL, van Rossum EF. LC-MS/MS-based method for long-term steroid profiling in human scalp hair. Clin Endocrinol (Oxf). 2015;83(2):162–166.
21. Wester VL, Noppe G, Savas M, van den Akker ELT, de Rijke YB, van Rossum EFC. Hair analysis reveals subtle HPA axis sup- pression associated with use of local corticosteroids: the Lifelines cohort study. Psychoneuroendocrinology. 2017;80:1–6.
22. Stolk RP, Rosmalen JG, Postma DS, de Boer RA, Navis G, Slaets JP, Ormel J, Wolffenbuttel BH. Universal risk factors for multifactorial diseases. LifeLines: a three-generation population-based study. Eur J Epidemiol. 2008;23(1):67–74.
23. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983;24(4):385–396.
24. Vercoulen JH, Swanink CM, Fennis JF, Galama JM, van der Meer JW, Bleijenberg G. Dimensional assessment of chronic fatigue syndrome. J Psychosom Res. 1994;38(5):383–392.
25. Vercoulen JHMM, Alberts M, Bleijenberg G. De checklist individuele spankracht (CIS). Gedragstherapie. 1999;32:6.
26. Hays RD, Sherbourne CD, Mazel RM. The RAND 36-Item Health Survey 1.0. Health Econ. 1993;2(3):217–227.
27. Van der Zee K, Sanderman R. Het Meten van de Algemene Gezondheidstoestand Met de RAND-36, een Handleiding. Tweede Herziene Druk. Groningen, the Netherlands: UMCG/ Rijksuniversiteit Groningen, Research Institute SHARE; 2012.

28. Ware JE, Kosinski M, Keller SD. SF-36 Physical and Mental Component Summary Measures: A User's Manual. Boston, MA: The Health Institute, New England Medical Center; 1994.
29. Aaronson NK, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, Sprangers MA, te Velde A, Verrips E. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. J Clin Epidemiol. 1998;51(11):1055–1068.
30. Ho DE, Imai K, King G, Stuart EA. MatchIt: nonparametric preprocessing for parametric causal inference. J Stat Softw. 2011; 42(8):1–28.
31. Di Dalmazi G, Vicennati V, Garelli S, Casadio E, Rinaldi E, Giampalma E, Mosconi C, Golfieri R, Paccapelo A, Pagotto U, Pasquali R. Cardiovascular events and mortality in patients with adrenal incidentalomas that are either non-secreting or associated with intermediate phenotype or subclinical Cushing's syndrome: a 15-year retrospective study. Lancet Diabetes Endocrinol. 2014; 2(5):396–405.
32. Pereg D, Gow R, Mosseri M, Lishner M, Rieder M, Van Uum S, Koren G. Hair cortisol and the risk for acute myocardial infarction in adult men. Stress. 2011;14(1):73–81.
33. Manenschijn L, Schaap L, van Schoor NM, van der Pas S, Peeters GM, Lips P, Koper JW, van Rossum EF. High long-term cortisol levels, measured in scalp hair, are associated with a history of cardiovascular disease. J Clin Endocrinol Metab. 2013;98(5): 2078–2083.
34. Stratakis CA. Cushing syndrome in pediatrics. Endocrinol Metab Clin North Am. 2012;41(4):793–803.
35. Rovet J, Ireland L. Behavioral phenotype in children with Turner syndrome. J Pediatr Psychol. 1994;19(6):779–790.
36. Lagrou K, Xhrouet-Heinrichs D, Heinrichs C, Craen M, Chanoine JP, Malvaux P, Bourguignon JP. Age-related perception of stature, acceptance of therapy, and psychosocial functioning in human growth hormone-treated girls with Turner's syndrome. J Clin Endocrinol Metab. 1998;83(5):1494–1501.
37. Rovet J, Holland J; The Canadian Growth Hormone Advisory Group. Psychological aspects of the Canadian randomized con- trolled trial of human growth hormone and low-dose ethinyl oestradiol in children with Turner syndrome. Horm Res. 1993; 39(Suppl 2):60–64.
38. Siegel PT, Clopper R, Stabler B. The psychological consequences of Turner syndrome and review of the National Cooperative Growth Study psychological substudy. Pediatrics. 1998;102(2 Pt 3): 488–491.
39. McCauley E, Ito J, Kay T. Psychosocial functioning in girls with Turner's syndrome and short stature: social skills, behavior problems, and self-concept. J Am Acad Child Psychiatry. 1986; 25(1):105–112.
40. McCauley E, Kay T, Ito J, Treder R. The Turner syndrome: cognitive deficits, affective discrimination, and behavior problems. Child Dev. 1987;58(2):464–473.

41. Rickert VI, Hassed SJ, Hendon AE, Cunniff C. The effects of peer ridicule on depression and self-image among adolescent females with Turner syndrome. J Adolesc Health. 1996;19(1):34–38.
42. Pavlidis K, McCauley E, Sybert VP. Psychosocial and sexual functioning in women with Turner syndrome. Clin Genet. 1995; 47(2):85–89.
43. Schmidt PJ, Cardoso GM, Ross JL, Haq N, Rubinow DR, Bondy CA. Shyness, social anxiety, and impaired self-esteem in Turner syndrome and premature ovarian failure. JAMA. 2006;295(12): 1374–1376.
44. Noppe G, van den Akker EL, de Rijke YB, Koper JW, Jaddoe VW, van Rossum EF. Long-term glucocorticoid concentrations as a risk factor for childhood obesity and adverse body-fat distribution. Int J Obes. 2016;40(10):1503–1509.
45. Gravholt CH, Hjerrild BE, Mosekilde L, Hansen TK, Rasmussen LM, Frystyk J, Flyvbjerg A, Christiansen JS. Body composition is distinctly altered in Turner syndrome: relations to glucose meta- bolism, circulating adipokines, and endothelial adhesion mole- cules. Eur J Endocrinol. 2006;155(4):583–592.
46. Roulot D. Liver involvement in Turner syndrome. Liver Int. 2013; 33(1):24–30.
47. Papanastasiou L, Fountoulakis S, Vatalas IA. Adrenal disorders and non-alcoholic fatty liver disease. Minerva Endocrinol. 2017; 42(2):151–163.
48. Targher G, Bertolini L, Rodella S, Zoppini G, Zenari L, Falezza G. Associations between liver histology and cortisol secretion in subjects with nonalcoholic fatty liver disease. Clin Endocrinol (Oxf). 2006;64(3):337–341.

Chapter 10

Long-Term Cortisol Levels Are Elevated in Erythropoietic Protoporphyria Patients and Correlate With Body Mass Index and Quality of Life

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DEAR EDITOR, Erythropoietic protoporphyria (EPP) is a rare, inherited disorder of haem biosynthesis, characterized by severe photosensitivity from early childhood.[1] In most countries, no effective treatment is available and the behavioural adaptations needed to avoid sunlight, in addition to pain and sleep deprivation associated with phototoxic episodes, are important stressors in patients with EPP.[2] This might be reflected in increased long-term cortisol levels, which can be measured in scalp hair. Hair cortisol concentrations (HCC) have previously been shown to be positively correlated with chronic stress.[3] In this study, we investigated HCC in patients with EPP and the possible relationship with body mass index (BMI), self-reported perceived stress, quality of life (QoL) and disease severity.

Adults with a confirmed diagnosis of EPP, attending our Porphyria Center at the Erasmus Medical Center (Rotterdam, the Netherlands), were invited to participate in the study. At the time of inclusion, patients were not receiving any treatment for EPP. Patients were age- and sex-matched to controls from our historical cohort[4] with a ratio of 1 : 3. Participants with insufficient hair growth, concomitant disorders of the hypothalamus-pituitary-adrenal (HPA) axis or continuous exogenous corticosteroids use in the past 3 months were excluded.

In both groups, a lock of scalp hair was cut from the posterior vertex and processed for determination of long-term cortisol exposure as described elsewhere.[4] We further collected data on age, sex, BMI, medication use and hair-related factors in all participants. In addition, the EPP group were asked to fill out the 14-item Perceived Stress Scale (PSS),[5] the EPP-specific QoL questionnaire (EPP-QoL)[6] and to report the time they could spend in direct sunlight without symptoms.

This study was approved by the local medical ethics committee and was conducted in accordance with the Declaration of Helsinki; all participants gave written informed consent. Statistical analyses were performed with IBM SPPS Statistics version 21 (IBM, Armonk, NY, U.S.A.). Differences between groups were assessed with ANCOVA and correlations were tested using Pearson's or Spearman's rho correlation coefficient. The level of significance was set at $\alpha = 0{\cdot}05$.

Fifteen participants with EPP and 45 controls were included. There were no significant differences between the groups in baseline or hair characteristics, except for natural hair colour ($P = 0{\cdot}022$). The EPP group had significantly higher HCC than matched controls [geometric mean, 17·06 pg mg^{-1}, 95% confidence interval (CI) 13·02–22·35 vs. 8·28 pg mg^{-1}, 95% CI 5·88–11·64, Cohen's $d = 0{\cdot}83$, $P = 0{\cdot}021$, Fig. 1]. Adjustments for age, sex and hair colour did not change the results. Scores on the EPP-QoL, PSS-14 and sunlight sensitivity were available

in nine, 15 and 13 patients, respectively. There was a strong inverse association between long-term cortisol exposure and EPP-QoL scores (q = -0·703, P = 0·035). No correlation was found between HCC and PSS scores, or between HCC and the time that could be spent in sunlight. In the EPP group, we additionally observed a positive correlation between HCC and BMI (r = 0·672, P = 0·012).

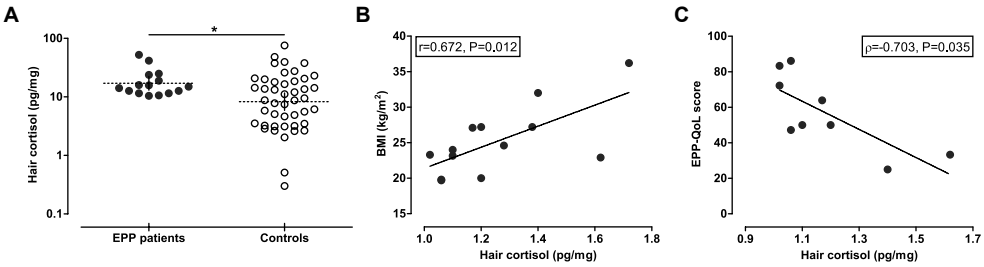

Figure 1: Long-term cortisol exposure as measured in scalp hair of study and control participants.

Hair cortisol concentrations in the erythropoietic protoporphyria (EPP) group and the age-and sex-matched control group (a), and their associations with body mass index (BMI) (b) and EPP-specific quality of life (EPP-QoL) questionnaire score (c) in the EPP group. Hair cortisol concentrations are shown as geometric mean (95% confidence interval) on a logarithmic scale or as log-transformed data. *P < 0·05

In this study, we have demonstrated that patients with EPP have higher long-term cortisol levels than age- and sex-matched controls and that this was correlated with lower QoL and higher BMI. Stress in patients with EPP is related to limitations in their social and professional life, but also to pain from phototoxic reactions and a deep-rooted fear of sunlight.[6] In addition, reduced light exposure and alterations in the day-night rhythm, common in these patients,[2] could disturb the circadian cortisol rhythm and induce alterations in HPA-axis activity.

The strong correlation between HCC and scores on the EPP-QoL suggests that the measurement of hair cortisol could be a useful addition in the study of EPP and the efficacy of new treatments, such as afamelanotide,[6] as it assesses all aspects of stress, including biological stress. Furthermore, the association between HCC and BMI, as found in the EPP group, has previously been described in other study participants.[7,8] It could be suggested that there is a vicious circle in patients with EPP in which weight gain because of stress, pain or reduced physical activity may lead to greater stress-induced cortisol production, thereby promoting further weight gain.

The main limitation of this study is the small sample size, which is related to the rarity of EPP.[1] Furthermore, we did not take into consideration the seasonal variance of EPP-related symptoms or potential seasonal effects on hair cortisol levels in both groups. Whether EPP-related symptoms affect cortisol production should be assessed in further studies.

In conclusion, we show that long-term cortisol levels, as measured in hair, are elevated in patients with EPP and that these are associated with a lower QoL and higher BMI. Hair cortisol analysis might be a valuable tool to monitor stress-related comorbidities and disease-related QoL in patients with EPP.

References

1 Lecha M, Puy H, Deybach JC. Erythropoietic protoporphyria. Orphanet J Rare Dis 2009; 4:19.
2 Holme SA, Anstey AV, Finlay AY et al. Erythropoietic protoporphyria in the U.K.: clinical features and effect on quality of life. Br J Dermatol 2006; 155:574–81.
3 Staufenbiel SM, Penninx BW, Spijker AT et al. Hair cortisol, stress exposure, and mental health in humans: a systematic review. Psychoneuroendocrinology 2013; 38:1220–35.
4 Manenschijn L, Koper JW, Lamberts SW et al. Evaluation of a method to measure long term cortisol levels. Steroids 2011; 76:1032–6.
5 Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983; 24:385–96.
6 Langendonk JG, Balwani M, Anderson KE et al. Afamelanotide for erythropoietic protoporphyria. N Engl J Med 2015; 373:48–59.
7 Wester VL, Staufenbiel SM, Veldhorst MA et al. Long-term cortisol levels measured in scalp hair of obese patients. Obesity 2014; 22:1956–8.
8 Stalder T, Steudte S, Alexander N et al. Cortisol in hair, body mass index and stress-related measures. Biol Psychol 2012; 90:218–23.

Chapter 11

Gender-Specific Effects of Raising First-Year Standards on Medical Student's Performance and Stress Levels

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Abstract

Context: *Medical schools are challenged to create academic environments that stimulate students to improve their study progress without compromising their well-being.*

Objectives: *This prospective comparative cohort study investigated the effects of raising Year-1 standards on academic performance and on students' chronic psychological and biological stress levels.*

Methods: *In a Dutch medical school, students within the last Bachelor's degree cohort (n = 410) exposed to the 40/60 (67%) credit Year-1 standard (67%-credit cohort) were compared with students within the first cohort (n = 413) exposed to a 60/60 (100%) credit standard (100%-credit cohort). Main outcome measures were Year-1 pass rate (academic performance), mean score on the Perceived Stress Scale (PSS, psychological stress) and hair cortisol concentration (HCC, biological stress).*

Results: *Year-1 pass rates were significantly higher in the 100%-credit cohort (odds ratio [OR] 4.65). Interestingly, there was a significant interaction effect (OR 0.46), indicating that raising the standard was more effective for male than for female students. PSS scores (n = 234 [response rate [RR]: 57%] and n = 244 [RR: 59%] in the 67%- and 100%-credit cohorts, respectively) were also significantly higher in the 100%-credit cohort ($F(1,474) = 15.08$, $P < .001$). This applied specifically to female students in the 100%-credit cohort. Levels of HCC (n = 181 [RR: 44%] and n = 162 [RR: 39%] respectively) did not differ between cohorts, but were significantly higher in female students ($F(1,332) = 7.93$, $P < .01$). In separate models including cohort and gender, both PSS score (OR 0.91) and HCC (OR 0.38) were significantly associated with Year-1 performance. Only students with both high PSS scores and high HCC values were significantly at risk of lower Year-1 pass rates (OR 0.27), particularly male students.*

Conclusions: *Raising the Year-1 performance standard increased academic performance, most notably in male students. However, it also increased levels of perceived stress, especially in female students. In particular, the combination of high levels of perceived stress and biological stress, as measured by long-term cortisol, was related to poor academic performance. The study suggests a relationship between raising performance standards and student well-being, with differential effects in male and female students.*

Introduction

The challenge for medical schools worldwide is to create academic environments that stimulate students to improve their study progress,[1] without compromising their health.[2] The urge to seek measures to improve student progress is driven by the substantial investment in students made by both the students themselves and society.[3,4] A possible strategy for achieving this involves the implementation of academic dismissal policies that require students to make satisfactory study progress.[1] Failure to meet set standards leads to significant delay in a student's progress (eg, in systems in which students are unable to proceed to the subsequent year if they fail to achieve the required credits, such as in year classes) or academic dismissal. Academic dismissal policies are common at universities in the USA and have been applied at Dutch universities for the last two decades. However, the literature on academic dismissal policies is scarce and the limited evidence regarding their impact on study progress is inconclusive.[5,6] Furthermore, policy interventions shown to be effective in some schools have proved unsuccessful in other disciplines,[1,5] and their effectiveness depends on characteristics of the student population.[5] Additionally, although data on the possible side-effects of these policy interventions are lacking, there is increasing fear that such measures imply a cost to student well-being.[7]

The introduction of an academic dismissal policy that required students to obtain at least two-thirds of the total number of Year-1 credits was found not to affect dropout, completion and study rates during the first 2 years of medical school, but was accompanied by higher rates of attendance at support sessions.[1] The lack of effect on study progress may be explained by the fact that an academic dismissal policy focuses on minimum standards rather than on the benefits of an optimal study rate. This raises the question of what might happen if the minimum requirements were to be set to the maximum, or, in other words, if students were expected to obtain all Year-1 credits within 1 year.[8] To the best of our knowledge, the impacts of a stricter dismissal policy on student well-being and academic performance in general, and within medical school more specifically, remain unknown.

Studies have found high prevalences of distress amongst medical students in comparison with age-matched controls including non-medical student peers,[9,10] which hampers learning, interferes with professional development and, in the long term, affects personal well-being and patient care.[11] Previous research has shown that not only student-related factors, such as gender, but also school-related factors, such as evaluation or grading systems and learning environments, affect student distress[12] and consequently influence student well-being.[13] An

important issue concerns whether there is an optimum level of stress for academic performance. Whereas acute stress may have some metabolic, immunological and cognitive benefits, chronic stress may cause cognitive decline, adverse effects in the hippocampus, and increase the risk for neurodegenerative disease, as well as cardiometabolic disease.[14,15] To date the scarce research in medical students has focused mainly on acute perceived stress and less on biological stress.[12] Additionally, the methods used previously to measure levels of cortisol, the main stress hormone (eg, in blood, urine and saliva) are complicated by the circadian rhythm and pulsatile process of cortisol secretion, and by the influence of acute stress. Therefore, little is known about the relationship between chronic stress and academic performance. Current models of emotions, based on appraisal processes, emphasise the individualistic way in which people respond to stressful circumstances.[13] An individual's responses (psychological and biological) to demands (eg, the difficulty of an examination) that threaten an important goal (eg, becoming a doctor or a lawyer) are highly dependent on that individual's perceptions of the demands and the resources (eg, student characteristics) that person has available to meet those demands. Given the high prevalences of distress amongst medical students in comparison with their age-matched controls,[9,10,16,17] it is vital to gain understanding of how the (increased) use of academic dismissal policies relates to stress and performance amongst students. In view of the differential individual responses to stressful circumstances, we consider it crucial to also take student characteristics into account. More specifically, we will look at differences between the genders as a recent review suggested that female medical students tend to experience higher levels of stress invoked by assessment than male students, although this finding was not consistent across all studies.[12] Our basic claim is that for a proper understanding of the impacts of implementing academic dismissal policies, the potential for these policies both to positively affect academic outcomes and to induce chronic stress, and consequently a decline in student well-being and academic outcomes, must be investigated. It is, therefore, imperative to take both academic outcomes and student stress levels into account in order to uncover the impact and relevance of academic progress policies.

The present study investigated the effects of raising Year-1 standards on academic performance and on students' chronic psychological and biological stress levels. The changes in policy at our medical school offered us the rare opportunity to respond to calls for research that compares differential effects for assessments with different stakes (high and even higher[18]), has relatively long follow-up durations and looks at the long-term effects of ongoing exposure to assessment.[12] In this study, we used a relatively novel parameter by measuring cortisol concentrations in scalp hair because these reflect the long-term cortisol levels of

recent months. This method has been well validated.[19] We and others have shown that this method provides a unique opportunity to reliably measure the biological effects of stressful circumstances in humans (cf. Groeneveld et al,[20] Staufenbiel et al,[21] Stalder et al[22]).

We aimed to answer the following research questions: (a) What are the effects of raising Year-1 standards on academic performance and on medical students' chronic perceived and biological stress levels?, and (b) Is there a differential effect for male and female students?

Methods

Context

The present study was carried out at the Erasmus MC Medical School in Rotterdam, the Netherlands. The first year of the integrated and theme-oriented Bachelor curriculum at this school is composed of thematic blocks and competence-based learning lines for which students can obtain a maximum of 60 credits under the European Credits Transfer System. From 2005 the Erasmus MC Medical School implemented an academic dismissal policy whereby substandard progress resulted in academic probation (at 12 months) or academic dismissal (at 24 months) (Table 1). Until 2014, students whose progress was substandard (ie, students who achieved less than 40 credits) at 12 months were allowed to repeat Year 1 (probation), whereas students with 41-59 credits at 12 months were allowed to engage in Year-2 modules alongside their remaining Year-1 module(s).

Table 1: Academic probation and dismissal policies.

Time from enrolment, months	Type of action		Standard (maximum)
	67%-credit cohort[a]	100%-credit cohort[b]	
12	Academic probation		<40 credits (60)
12		Academic dismissal[c]	<60 credits (60)
24	Academic dismissal[c]		<60 credits (120)

[a] Lowest grade allowed: 5.5, minimum grade point average (GPA);
[b] Two grades of 5.0-5.49 were allowed, minimum GPA: 6.0;
[c] Dispensation possible for 1 year for temporary personal circumstances.

Credits were awarded for each module provided the student obtained a sufficient grade (ie, ≥ 5.5 out of a maximum of 10.0) on the examination. In 2014, the Year-1 credit standard was raised from 67% (40/60 credits) to 100% (60/60 credits). Students were required to achieve an average grade of at least 6.0 on the nine examinations, but two grades of 5.0-5.49 were allowed under the condition that they were not obtained in the same thematic block. The intention of raising the

standard was to increase the academic progress of Bachelor students.[8] Students who failed to earn the required number of credits at the end of the first year (12 months) were not allowed to repeat Year 1 but were immediately subject to academic dismissal. The change in the assessment policy was the only major curriculum alteration in recent years.

Participants and procedure

Participants in this study were students in two consecutive cohorts, which included the last cohort to be subject to the requirement to obtain 67% of credits (entering in 2013, 67%-credit cohort) and the first cohort to be subject to the requirement to obtain 100% of credits (entering in 2014, 100%-credit cohort) and comprised 410 and 413 students, respectively. In order to collect data on psychological stress, all students in both cohorts were invited to complete a survey at 1.5 months before the final Year-1 examination, which is taken in early July. Students were recruited during a single large-scale lecture and online. To determine average biological stress levels during the last 3 months of the academic year, scalp hair samples were collected from student volunteers in both cohorts on the last day of the academic year. Students were recruited immediately after completing their final examination. We deliberately planned to administer the survey on psychological stress in the middle of the 3-month period covered by the hair samples.

Data on academic performance were derived from the university student administration system and confidentiality was guaranteed. As data were collected as part of regular academic activities and only aggregate data are reported, individual consent was not necessary. For the measures of psychological and biological stress, written informed consent was obtained from all participants and confidentiality was guaranteed. Students were able to participate voluntarily and were not given incentives for participation. Prior to the analyses, all data were coded and saved without direct identification information. The current study was carried out in accordance with the Declaration of Helsinki and was deemed exempt from review after evaluation by the Medical Ethics Committee of Erasmus MC University Medical Centre Rotterdam.

Outcome measures

Perceived stress

The Perceived Stress Scale (PSS) questionnaire[23] consists of 14 items assessing both general distress and inability to deal with stress. Example items are: 'In the last month, how often have you felt nervous and stressed?' and 'In the last month, how often have you felt that you could not cope with all the things that you had to do?' Items are scored on a 5-point Likert scale (0 = never; 4 = very often). Higher

scores reflect a higher level of perceived stress (total score range: 0-56). We used a validated Dutch version of this questionnaire.[21]

Biological stress

To assess biological stress levels, we collected scalp hair samples from the posterior vertex. From each hair sample, the 3 cms most proximal to the scalp was analysed to provide data on average cortisol exposure in the preceding 3 months. Cortisol was extracted from scalp hair using methanol and hair cortisol concentration (HCC) was measured using an enzyme-linked immunosorbent assay (ELISA) kit (DRG Instruments GmbH, Marburg, Germany) as described previously.[24] Additionally, students completed a questionnaire on hair-related factors that could potentially affect cortisol concentration, such as hair colour, washing frequency, use of corticosteroids during the previous 6 months, other medication use and distressing life events (herein referred to as the 'hair questionnaire'). Hair cortisol values were log-transformed to normalise the distribution.

Academic Performance

The academic performance indicator used in this study was the Year-1 curriculum pass rate, which was defined as the proportion of students in each cohort who earned all 60 credits in the Year-1 curriculum within 12 months after enrolment.

Statistical analysis

To enable valid comparisons, the 67%- and 100%-credit cohorts were compared on the pre-admission variables of gender, using chi-squared tests, and on age and pre-university education grade point average (pu-GPA), using analyses of variance (ANOVAs). Pre-university GPA represented the mean grade obtained by a student during the final year of pre-university education. Final pu-GPAs were based half on school examinations and half on the national examination. Additionally, the cohorts were compared on the different variables measured in the hair questionnaire using chi-squared tests. We first conducted exploratory analyses comparing the 67%- and 100%-credit cohorts and male and female students on the three outcome measures. Differences in percentages were tested using chi-squared tests and differences in means using Student's *t*-test. As measures of effect size, we included odds ratios (ORs) (values of 1.22, 1.86 and 3.00 represent small, medium and large effects, respectively) or inverse equivalents (values of 0.82, 0.54 and 0.33 represent small, medium and large effects, respectively)[25] and Cohen's d (values of 0.20, 0.50 and 0.80 represent small, medium and large effect sizes, respectively).[26]

Next, we used logistic regression to calculate an OR for the effect of the academic dismissal policy (67%-credit versus 100%-credit requirement) on Year-1 pass rate. Statistical interaction terms were used to study the potentially differential effects of the academic dismissal policy by gender. We included ORs as measures of effect size.[25] We used a two-way ANOVA to examine the effect of the academic dismissal policy and gender on PSS sum scores and on HCC values. Generalised omega-squared was computed as a measure of effect size as recommended by Olejnik and Algina,[27] with values of 0.01, 0.06 and 0.14 indicating small, medium and large effects, respectively.

Finally, we used logistic regression to test three models: (a) a model including the academic dismissal policy, gender and PSS; (b) a model including the academic dismissal policy, gender and HCC, and (c) a model including the academic dismissal policy, gender and a compound score based on median values for PSS score and HCC. The compound score divided participants into four groups: (a) LowLow (≤ median for both PSS score and HCC value); (b) HighLow (> median for PSS score and ≤ median for HCC value; (c) LowHigh (≤ median for PSS score and > median for HCC value), and (d) HighHigh (> median for both PSS score and HCC value). All variables were entered simultaneously in a multivariable logistic regression model. Analyses were performed in spss Version 21.0 (IBM Corp., Armonk, NY, USA). A *P*-value of <.05 was considered to indicate differences of statistical significance.

Results

Student characteristics

The PSS was completed by the majority of the students (67%-credit cohort, n = 234 [57%]; 100%-credit cohort, n = 244 [59%]). All respondents answered all items on the questionnaire. With respect to biological stress, we collected scalp hair samples from 181 students in the 67%-credit cohort (44%) and from 162 students in the 100%-credit cohort (39%). All of these students also completed the hair questionnaire.

The 67%- and 100%-credit cohorts did not show significant differences with respect to gender (66% female and 67% female, respectively), mean age (19.27 years and 19.26 years, respectively) and pu-GPA (7.15 and 7.16, respectively). The only significant difference on the hair questionnaire was a higher score in the 100%-credit cohort for distressing life events, most of which referred to examinations as indicated by the students (41% and 71% in the 67%- and 100%-credit cohorts, respectively; $x2(1) = 30.25$, $P < .001$; OR 3.47, 95% confidence interval [CI] 2.21-5.45).

Academic performance

The exploratory analyses showed significantly higher Year-1 pass rates in the 100%-credit cohort compared with the 67%-credit cohort, both for the total cohorts and for men and women separately (Table 2). Female students had a significantly higher Year-1 pass rate than male students (Table 3).

Table 2: Academic performance and stress measures for the 67%- and 100%-credit cohorts.

	Cohort				Statistics		
	67%		100%				
	n	%	n	%	x^2	*P*-value	ES[a]
Year-1 completion[b]							
Total	203	49.5	302	73.1	48.38	<.001	2.77
Male	53	37.9	102	73.9	36.62	<.001	4.65
Female	150	55.6	200	72.7	17.48	<.001	2.13
	n	**Mean**	**n**	**Mean**	***t***	***P*-value**	**ES[c]**
PSS							
Total	234	24.10	244	27.82	-4.93	<.001	0.45
Male	68	22.91	75	24.47	-1.03	.31	-
Female	166	24.59	169	29.31	-5.65	<.001	0.62
HCC							
Total	181	23.78	162	22.65	0.60	.55	-
Male	67	21.03	66	19.41	0.49	.62	-
Female	114	25.57	96	25.19	0.18	.86	-

[a] Odds ratio;
[b] Percentage of all students from initial cohort;
[c] Cohen's d.
Abbreviations: *ES*, effect size; *HCC*, hair cortisol concentrations; *PSS*, Perceived Stress Scale.

Table 3: Academic performance and stress measures in male and female students.

	Gender				Statistics		
	Male		Female				
	n	%	n	%	x^2	*P*-value	ES[a]
Year-1 completion[b]	155	56	350	64	5.56	<.05	1.42
	n	**Mean**	**n**	**Mean**	***t***	***P*-value**	**ES[c]**
PSS	143	23.72	335	26.97	3.72	<.001	0.38
HCC	133	20.21	210	25.39	2.51	<.05	0.26
	n	**%**	**n**	**%**	**x^2**	***P*-value**	**ES[a]**
Compound Score							
PSS Low HCC Low	35	39	45	25	8.68	<0.05	1.88
PSS High HCC Low	14	15	43	24			-
PSS Low HCC High	25	28	41	23			-
PSS High HCC High	16	18	49	28			-

[a] Odds ratio;
[b] Percentage of all students from initial cohort;
[c] Cohen's d.
Abbreviations: *ES*, effect size; *HCC*, hair cortisol concentrations; *PSS*, Perceived Stress Scale.

The logistic regression analysis revealed that Year-1 pass rates were significantly higher in the 100%-credit cohort (Wald x2(1) = 34.77, *P* < .001; OR 4.65, 95% CI 2.79-7.75) and in female students (Wald x2(1) = 11.39, *P* < .001; OR 2.05, 95% CI 1.35-3.11). Furthermore, a significant interaction effect (Wald x2(1) = 6.00, *P* < .05; OR 0.46, 95% CI 0.25-0.86) indicates that raising the standard was more effective for male than for female students (Figure 1A).

Stress

Students in the 100%-credit cohort scored significantly higher on the PSS than students in the 67%-credit cohort (Table 2). Subanalyses by gender revealed that only female students had significantly higher PSS scores in the 100%-credit cohort compared with the 67%-credit cohort. In general, female students had significantly higher PSS scores than male students (Table 3).

In line with these findings, the two-way ANOVA revealed significantly higher PSS scores in the 100%-credit cohort (*F*(1,474) = 15.08, *P* < .001, ωG2 = 0.03) and in female students (*F*(1,474) = 16.29, *P* < .001, ωG2 = 0.03). There was no significant interaction effect (*F*(1,474) = 3.84, *P* = .051, ωG2 = 0.01) (Figure 1B).

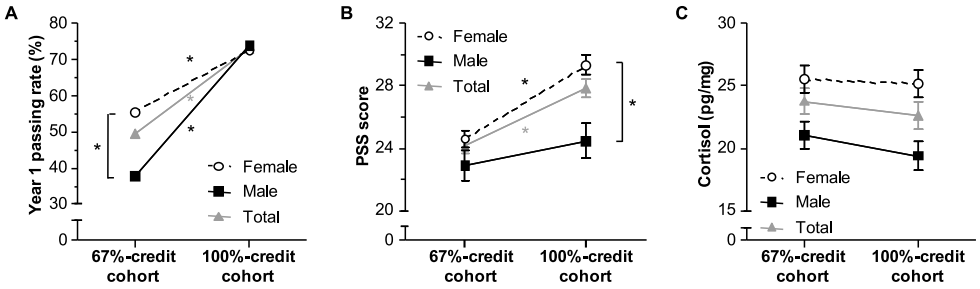

Figure 1: Year-1 performance and stress outcomes in study cohorts of medical students tasked with achieving 67% and 100% of Year-1 credits, respectively.

A, Year-1 pass rates in the total 67%-credit (n = 410) and 100%-credit (n = 413) cohorts, and separately in each cohort for female (n = 270 and n = 275, respectively) and male (n = 140 and n = 138, respectively) students. B, Mean ± standard error (SE) scores on the Perceived Stress Scale (PSS) for all participants in the 67%-credit (n = 234) and 100%-credit (n = 244) cohorts, and for female (n = 166 and n = 169, respectively) and male (n = 68 and n = 75, respectively) students. C, Mean ± SE untransformed hair cortisol concentration (HCC) in all participants in the 67%-credit (n = 181) and 100%-credit (n = 162) cohorts, and in female (n = 114 and n = 96, respectively) and male (n = 67 and n = 66, respectively) students. Statistical analyses were performed to show differences between cohorts (total or subgroup) or between male and female students within a cohort. *P < .05

Students in the two cohorts did not significantly differ in HCC values (Table 2). However, female students had higher HCC levels than male students (Table 3). These findings were confirmed by the two-way ANOVA. Hair cortisol concentrations did not differ between the cohorts ($F(1,343) = 0.33$, $P = .57$), but were significantly higher in female students ($F(1,343) = 7.55$, $P < .01$, $\omega G2 = 0.02$); there was no interaction effect ($F(1,343) = 0.15$, $P = .69$) (Figure 1C).

Stress and academic performance

Both PSS and HCC data were available for 135 students (33%) in the 67%-credit cohort and for 133 students (32%) in the 100%-credit cohort. There was no significant correlation between PSS score and HCC ($r(268) = .11$, $P = .07$). In separate models including cohort and gender, both PSS (Wald x2(1) = 33.35, $P < .001$; OR 0.91, 95% CI 0.89-0.94) and HCC (Wald x2(1) = 4.17, $P < .05$; OR 0.39, 95% CI 0.16-0.96) were significantly associated with Year-1 performance. Only students with high values (above median) on both the PSS and HCC were significantly at risk of lower Year-1 academic performance (Wald x2(1) = 9.22, $P < .01$; OR 0.26, 95% CI 0.11-0.62), particularly male students (Figure 2). Notably, the gender proportion was not comparable within the four compound score groups (x2(3) = 8.68, $P < .05$) as male students were more likely to have both low PSS scores and low HCC values than female students (39% and 25%, respectively; OR 1.88) (Table 3).

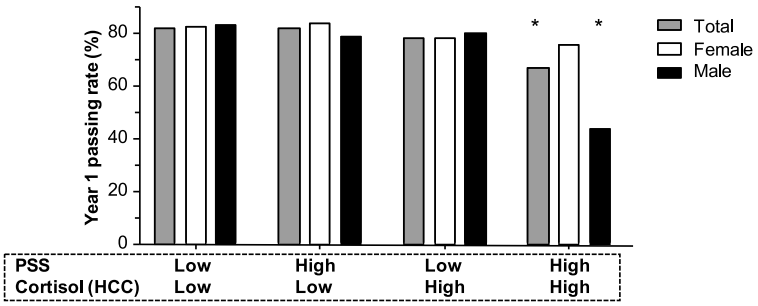

Figure 2: Year-1 performance and compound Perceived Stress Scale (PSS) scores and hair cortisol concentration (HCC) values.

Year-1 pass rates for all participants combined (n = 268) and by gender (ie, female [n = 178] and male [n = 90]) divided according to compound score based on median values for PSS (26.00) and HCC (25.30). Reference group: LowLow (≤ median for both PSS score and HCC value). *$P < .05$

Discussion

This study shows that raising the Year-1 performance standard increased academic performance, most prominently in male students. However, it also increased levels of perceived stress, especially in female students. There was no effect on levels of biological stress as measured by long-term cortisol secretion. Nevertheless, the combination of high perceived stress and high biological stress was found to be related to poor academic performance.

It is not surprising that Year-1 performance improved after the Year-1 standard was raised because this is in line with findings in previous studies that have shown superior performance on tests with higher stakes[28-30] (ie, higher consequences of performance) or with higher performance standards[31,32] (ie, higher demands in order to pass). However, it is not in line with previous findings by ourselves and others on the effectiveness of implementing academic dismissal policies.[1,5,6,33] An important difference between the current and these previous studies is that the present study is the first to investigate the effect of setting the minimum standard to be equivalent to the maximum. To date, two possible explanations have been suggested for the limited effects of academic dismissal policies on medical student performance: (a) a threshold effect, which assumes that students may reduce their efforts after obtaining the minimum number of credits required, and (b) a ceiling effect based on the assumption that there is little room for improvement given the already high Year-1 pass rates of medical students.[8] Our study suggests that the first explanation is more plausible because some students were apparently able to improve their study progress after they were (strongly) encouraged to do so.

A striking finding concerned the gender-related difference in the effectiveness of the measure and the observation that male students were able to surpass female students in Year-1 performance. It is possible that the threshold effect applies more to male than to female students. Previously, it has been suggested that despite the importance of intrinsic motivation, external triggers (ie, higher performance standards) may have a powerful additional effect on academic motivation.[18] Our data suggest that this additional effect may be stronger for male than for female students. This is in line with findings in previous studies, which have shown that male students tend to have higher extrinsic or controlled motivation and lower intrinsic or autonomous motivation than female students.[34,35]

The increased academic performance coincided with increased levels of perceived stress, especially in female students. Higher levels of assessment stress or anxiety in female students than in male students have been reported previously, but this

gender effect was not consistent across the studies included in the review by Lyndon et al.[12] One possible explanation for the gender-related differences in perceived stress refers to personality traits, of which the combination of neuroticism and conscientiousness in particular has been found to be more commonly present in female medical students and to be associated with higher levels of stress.[36] Other potential explanations for the gender-related differences in perceived stress refer to previously identified gender-based differences in levels of overestimation[37] and of rumination.[38] Despite the increase in perceived stress brought about by the implementation of the new policy, our students generally reported lower levels of perceived stress than medical students in the USA[39] and Pakistan.[40]

Raising the standard did not have an effect on levels of biological stress. However, students who scored highly on both stress outcomes, particularly male students, showed worse study performance. This finding emphasises the individual approaches of students in evaluating their well-being during medical school. Furthermore, differences in dynamics between psychological and biological stress may explain the non-significant relationship between the two stress outcomes. Future studies may want to investigate the differential consequences of high levels of both biological and psychological stress in male and female students.

The current study has several strengths and limitations that should be mentioned. A first strength is that we included a rather large sample size in both cohorts, which increased the power to identify differences and allowed us to perform multiple group comparisons. Nevertheless, it may be that our subsamples were not representative of the total cohorts. However, we do not have any reason to suspect differences in non-participants between the two cohorts. Focusing on the participants from whom we collected hair samples revealed no significant differences with respect to gender, mean age and pu-GPA between the two cohorts, and similar conclusions with respect to academic performance and perceived stress. Another strength of our study is that the students were well characterised for both individual parameters at admission and different stress parameters at the end of Year 1. An important limitation is that no data were collected on stress-related psychological and physical effects, which makes it difficult to infer anything about the consequences of higher levels of stress in this population. Additionally, it is not possible to infer causality on the basis of our data, despite the fact that data on stress and academic performance were collected at different time-points. To ascertain definitive causal pathways, further studies that measure stress levels throughout the first year are required. Although the groups were quite similar regarding pre-admission variables and the 100%-credit cohort more frequently reported examination-related life events, the use of

historical control subjects in the study design prevents us from drawing definitive conclusions about the effects of the academic progress policy on the outcomes. This study has some practical implications for medical schools that aim to improve their students' progress and offers some directions for future research. First, our findings suggest a relationship between the raising of performance standards and student well-being. As we noted earlier, an important aspect of the relationship between stress and academic performance relates to the issue of whether there is an optimum level at which students can perform best. In this study, we found that an increased academic demand was associated with better performance, as well as relatively higher PSS scores, reflecting psychological stress levels in the past month, whereas no differences were found in average long-term biological stress experienced over the preceding 3 months. Despite the use of measurements to detect chronic stress, it remains uncertain whether the higher levels of perceived stress observed were present during the whole of the first year and continued into the second year. This is of particular importance given the relatively high frequencies of depression, as well as suicidal thoughts, in medical students.[41] Therefore, we recommend that medical schools monitor their students' stress levels when implementing measures to increase study progress and consider implementing interventions to improve student well-being, such as wellness programmes that teach mind- and body-based stress reduction skills and formal faculty advisor/mentor programmes for small groups.[2]

Second, our study revealed gender-related differences in the effects of the raising of standards. This suggests that changes in the academic environment may have differential effects in male and female students. Therefore, as in medical practice, we urge medical educationalists to take differential effects in subgroups into account, both in designing and implementing, and in evaluating the effects of educational innovations. This may be particularly important for educational innovations that influence feelings of autonomy. Generally, autonomous motivation is reported to be associated with greater psychological well-being than controlled motivation.[42] Further research is required to explore possible gender-based differences in that pattern, especially in an academic environment.

Conclusions

Raising the Year-1 performance standard increased academic performance, most prominently in male students. However, it also increased levels of perceived stress, especially in female students. In particular, the combination of a high level of perceived stress and a high level of biological stress was related to poor academic performance. Our study suggests a relationship between the raising of performance standards and student well-being, with differential effects in male

and female students. Medical schools should take these differences into account when trying to strike a balance between optimising study progress and supporting student well-being.

References

1. Stegers-Jager K, Cohen-Schotanus J, Splinter T, Themmen A. Academic dismissal policy for medical students: effect on study progress and help-seeking behaviour. *Med Educ*. 2011;45(10):987-994.
2. Wasson L, Cusmano A, Meli L, et al. Association between learning environment interventions and medical student well-being: a systematic review. *JAMA*. 2016;316(21):2237-2252.
3. Johnson C, Johnson R, McKee J, Kim M. Using the personal background preparation survey to identify health science professions students at risk for adverse academic events. *Adv Health Sci Educ Theory Pract*. 2009;14(5):739-752.
4. Yates J, James D. Risk factors for poor performance on the undergraduate medical course: cohort study at Nottingham University. *Med Educ*. 2007;41(1):65-73.
5. Arnold I. The effectiveness of academic dismissal policies in Dutch university education: an empirical investigation. *Stud High Educ*. 2015;40(6):1068-1084.
6. Sneyers E, De Witte K. Interventions in higher education and their effect on study success: a meta-analysis. *Educ Rev*. 2018;70(2):208-228.
7. Sneyers E, De Witte K. The effect of an academic dismissal policy on dropout, graduation rates and student satisfaction. Evidence from the Netherlands. *Stud High Educ*. 2017;42(2):354-389.
8. Stegers-Jager K, Themmen A. Binding study advice: effect of raising the standards? *Perspect Med Educ*. 2015;4(3):160-162.
9. Dyrbye LN, Thomas MR, Shanafelt TD. Systematic review of depression, anxiety, and other indicators of psychological distress among US and Canadian medical students. *Acad Med*. 2006;81(4):354-373.
10. Hope V, Henderson M. Medical student depression, anxiety and distress outside North America: a systematic review. *Med Educ*. 2014;48(10):963-979.
11. Dyrbye LN, Massie FS Jr, Eacker A, et al. Relationship between burnout and professional conduct and attitudes among US medical students. *JAMA*. 2010;304(11):1173-1180.
12. Lyndon M, Strom J, Alyami H, et al. The relationship between academic assessment and psychological distress among medical students: a systematic review. *Perspect Med Educ*. 2014;3(6):405-418.
13. LeBlanc VR. The effects of acute stress on performance: implications for health professions education. *Acad Med*. 2009;84(10 Suppl):S25-S33.
14. Everson-Rose SA, Lewis TT. Psychosocial factors and cardiovascular diseases. *Annu Rev Public Health*. 2005;26(1):469-500.
15. Oken BS, Chamine I, Wakeland W. A systems approach to stress, stressors and resilience in humans. *Behav Brain Res*. 2015;282:144-154.
16. Ibrahim AK, Kelly SJ, Adams CE, Glazebrook C. A systematic review of studies of depression prevalence in university students. *J Psychiatr Res*. 2013;47(3):391-400.

17. Regehr C, Glancy D, Pitts A, LeBlanc VR. Interventions to reduce the consequences of stress in physicians a review and meta-analysis. *J Nerv Ment Dis*. 2014;202(5):353-359.
18. Kickert R, Stegers-Jager KM, Meeuwisse M, Prinzie P, Arends LR. The role of the assessment policy in the relation between learning and performance. *Med Educ*. 2018;52(3):324-335.
19. Russell E, Kirschbaum C, Laudenslager ML, et al. Toward standardization of hair cortisol measurement: results of the first international interlaboratory round robin. *Ther Drug Monit*. 2015;37(1):71-75.
20. Groeneveld MG, Vermeer HJ, Linting M, Noppe G, van Rossum EF, van IJzendoorn MH. Children's hair cortisol as a biomarker of stress at school entry. *Stress*. 2013;16(6):711-715.
21. Staufenbiel SM, Penninx BW, Spijker AT, Elzinga BM, van Rossum EF. Hair cortisol, stress exposure, and mental health in humans: a systematic review. *Psychoneuroendocrinology*. 2013;38(8):1220-1235.
22. Stalder T, Steudte-Schmiedgen S, Alexander N, et al. Stress-related and basic determinants of hair cortisol in humans: a meta-analysis. *Psychoneuroendocrinology*. 2017;77:261-274.
23. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. 1983;24(4):385-396.
24. Manenschijn L, Koper JW, Lamberts SW, van Rossum EF. Evaluation of a method to measure long term cortisol levels. *Steroids*. 2011;76(10- 11):1032-1036.
25. Olivier J, Bell M. Effect sizes for 2×2 contingency tables. *PLoS One*. 2013;8(3):e58777.
26. Cohen J. A power primer. *Psychol Bull*. 1992;112(1):155-159.
27. Olejnik S, Algina J. Generalized eta and omega squared statistics: measures of effect size for some common research designs. *Psychol Methods*. 2003;8(4):434-447.
28. Cole JS, Osterlind SJ. Investigating differences between low- and high-stakes test performance on a general education exam. *J Gen Educ*. 2008;57(2):119-130.
29. Sundre D, Kitsantas A. An exploration of the psychology of the examinee: can examinee self-regulation and test-taking motivation predict consequential and non-consequential test performance? *Contemp Educ Psychol*. 2004;29(1):6-26.
30. Wolf L, Smith J. The consequence of consequence: motivation, anxiety, and test performance. *Appl Meas Educ*. 1995;8(3):227-242.
31. Elikai F, Schuhmann P. An examination of the impact of grading policies on students' achievement. *Issues Account Educ*. 2010;25(4):677-693.
32. Johnson B, Beck H. Strict and lenient grading scales: how do they affect the performance of college students with high and low SAT scores? *Teach Psychol*. 1988;15(3):127-131.
33. de Koning BB, Loyens SMM, Rikers R, Smeets G, van der Molen HT. Impact of binding study advice on study behavior and pre-university education qualification factors in a problem-based psychology bachelor program. *Stud High Educ*. 2014;39(5):835-847.

34. Buddeberg-Fischer B, Klaghofer R, Abel T, Buddeberg C. The influence of gender and personality traits on the career planning of Swiss medical students. *Swiss Med Wkly*. 2003;133(39-40):535-540.
35. Kusurkar RA, Croiset G, Galindo-Garré F, ten Cate O. Motivational profiles of medical students: association with study effort, academic performance and exhaustion. *BMC Med Educ*. 2013;13(1):87.
36. Tyssen R, Dolatowski FC, Rovik JO, et al. Personality traits and types predict medical school stress: a six-year longitudinal and nationwide study. *Med Educ*. 2007;41(8):781-787.
37. Colbert-Getz JM, Fleishman C, Jung J, Shilkofski N. How do gender and anxiety affect students' self-assessment and actual performance on a high-stakes clinical skills examination? *Acad Med*. 2013;88(1):44-48.
38. Calmes CA, Roberts J. Rumination in interpersonal relationships: does co-rumination explain gender differences in emotional distress and relationship satisfaction among college students? *Cog Ther Res*. 2008;32(4):577-590.
39. Tucker P, Jeon-Slaughter H, Sener U, Arvidson M, Khalafian A. Do medical student stress, health, or quality of life foretell step 1 scores? A comparison of students in traditional and revised preclinical curricula. *Teach Learn Med*. 2015;27(1):63-70.
40. Waqas A, Khan S, Sharif W, Khalid U, Ali A. Association of academic stress with sleeping difficulties in medical students of a Pakistani medical school: a cross sectional survey. *PeerJ*. 2015;3:e840.
41. Rotenstein LS, Ramos MA, Torre M, et al. Prevalence of depression, depressive symptoms, and suicidal ideation among medical students: a systematic review and meta-analysis. *JAMA*. 2016;316(21):2214-2236.
42. Deci E, Ryan R. Facilitating optimal motivation and psychological well-being across life's domains. *Can Psychol*. 2008;49(1):14-23.

Chapter 12

Children's Hair Cortisol as a Biomarker of Stress at School: A Follow-Up Study

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Abstract

In a previous study, we examined hair cortisol concentrations (HCC) in children when first entering elementary school (at 4 years). In this follow-up study, we examined their HCC when they entered third grade (at 6 years), where the more playful first grades proceed into a more formal learning setting. Participants were 30 six-year-old children (14 boys). Hair samples (≥ 5 cm) were collected two months after the summer holidays. Hair analysis was conducted using two 2-cm long segments, reflecting the first two months of school attendance in grade 3 (the scalp-near segment), and two months prior to the start in grade 3. Between these two sections, we left a gap of 1 cm to avoid overlap of periods (due to differences in hair growth rate). Children showed a significant increase in cortisol levels when they entered third grade. This increase was not associated with social fearfulness or academic achievement, but did show significant associations with inhibitory control: children with less inhibitory control had higher cortisol levels after entering third grade, and larger increases in cortisol than children with higher scores on inhibitory control. This suggests that the ability to inhibit or control impulsive responsivity is important for children's stress regulation when making the transition to a more formal school environment.

Introduction

In a previous study, we showed that starting elementary school is accompanied by increased stress hormone levels in four-year-old children, by analyzing their hair cortisol concentrations (HCC; Groeneveld et al., 2013). Cortisol is a well-known stress hormone which in humans is the final product of activation of the hypothalamic-pituitary-adrenal axis. In the past, cortisol levels have mainly been determined in urine, blood or saliva. To assess cortisol levels over a prolonged period of time, repeated sampling is needed at different daily time points over several days. Currently, cortisol can also be determined in human scalp hair to determine long-term cortisol levels (Bates, Salsberry, & Ford, 2017; Russell et al., 2012; Stalder & Kirschbaum, 2012). Because hair grows at an average of 1 cm/month, assessment of hair cortisol can reflect changes over time. In our previous study, we showed that HCC were higher after school entry than before, especially for fearful children. This finding supported our hypothesis that a rise in HCC can be specifically linked to a stress-related transition: the first entry into elementary school. In this follow-up study, we examined the same children's HCC when they entered third grade at six years of age, where the more playful first grades proceed into a more formal learning setting.

Although researchers have used HCC widely to approximate stress levels in adults (Sauve et al., 2007; Stalder & Kirschbaum, 2012; Stalder et al., 2017; Staufenbiel et al., 2013), this measure is less often used in early childhood (Bates et al., 2017; Golub et al. 2019; Gray et al., 2018). The studies that measured HCC in children especially focused on chronic stress, not on stress during transitions (Yamada et al., 2007). Because HCC allows a retrospective assessment of cortisol exposure, this method seems very valuable when studying the effects of major transitions in life. Previous studies, using saliva measures, showed that it can be expected that the transition from grades is stressful (Bruce et al., 2002). We expect that especially the transition from the more playful first grades into a phase with more formal requirements can be stressful for young children. In the third grade children have to focus on reading, writing, and math skills, working at their own desk, and experience ratings on tests (Smeets, 2014; Smeets & Resing, 2013). The main research question of this study is: Do cortisol levels increase when children make this transition to formal learning in grade 3? These increased stress levels might have a negative effect on the development of children. Although stress responses are necessary for survival, chronic exposure to stress can change from adaptive into maladaptive (De Kloet et al 1999; Segerstrom & Miller, 2004). Previous studies have shown that higher stress levels in children are related to behavior changes, anxiety, sleep problems, and illness (Alink et al. 2018; Forbes et al. 2006; Golub et

al. 2019; Kenny et al. 2002, Lipton et al. 2005; Turner Cobb & Steptoe, 1998; Windle & Windle, 1996).

It can be expected that the transition to formal learning is more stressful for a subgroup of children, for example, children with a difficult temperament. In our previous study, we showed that especially children scoring high on social fearfulness showed an increase of cortisol levels when entering elementary school (Groeneveld et al., 2013), probably due to the new situation, with unfamiliar children and teachers. This temperamental characteristic has more often been linked to individual differences in children's stress reactivity in young children in childcare (Talge et al., 2008; Vermeer & Groeneveld, 2017; Watamura et al., 2003), although other studies did not find any associations between a fearful temperament and cortisol reactivity in childcare (Watamura, Sebanc, & Gunnar, 2002; Ahnert et al., 2004; Groeneveld et al, 2012) or during the start of a new school year (Davis, Donzella, Krueger, & Gunnar, 1999).

In addition to temperament, academic skills can also be an important factor in explaining individual differences in cortisol levels in a school setting. It could be argued that especially low achievers show an increase in cortisol levels when they enter third grade. In a study conducted with adolescents, it was shown that those students who were at the bottom of the scholastic hierarchy in the classroom, defined as students with less academic success and more troublesome behaviors, had higher cortisol levels (West et al., 2010) compared to their peers.

Finally, behavioral inhibition, i.e. the ability to regulate and control behavioral impulses, might be related to higher cortisol levels after the transition to third grade at age six. This executive cognitive ability is important since other executive functions appear to depend on it: working memory, self-regulation of affect, motivation, and arousal, internalization of speech, and reconstitution (Barkley, 1997). Individual differences in children's inhibitory control have been found to be related to internalizing and externalizing behavior of children (Eisenberg et al., 2001) and teacher-child conflict (Berry, 2012). Moreover, it has been shown that inhibitory control is related to (salivary) cortisol responses in preschoolers (Blair, Granger, & Razza, 2005). Overactivity of the stress systems may impact the development of prefrontal regulatory systems, and as a consequence increase the risk for both attention- and emotion-regulatory problems (for a review, see Loman & Gunnar, 2010). With the transition to formal learning, it seems important to be able to inhibit behavioral responses that enable children to direct their attention and behavior. Thus, higher levels of inhibitory control would be expected to be associated with lower cortisol levels when making the transition to formal learning.

To summarize, in the current study, we measure HCC alterations in young children before and after a potential stressful transition, that is, their entry into formal learning at school. We hypothesize that children's HCC are higher after entry in third grade than before entry. Furthermore, we study whether alterations in HCC are moderated by temperament (social fearfulness), academic skills, and executive functioning (inhibitory control).

Methods

Subjects

The current study is a follow-up of the (Groeneveld et al., 2013) study that started in 2012. A total of 284 families from Leiden (the Netherlands) were invited by postal mail to participate. Recruitment material was in Dutch. Registration for the study was closed after agreement from 50 families. In 2012, eight children were excluded for analyses, (n = 4 hair was too short and n = 4 extremely high cortisol values with > 3 SD above the mean). The remaining 42 families were again invited to participate in the study of 2014. An additional 9 families declined participation (n = 3 moved/ not able to contact, n = 3 did not want to cut hair, n = 3 were too busy). After the home visits another three children were excluded (n = 2 hair too short, n = 1 extremely high cortisol values with > 3 SD above the mean). This resulted in a final sample of 30 children (45% boys). There were no significant differences between the families who participated in the first study and the follow up study (e.g. maternal or paternal education or age, child fearfulness, time spent in childcare, or children's HCC). In the follow up study, children's age ranged from 6.5 to 6.8 years during the home visit (M = 6.6, SD = 0.08). Parents' educational level was coded as the number of years of education after age 6. Mean educational level across mothers and fathers was 14.90 years (SD = 1.90). All children had at least one Dutch parent with the Dutch nationality, six children had one Dutch parent, and one parent with a different nationality (Egypt, Afghanistan, Hungarian, Chilean, German, and Japanese).

Home visit

All children were visited at home, in a two week period, approximately two months after they started in grade 3. Prior to the home-visit, parents received a questionnaire, with questions about the background of the parents and the children and the temperament of the children. During the home visit children's hair was collected. In addition, children administered tasks to measure their academic skills and a computerized inhibition control task. All procedures were carried out with adequate understanding and written consent of the parents. Ethical approval for this study was provided by the Research Ethics Committee of the Institute of Education and Child Studies of Groeneveld et al., 2013.

Temperament

The Child Behavior Questionnaire (CBQ) short form was used to measure the child's temperament (Rothbart, Ahadi, & Hershey, 1994; Rothbart, Ahadi, Hershey, & Fisher, 2001). Parents were asked to fill out the CBQ based on their child's behaviour during the last six months. The questionnaire was sent to them a few weeks before the home visit. The CBQ is a 7-point rating scale for the assessment of different aspects of temperament in children aged 3-7 years. For this study, we used the subscale fearfulness (12 items, Cronbach's alpha = .86). An example item is 'How often was your child afraid of the dark?' answers can range from '1 never' to '7 always'. Higher scores on the fear scale represent more fearfulness in children. In 2012, parents were also asked to fill out the CBQ about their child when they were four years of age. Scores of the two questionnaires were significantly correlated, $r = .71$, $p < .01$.

Academic skills

The Rapid Automatized Naming (RAN; CB&WL [Continu Benoemen en Woorden Lezen]; Van den Bos & Lutje Spelberg, 2007) task was used to measure children's academic skills. The CB task consists of four components: colors, letters, numbers, and objects. For each component, children were asked to name 50 symbols or objects as rapid as they can, from paper forms. The time was recorded per component using a stopwatch. A mean score of the time of these four components represents children's academic skills. Higher scores represent lower academic skills, since the children took longer to finish the naming tasks. The RAN is a reliable instrument for children aged 5 to 16 years, judged by the COTAN (Dutch Commission for Test Materials of the Dutch Institute of Psychologists that test the quality of psychodiagnostic instruments).

Inhibitory control

A computerized Go/NoGo task was administered during the home visit to measure inhibitory control on a laptop. In this task, children had to catch all the mice that appeared on the screen (Go-stimuli) by pressing a red button. When a cat appeared, children had to inhibit their reaction, and should not press the button (NoGo-stimuli). The task consisted of a practice session, in which five mice and five cats were presented (in alternating order), and a test session, in which 30 mice and 10 cats were displayed in random order. Only during the practice session the child received feedback. Commission errors (responses to NoGo-stimuli) were used as a measure for a lack of inhibitory control (Groot, De Sonneville, Stins, & Boomsma, 2004). To generate a measure for inhibitory control the sum score of the commission errors was subtracted from the total number of NoGo-stimuli (10 – number of commission errors), with higher scores representing more inhibitory control.

Scalp hair collection

Hair was collected during home visits, which were planned approximately two months after the children had started school. Visits were conducted by the first author or trained (under)graduate students. Hair collection was performed as described previously (Manenschijn et al., 2011). A sample containing around 300 hairs was cut from the posterior vertex as close to the scalp as possible. The sample was taped to a piece of paper, the scalp end marked, and stored in an envelope at room temperature until analysis.

Prior to the home-visit, parents received a questionnaire, with questions about age, educational level, and birth country of the parents, and the following information concerning the participating child: after-school care, hair colour, hair washing frequency, use (and type) of corticosteroids during last six months, other medication use, and chronic diseases.

Hair processing and analysis

Hair analysis was conducted using two 2-cm long segments, reflecting the first two months of school attendance in grade 3 (the scalp-near segment), and two months prior to the start in grade 3. Between these two sections, we left a gap of 1 cm to avoid overlap of periods (due to differences in hair growth rate). As described previously (Manenschijn et al 2011), we weighed and cut the hair segments into small pieces in glass vials using surgical scissors. Cortisol was extracted by 16h incubation in one mL methanol at 52°C while gently shaking. Subsequently, the methanol was transferred to disposable glass vials, and evaporated under a constant stream of nitrogen. The samples were then dissolved in 250 µL phosphate buffered saline, pH 8.0, and vortexed for one min. Prior to analysis, samples were vortexed again for 30s. Cortisol concentrations were measured using a commercially available salivary cortisol ELISA kit (DRG Instruments GmbH, Marburg, Germany) according to manufacturer's directions. We applied a correction factor to take background signal into account. As stated by the manufacturer, antibody cross reactivity is as follows: corticosterone 29%, cortisone 3%, 11-deoxycortisol <1%, 17-OHP <0.5%, prednisone <0.1%. Intra-assay variation was 2.6% and the inter-assay variation was 6.7%, as stated by the manufacturer.

Data analysis

Because the distributions of the cortisol measurements were positively skewed, $\log_{10}$ transformations were used for analysis. After $\log_{10}$ transformation, HCC did not deviate from normality. To test whether HCC differed before and after school entry, we conducted a repeated measure MANCOVA with time as within-subject

variable. To analyse associations with temperament, academic skills, and inhibitory control, multivariate regression analyses were conducted, using interaction terms.

Results

Descriptive statistics of HCC during wave 1 (transition to grade 1) and wave 2 (transition to grade 3) are shown in Table 1. HCC before and after entering a new grade showed stability across time (wave 1 $r = .80$, $p < .001$; wave 2 $r = .52$, $p = .004$). In addition, HCC prior to entering third grade (wave 2, pre HCC) were related to HCC in wave 1 (pre $r = .36$, $p = .05$; post $r = .37$, $p = .04$), while HCC after entering third grade (wave 2, post HCC) were not related to HCC in wave 1. HCC in wave 2 were not significantly related to any of the child characteristics (gender, hair color, hair washing frequency, use of corticosteroids, other medication, hours in group care), or parent characteristics (educational levels fathers and mothers).

Table 1: Descriptive statistics of HCC in wave 1 (transition grade) and wave 2 (transition grade 3).

			Correlations			
	Mean	SD	W1 Pre	W1 Post	W2 Pre	W2 Post
Wave 1						
Pre	29.29	14.81	-			
Post	32.01	12.84	.80**			
Wave 2						
Pre	12.61	10.11	.36*	.37*		
Post	16.54	12.78	.22	.24	.52**	

HCC: hair cortisol concentrations; W1: Wave 1; W2: Wave 2
*p <.05; **p <.01

Change in HCC

A repeated measures MANCOVA on children's HCC yielded a significant main effect of time (Pillais, $F[1, 29] = 5.22$, $p = .03$, η^2partial = .15). Children's HCC were significantly lower during the holiday (M = 12.61 SD = 10.11) than after the start in third grade (M = 16.54, SD = 12.78). The Ratio of Change (RC) ((Post HCC – pre HCC)/pre HCC) was not correlated between the two waves ($r = .11$, $p = 57$), which means that an increase in HCC when entering first grade was not associated with an increase in HCC when entering third grade. Nevertheless, almost half of the children (46.7%) showed an increase in HCC after entering both first and third grade (Figure 1).

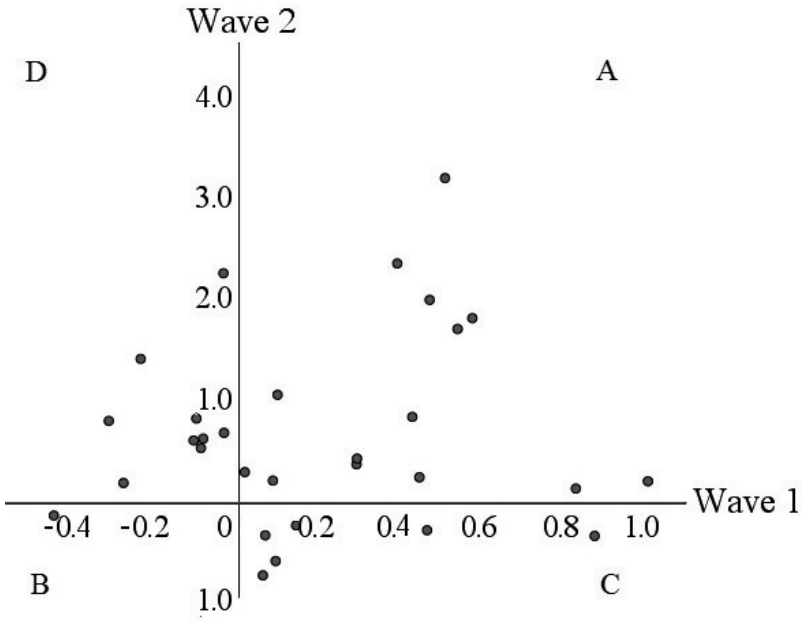

Figure 1: HCC ratio of change in waves 1 and 2.

Note: A = increase in HCC in waves 1 and 2, *n* = 14. B = decrease in HCC in waves 1 and 2, *n* = 1. C = increase in wave 1, but decrease in wave 2, *n* = 6. D = decrease in wave 1 but increase in wave 2, *n* = 9. HCC: hair cortisol concentrations.

Child characteristics

The correlations between HCC in wave 2 and social fearfulness, academic achievement, and inhibitory control are shown in Table 2. There were no significant correlations between these child characteristics and pre HCC, post HCC, or change in HCC. In addition, we found no interaction effect for social fearfulness (B = -0.17, SEB = 0.19, β = -0.15, p = .37, R^2 = .32) or academic skills (B = -0.01, SEB = 0.01, β = -0.25, p = .14, R^2 = .34) when predicting post HCC.

Table 2: Descriptive statistics of child characteristics and correlations with hair cortisol concentrations wave 2.

			Correlations cortisol wave 2		
	M	SD	Pre	Post	Change
Social fearfulness	2.52	.90	-.12	-.19	-.05
Academic achievement	64.63	21.83	.12	-.08	-.19
Inhibitory control	8.50	1.38	.11	-.24	-.31

For inhibitory control, significant main effects and an interaction effect were present. In Table 3 it is shown that pre HCC and inhibitory control predicted post HCC (R^2 = 0.36, p < .01). The interaction between pre HCC and inhibitory control significantly improved the model (ΔR^2 = .14, p < .05).

For graphic concerns, we made a median split in inhibitory control (Median=8). In Figure 2 it is shown that children scoring low on inhibitory control show a large increase in their HCC after entering grade 3 (Pillais, F[1, 14] = 14.70, p < .01, η^2partial = .51), while HCC from children scoring high on inhibitory control do not change (Pillais, F[1, 14] = 0.02, p = .89, η^2partial < .01).

Table 3: Inhibitory control predicting post HCC.

	Step 1 (R^2 = .36*)				Step 2 (R^2 = .50*)			
	B	SEB	β	p	B	SEB	β	p
Constant	1.13	0.05			1.14	0.04		
Pre HCC	0.57	0.16	0.55	<.01	0.47	0.15	0.46	<.01
Inhibitory control	0.07	0.03	0.30	.06	0.07	0.03	0.30	.04
Pre HCC * inhibitory control					0.31	0.11	0.40	.01

HCC: hair cortisol concentrations.
*p < .05.

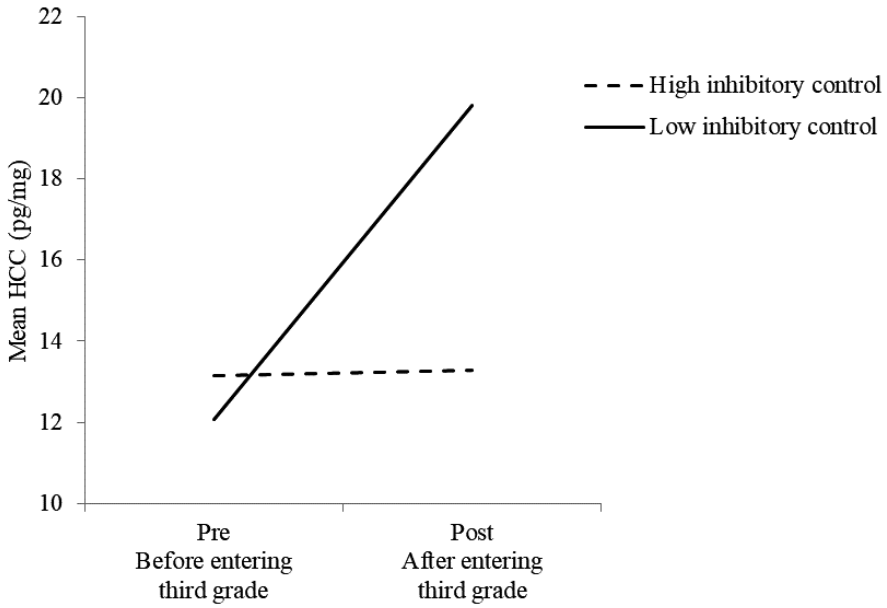

Figure 2: HCC levels of children scoring high or low on inhibitory control.

HCC: hair cortisol concentrations.

Discussion

In this study, we showed that on average HCC increase when children enter third grade at six years of age. This increase was not associated with social fearfulness or academic achievement, but we did find associations with inhibitory control: children with less inhibitory control had higher cortisol levels after entering third grade, and larger increases in cortisol, than children with higher scores on inhibitory control.

As expected, children show an increase in HCC when they entered grade three, where the more playful first grades proceed into a more formal learning setting. This corresponds to our earlier finding of HCC increases after entering grade one (Groeneveld et al., 2013). HCC in this follow up study were lower in terms of absolute values (pre and post) than in the previous study. Because we measured differences between the manufacturer's standards (optimized for measuring cortisol in saliva) and our own standard curve (prepared in PBS, like the HCC samples), we computed a correction factor and corrected the absorbance values of the samples based on the standards from the kit. This is something we have decided to apply with increased knowledge over time and could probably be one of the reasons for the difference with the earlier study.

When studying child characteristics, we focused on inhibitory control, temperament, and academic skills. First, we found that children scoring low on inhibitory control showed an increase in their HCC after entering grade 3, while HCC from children scoring high on inhibitory control do not change. This implies that especially children who find it difficult to inhibit reactions experience the transition to formal learning as more stressful. This seems logical because the dynamics change from the more playful first years, to the more scholastic environment. For example, in third grade the time children spend playing inside and outside decreases considerably, and at the same time they are requested to sit still at their desk, and focus on fine motoric skills, learning how to write, read, and do math (Smeets, 2014; Smeets & Resing, 2013). On the other hand, it might also be possible that cortisol levels influenced the inhibitory control levels of the children. It has been shown that overactivity of the stress systems may impact the development of prefrontal regulatory systems, and as a consequence increase the risk for emotion-regulatory problems (for a review, see Loman et al. 2010), but these studies are primarily based on animal-models (Brake et al. 20004), or on children with severe early life stress (Nemerof, 2004), so the relevance for humans is not clear yet. Previous studies with humans, focusing on the effect of cortisol levels on inhibition showed a positive association: higher cortisol levels increase inhibitory control (Shields, Bonner, & Moons, 2015). But these studies mainly focus on short term increases in (salivary) cortisol. A meta-analysis has shown that cortisol improves inhibition from 15 min to 135 minutes

post-administration, but cortisol begins to impair inhibition after 136 minutes (Shields et al., 2015). This could imply that the increases in cortisol levels with the transfer to third grade have resulted in impaired inhibition. A pretest measure of inhibition scores before entry in third grade should be conducted to test causality.

Secondly, we tested the moderation effect of social fearfulness, because this temperamental subscale was a significant moderator in our previous study (Groeneveld et al., 2013). High fearful children showed the highest rise in HCC after school entry in grade 1. In the current study, it seemed conceivable that the same pattern would be present when entering grade 3, with new unfamiliar teachers, and more focus on formal learning. This hypothesis was however not confirmed. The increase in cortisol levels of fearful children was comparable to the increases of HCC of their less fearful peers. It is possible that, although children entered a new classroom with new peers and teachers, the presence of familiar peers and the familiar school environment and routine is a buffer for these children.

Third, no association was found between cortisol levels and academic skills, although some studies did find this association for older children (West et al., 2010). Stress may interfere with academic performance by diverting attention from cognitive tasks to worry and feelings of being overwhelmed (Matheny, Aycock, & McCarthy, 1993). It might be possible that children were not yet aware of (and thus stressed by) their lower academic skills in the first weeks after they entered grade 3. This effect might be present a few months later when children have experienced more academic tests. In addition, parental educational levels were quite high. A larger, more diverse sample including families from lower SES is needed to further explore these associations.

To conclude, in this study, we showed that cortisol levels increase when children make the transition to a school phase with formal learning. It is important to study this transition, since higher levels of stress in the school and classroom are related to more mental health problems, adjustment problems in school, and lower academic achievement (Kaplan et al., 2005; Kenny et al., 2002, Windle & Windle, 1996). It might be helpful for children to learn strategies to cope with the transition, or even to cope with stress. Previous studies have shown a positive effect of interventions to decrease stress in children (Bothe et al. 2014; Haraldsson et al. 2008). Bothe et al (2014) showed that a 10-minute daily stress management technique was helpful for reducing anxiety scores and improving the ability to relax in school-age children. These daily sessions included deep breathing, movement, and guided imagery and were provided by the teacher, in the classroom for four months. In addition, there are several other resources that seem to help students

to cope with stress, such as improved social skills, problem-solving orientation, and social support. An important source of support in the educational setting is the teacher. In childcare studies, it has been shown that higher caregiver sensitivity and quality of care are associated with lower diurnal cortisol production in preschoolers (Vermeer & Groeneveld, 2017). In addition, lowering teachers' occupational stress may be effective, since this occupational stress has been linked with children's physiological stress regulation as well (Oberle & Schonert-Reichl, 2016).

We showed that especially children scoring low on inhibitory control showed these increased cortisol levels. This means that this group of children deserves extra attention in schools: how can we support these children to regulate their inhibitory control (for specific tasks) and how can we adapt the school environment to support these children? Several intervention use self-regulation techniques such as relaxation, yoga, and imagery to improve well-being, health related issues, and cognitive or emotional control in children (Bell & Deater-Deckard, 2007; Bothe et al 2014; Ehud et al. 2010; Goldbek & Schmid, 2003; Lee & Olness, 1996). The Bothe et al (2014) intervention with the 10-minute daily stress management technique also included these self-regulation techniques. It would be interesting to test whether this intervention might positively affect children's inhibitory control as well. In addition, the school environment might also be adapted, for example by giving children more room in the third grade to move around. It has been shown that physical activity can positively affect important brain areas, which might lead to increases in working memory, planning skills, and cognitive control in the short and long term (Hillman et al. 2009; Davis et al. 2011; Mullender-Wijnsma et al. 2015). A recent intervention in third grade children, including physically active academic lessons, showed an increase in mathematics and reading scores of children who participated in the intervention compared to children in a control group who received regular lessons (Mullender-Wijnsma et al. 2015). These physically active academic lessons might be especially effective for children scoring low on inhibitory control. More research is needed for this group of children.

To summarize, the current study showed that cortisol levels increase when children make the transition to formal learning, especially children scoring low on inhibitory control. It is important to study this transition to grade three and possible resources to regulate the stress responses of children, because this transition is the basis of the academic career of all children, and stress can have a negative effect on this career.

References

Ahnert, L., Gunnar, M.R., Lamb, M.E., & Barthel, M. (2004). Transition to child care: Associations with infant-mother attachment, infant negative emotion, and cortisol elevations. *Child Development, 75,* 639–650.

Barkley, R.A. (1997). Behavioral inhibition, sustained attention, and executive functions: Construction a unifying theory of ADHA. *Psychological Bulletin, 121,* 65–94.

Bates, R., Salsberry, P., & Ford, J. (2017). Measuring stress in young children using hair cortisol: The state of the science. *Biological Research for Nursing, 19,* 499–510.

Bäumler, D., Voigt, B., Miller, R., Stalder, T., Kirschbaum, C., & Kliegel, M. (2014). The relation of the cortisol awakening response and prospective memory functioning in young children. *Biological Psychology, 99,* 41–46.

Bell, M.A., & Deater-Deckard, K. (2007). Biological systems and the development of self-regulation: Integrating behavior, genetics, and psychophysiology. *Journal of Developmental & Behavioral Pediatrics, 28,* 409–420.

Berry, D. (2012). Inhibitory control and teacher-child conflict: Reciproval associations across the elementary-school years. *Journal of Applied Developmental Psychology, 33,* 66–76.

Blair, C., Granger, D., & Razza, R.P. (2005). Cortisol reactivity is positively related to executive function in preschool children attending Head Start. *Child Development, 76,* 554–567.

Bothe, D.A., Grignon, J.B., & Olness, K.N. (2014). The effects of a stress management intervention in elementary school children. *Journal of Developmental and Behavioral Pediatrics : JDBP, 35,* 62–67.

Brake, W.G., Zhang, T.Y., Diorio, J., Meaney, M.J., & Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *European Journal of Neuroscience, 19,* 1863–1874.

Bruce, J., Poggi Davis, E., & Gunnar, M.R. (2002). Individual differences in children's cortisol response to the beginning of a new school year. *Psychoneuroendocrinology, 27,* 635–650.

Davis, C.L., Tomporowski, P.D., McDowell, J.E., Austin, B.P., Miller, P.H., Yanasak, N.E., … Naglieri, J.A. (2011). Exercise improves executive function and achievement and alters brain activation in overweight children: A randomized, controlled trial. *Health Psychology, 30,* 91–98.

Davis, E.P., Donzella, B., Krueger, W.K., & Gunnar, M.R., (2001). The start of a new school year: Individual differences in salivary cortisol response in relation to child temperament. *Developmental Psychobiology, 35,* 188–196.

De Kloet, E.R., Oitzl, M.S., & Joëls, M. (1999). Stress and cognition: Are corticosteroids good or bad guys? *Trends in Neuroscience, 22,* 422–426.

Ehud, M., An, B.D., & Avshalom, S. (2010). Here and now: Yoga in Israeli schools. *International Journal of Yoga, 3,* 42–47.

Eisenberg, N., Cumberland, A., Spinrad, T.L., Fabes, R.A., Shepard, S.A., Reiser, M., Murphy, B.C., Losova, S.H., & Guthrie, I.K. (2001). The relations of regulation and emotionality

to children's externalizing and internalizing problem behavior. *Child Development, 72,* 1112–1134.

Forbes, E.E., Williamson, D.E., Ryan, N.D., Birmaher, B., Axelson, D.A., & Dahl, R.E., (2005). Peri-sleep-onset cortisol levels in children and ado lescents with affective disorders. *Biological Psychiatry, 59,* 24–30.

Goldbeck, L., & Schmid, K. (2003). Effectiveness of autogenic relaxation training on children and adolescents with behavioral and emotional problems. *Journal of the American Academy of Child & Adolescent Psychiatry, 42,* 1046–1054.

Golub, Y., Kuitunen-Paul, S., & Panaseth, K. (2019). Salivary and hair cortisol as biomarkers of emotional and behavioral symptoms in 6-9 year old children. *Physiology & Behavior, 209,* 1–10.

Gray, N.A., Dhana, A., Van Der Vyver, L., Van Wyk, J., Khumalo, N.P., & Stein, D.J. (2018). Determinants of hair cortisol concentration in children: A systematic review. *Psychoneuroendocrinology, 87,* 204–214.

Groeneveld, M.G., Vermeer, H.J., Linting, M., Noppe, G., van Rossum, E.F.C., & van IJzendoorn, M.H. (2013). Children's hair cortisol as a biomarker of stress at school entry. *Stress, 16,* 711–715.

Groeneveld, M.G., Vermeer, H.J., Van IJzendoorn, M.H., & Linting, M. (2012). Stress, cortisol and well-being of caregivers and children in home-based child care: A case for differential susceptibility. *Child: Care, Health and Development, 38,* 251–260.

Groot, A.S., De Sonneville, L.M.J., Stins, J.F., & Boomsma, D.I. (2004). Familial influences on sustained attention and inhibition in preschoolers. *Journal of Child Psychology and Psychiatry, 45,* 306–314.

Haraldsson, K.S., Lindgren, E.-C.M., Fridlund, B.G.A., Baigi, A.M.A.E., Lydell, M.C., & Marklund, B.R.G. (2008). Evaluation of a school-based health promotion programme for adolescents aged 12-15 years with focus on well-being related to stress. *Public Health, 122,* 25–33.

Hillman, C.H., Pontifex, M.B., Raine, L.B., Castelli, D.M., Hall, E.E., & Kramer, A.F. (2009). The effect of acute treadmill walking on cognitive control and academic achievement in preadolescent children. *Neuroscience, 159,* 1044–1054.

Kaplan, D.S., Liu, R.X., & Kaplan, H.B. (2005). School related stress in early adolescence and academic performance three years later: The conditional influence of self expectations. *Social Psychology of Education, 5,* 3–17.

Kenny, M.E., Gallagher, L.A., Alvarez-Salvat, R., & Silsby, J. (2002). Sources of support and psychological distress among academically successful inner-city youth. *Adolescence, 37,* 161–182.

Lee, L.H., & Olness, K.N. (1996). Effects of self-induced mental imagery on autonomic reactivity in children. *Journal of Developmental & Behavioral Pediatrics, 17,* 323–327.

Lipton, J., Becker, R.E., & Kothare, S.V. (2008). Insomnia of childhood. *Current Opinion in Pediatrics, 20,* 641–649.

Loman, M.M., & Gunnar, M.R. (2010). Early experience and the development of stress reactivity and regulation in children. *Neuroscience & Biobehavioral Reviews, 34,* 867–876.

Manenschijn, L., Koper, J.W., Lamberts, S.W.J., & van Rossum, E.F.C. (2011). Evaluation of a method to measure long term cortisol levels. *Steroids, 76,* 1031–1036.

Matheny, K.B., Aycock, D.W., & McCarthy, C.J. (1993). Stress in school-aged children and youth. *Educational Psychology Review, 5,* 109–134.

Mullender-Wijnsma, M.J., Hartman, E., de Greeff, J.W., Bosker, R.J., Doolaard, S., & Visscher, C. (2015). Improving academic performance of school-age children by physical activity in the classroom: 1-year program evaluation. *Journal of School Health, 85,* 365–371.

Nemeroff, C.B. (2004). Neurobiological consequences of childhood trauma. *The Journal of Clinical Psychiatry, 65,* 18–28.

Oberle, E., & Schonert-Reichl, K.A. (2016). Stress contagion in the class- room? The link between classroom teacher burnout and morning cortisol in elementary school students. *Social Science & Medicine, 159,* 30–37.

Rothbart, M.K., Ahadi, S.A., & Hershey, K.L. (1994). Temperament and social behavior in childhood. *Merrill-Palmer Quarterly, 40,* 21–39.

Rothbart, M.K., Ahadi, S.A., Hershey, K.L., & Fisher, P.A. (2001). Investigations of temperament at three to seven years: The children's behavior questionnaire. *Child Development, 72,* 1394–1408.

Russell, E., Koren, G., Rieder, M., & Van Uum, S. (2012). Hair cortisol as a biological marker of chronic stress: Current status, future directions and unanswered questions. *Psychoneuroendocrinology, 37,* 589–601.

Sauve, B., Koren, G., Walsh, G., Tokmakejian, S., & Van Uum, S.H. (2007). Measurement of cortisol in human hair as a biomarker of systemic exposure. *Clinical & Investigative Medicine, 30,* 183–191.

Segerstrom, S.C., & Miller, G.E. (2004). Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychological Bulletin, 130,* 1–27.

Shields, G.S., Bonner, J.C., & Moons, W.G. (2015). Does cortisol influence core executive functions? A meta-analysis of acute cortisol administration effects on working memory, inhibition, and set-shifting. *Psychoneuroendocrinology, 58,* 91–103.

Smeets J. (2014). *Zo werkt het met jonge kinderen in groep 3! [This is how it works with young children in grade 3!*] Retrieved from: http:// docplayer.nl/368497-Zo-werkt-het-met-jonge-kinderen-in-groep-3-jose-smeets-ipabo-amsterdam

Smeets, J., & Resing, W. (2013). Overgang van najaarsleerling naar groep 3 nader onderzocht. [Transition from fall students to grade 3, further investigated]. *Tijdschrift Voor Orthopedagogiek, 52,* 442–453.

Stalder, T., & Kirschbaum, C. (2012). Analysis of cortisol in hair – State of the art and future directions. *Brain, Behavior, and Immunity, 26,* 1019–1029.

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., … Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology, 77,* 261–274.

Staufenbiel, S.M., Penninx, B.W., Spijker, A.T., Elzinga, B.M., & Van Rossum, E.F. (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology, 38,* 1220–1235.

Talge, N.M., Donzella, B., & Gunnar, M.R. (2008). Fearful temperament and stress reactivity among preschool-aged children. *Infant and Child Development, 17,* 427–445.

Turner Cobb, J.M., & Steptoe, A. (1998). Psychosocial influences on upper respiratory infectious illness in children. *Journal of Psychosomatic Research, 45,* 319–330.

Vermeer, H.J., & Groeneveld, M.G. (2017). Children's physiological responses to childcare. *Current Opinion in Psychology, 15,* 201–206.

Van den Bos, K., & Lutje Spelberg, H.C. (2007). *Continu Benoemen en Woorden Lezen (CB&WL)*. Amsterdam: BoomTestuitgevers.

Watamura, S.E., Donzella, B., Alwin, J., & Gunnar, M.R. (2003). Morning-to-afternoon increases in cortisol concentrations for infants and toddlers at child care: Age differences and behavioral correlates. *Child Development, 74,* 1006–1020.

Watamura, S.E., Sebanc, A.M., & Gunnar, M.R. (2002). Rising cortisol at childcare: Relations with nap, rest, and temperament. *Developmental Psychobiology, 40,* 33–42.

West, P., Sweeting, H., Young, R., & Kelly, S. (2010). The relative importance of family socioeconomic status and school-based peer hierarchies for morning cortisol in youth: An exploratory study. *Social Science & Medicine, 70,* 1246–1253.

Windle, M., & Windle, R.C. (1996). Coping strategies, drinking motives, and stressful life events among middle adolescents: Associations with emotional and behavioral problems and with academic functioning. *Journal of Abnormal Psychology, 105,* 551–560.

Yamada, J., Stevens, B., de Silva, N., Gibbins, S., Beyene, J., Taddio, A., … Koren, G. (2007). Hair cortisol as a potential biologic marker of chronic stress in hospitalized neonates. *Neonatology, 92,* 42–49.

Chapter 13

Associations Among Hair Cortisol Concentrations, Posttraumatic Stress Disorder Status, and Amygdala Reactivity to Negative Affective Stimuli in Female Police Officers

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Abstract

Posttraumatic stress disorder (PTSD) is associated with altered hypothalamic-pituitary-adrenal (HPA) axis function. Measurement of hair cortisol concentrations (HCC) allows retrospective assessment of HPA axis regulation over prolonged periods of time. Currently, research investigating HCC in PTSD remains sparse. Previous cross-sectional studies have included only civilian populations, although it is known that trauma type moderates associations between PTSD status and HPA axis function. We investigated differences in HCC between trauma-exposed female police officers with current PTSD (n = 13) and without current and lifetime PTSD (n = 15). To investigate whether HCC was associated with neural correlates of PTSD, we additionally performed exploratory correlational analyses between HCC and amygdala reactivity to negative affective stimuli. We observed significantly lower HCC in participants with PTSD than in participants without PTSD, d = 0.89. Additionally, within participants with PTSD, we observed positive correlations between HCC and right amygdala reactivity to negative affective (vs. happy/neutral) faces, r = .806 (n = 11) and left amygdala reactivity to negative affective (vs. neutral) pictures, r = .663 (n = 10). Additionally, left amygdala reactivity to negative faces was positively correlated with HCC in trauma-exposed controls, r = .582 (n = 13). This indicates that lower HCC is associated with diminished amygdala differentiation between negative affective and neutral stimuli. Thus, we observed lower HCC in trauma-exposed noncivilian women with PTSD compared to those without PTSD, which likely reflects prolonged HPA axis dysregulation. Additionally, HCC was associated with hallmark neurobiological correlates of PTSD, providing additional insights into pathophysiological processes in PTSD.

Introduction

Posttraumatic stress disorder (PTSD) is associated with altered hypothalamic-pituitary-adrenal (HPA) axis function; this has mainly been investigated by assessing acute cortisol levels and glucocorticoid receptor (GR) function (Olff & van Zuiden, 2017). Insight into chronic HPA axis dysregulation may be gained by assessing cortisol in scalp hair, which allows retrospective assessment over prolonged periods of time (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013). Research on hair cortisol concentrations (HCC) in PTSD has been relatively sparse. In one cross-sectional study, higher levels of HCC were observed in individuals with PTSD compared to trauma-exposed controls (TCs) in a mixed-gender sample of refugees with ongoing distress (Steudte et al., 2011). In another study, the authors found lower levels of HCC in trauma-exposed individuals with and without PTSD compared to non-trauma-exposed controls in a predominantly female sample (Steudte et al., 2013). Additionally, no HCC differences were found between female refugees with stress-related disorders and TCs (Schalinski, Elbert, Steudte-Schmiedgen, & Kirschbaum, 2015) or between male refugees who had recently fled their country of origin with and without PTSD (Mewes, Reich, Skoluda, Seele, & Nater, 2017). In a longitudinal study, pretrauma HCC did not differ between female adolescent earthquake survivors with and without PTSD at 7 months after the earthquake (Luo et al., 2012). Acute posttrauma HCC was elevated in both groups but had normalized at 5–7 months after the earthquake in TCs. At the final assessments, survivors with subsequent PTSD had lower HCC than TCs (Luo et al., 2012). In a prospective study, high HCC in the acute posttrauma period predicted subsequent PTSD symptoms in a predominantly female sample of individuals hospitalized with physical injuries (Pacella, Hruska, Steudte-Schmiedgen, George, & Delahanty, 2017). In the only study to our knowledge that has used a noncivilian sample, low predeployment HCC predicted PTSD symptom development in male soldiers upon trauma exposure during deployment (Steudte-Schmiedgen et al., 2015). Trauma type has been shown to moderate the association between PTSD and acute cortisol levels (Meewisse, Reitsma, de Vries, Gersons, & Olff, 2007). To date, associations between HCC and PTSD have been predominantly investigated in female civilian populations. It remains unknown whether previous findings extend to noncivilian populations, particularly those at risk for cumulative exposure to work-related traumatic events.

To understand the pathophysiology and clinical and phenotypic heterogeneity of PTSD, it is of interest to investigate whether chronic HPA axis dysregulation is associated with neural correlates of PTSD. Glucocorticoid receptor and mineralocorticoid receptor (MR) activation affect amygdala excitability and synaptic connectivity and may induce anxiogenic responses, with exact effects

depending on duration, timing, and amount of current and prior glucocorticoid exposure (de Quervain, Schwabe, & Roozendaal, 2017). This is of relevance for PTSD as several meta-analyses have observed significant amygdala hyperreactivity in individuals with PTSD (Hayes, Hayes, & Mikedis, 2012; Koch et al., 2016a; Patel, Spreng, Shin, & Girard, 2012; Sartory et al., 2013; Stark et al., 2015). This fits with the predominant neurocircuitry model for PTSD, which posits a central role for increased salience and threat hypersensitivity by brain areas within the salience network, including the amygdala, that is paralleled by inadequate top-down regulation by ventromedial prefrontal areas (Koch et al., 2016a). In these meta-analyses, specifically those related to right amygdala hyperreactivity in response to non-trauma- related negative stimuli (Stark et al., 2015), differences in amygdala functioning between trauma-exposed individuals with and without PTSD were observed, whereas no differences in amygdala reactivity to trauma-related stimuli were observed between PTSD and TC groups (Sartory et al., 2013).

In the current study, we investigated HCC differences between trauma-exposed female police officers with (n = 13) and without PTSD (n = 15). We additionally performed exploratory analyses to assess associations between HCC and amygdala reactivity to non-trauma-related negative affective stimuli and a mixture of non-trauma-related and potentially trauma-related negative affective stimuli. The current study comprised a subsample of a larger neuroimaging study that included both women and men. In the larger study, we did not observe significant group differences in amygdala reactivity toward negative affective stimuli (Koch et al., 2016b). However, although TCs showed increased amygdala reactivity to negative compared to neutral or positive affective faces, this differential reactivity was absent in participants with PTSD, indicating there may be increased saliency attributed to potential affective aspects of faces (Koch et al., 2016b).

Methods

Participants

This study was part of a randomized controlled trial on neural effects of a single oxytocin administration in male and female trauma-exposed police officers with and without PTSD who were between 18 and 65 years of age (Koch et al., 2016b). Data presented in the current manuscript were collected at baseline or during the session in which they received a placebo. For the current study, we included female participants only, as the overlarge majority of male participants did not have sufficient hair length for HCC determination. Hair segments were available for 14 participants with PTSD and 16 TCs. One participant with PTSD and one TC were excluded due to extreme HCC values (described later), which resulted in 13 patients with PTSD and 15 TCs. Additionally, two patients with PTSD dropped out

of the study prior to the placebo-scanning session, and one additional participant with PTSD did not complete the picture task during this session. Thus, for participants with PTSD, imaging data were available for 11 participants for the faces task and 10 for the pictures task (tasks are described later in this article). Imaging data were available for all TCs, but one TC was excluded due to a scanning artefact in the temporal cortex.

Participants with PTSD fulfilled criteria given in the fourth edition of the Diagnostic and Statistical Manual of Mental Dis- orders (DSM-IV) for current PTSD, with a total score on the Clinician-Administered PTSD Scale for DSM-IV (CAPS) of 45 or higher (Blake et al., 1995). Exclusion criteria for participants with PTSD were current severe major depressive disorder (MDD) with psychotic symptoms and/or suicidal intent, suicidal ideation, alcohol/substance abuse (except smoking), bipolar disorder, and psychotic disorder. Individuals in the TC group had to have reported at least one DSM-IV Criterion A traumatic event and scored a 15 or less on the CAPS. They were matched to patients based on sex, age, education, and years of service. Exclusion criteria for TCs were lifetime MDD or PTSD or any current DSM-IV Axis I psychiatric disorder. Exclusion criteria for all participants were daily use of psychoactive medication (incidental use was allowed as long as it had not occurred less than 24 hr prior to scanning) or systemic glucocorticoids, serious medical conditions, a history of neurological disorders, and several common contraindications for magnetic resonance imaging (MRI) and oxytocin administration (Koch et al., 2016b).

We did not observe significant group differences in demographic or health characteristics (Table 1). There were 12 TCs (80.0%) and six participants with PTSD (50.0%) who were in active police duty, Fisher's exact test = 0.13, $p = .127$; this likely explained why TCs reported nominally more types of work-related traumatic events than participants with PTSD, $t(25) = 1.82$, $p = .080$. Hair color differed between groups— the majority of participants with PTSD had brown hair whereas more participants in the TC group had blond hair, Fisher's exact test = 7.56, $p = .045$.

Table 1: Demographic and clinical characteristics of study participants.

	PTSD (n=13)				Trauma-Exposed controls (n=15)				
Characteristic	***M***	***SD***	***n***	***%***	***M***	***SD***	***n***	***%***	***p***
Age (years)	42.00	7.96			38.00	9.98			.257[a]
Years of police service	15.88	9.91			18.73	8.13			.795[a]
Current active executive duty			6	50.0			12	80.0	.127[d]
PLES total score	12.92	8.38			18.73	8.13			.080[a]
ETI-SF total score	5.42	5.90			4.40	5.49			.648[b]
CAPS total score	69.38	10.91			4.00	4.60			<.001[a]
Major depressive order			4	30.8			0	0.0	.035[d]
Body mass index (kg/m^2)	25.00	4.14			26.43	3.26			.321[a]
Current smoker			3	25.0			5	33.3	.696[d]
AUDIT total score	3.17	4.13			3.60	1.64			.712[a]
Hormonal contraceptive use			5	41.7			7	46.7	1.000[c]
Local glucocorticoid use			2	15.4			0	0.0	.206[d]
Hair color									.045[d]
• Black			1	7.7			0	0.0	
• Brown			8	61.5			4	26.7	
• Blond			3	23.1			9	60.0	
• Grey			1	7.7			0	0.0	
• Red			0	0.0			2	13.3	
Hair washing frequency									.105[d]
• 0-2 times per week			2	15.4			4	26.7	
• 3-4 times per week			7	53.8			2	13.3	
• > 4 times per week			4	30.8			9	60.0	
Hair treatment within past 3 months[e]			7	53.8			7	46.7	.705[c]
Days between hair collection and scanning session	3.09	7.33			-0.33	15.24			.443[b]
Scanning session time of day (hh:mm)	14:09	1:38			15:12	2:05			.185[a]

Note. PLES = Police Life Events Scale; ETI = Early Trauma Inventory-Self-report form; AUDIT = Alcohol Use Disorder Identification Test; CAPS = Clinician-Administered PTSD Scale for *DSM-IV*.

[a] Independent samples t test used.
[b] Mann-Whitney *U* test used.
[c] Chi-square test used.
[d] Fisher's exact test used.
[e] Includes coloration, bleaching, and/or permanent wave.

Procedure

Participants were recruited through a diagnostic outpatient center for police personnel (PDC; Diemen, the Netherlands; *n* = 3 participants with PTSD) and advertisements (*n* = 11 participants with PTSD and all TCs). All participants provided verbal and written informed consent prior to study initiation. At baseline, inclusion and exclusion criteria were assessed using diagnostic clinical interviews and self-report questionnaires. For patients recruited via the diagnostic outpatient

center, clinical interviews administered during intake were used. After inclusion, participants completed two scanning sessions (described later). Participants were asked to abstain from alcohol and drugs 24 hr before scanning and from rigorous exercise, beverages except water, and nicotine for 2.5 hr before scanning. Prior to scanning, intranasal oxytocin (40 IU) and a placebo (0.9% saline) were administered in a randomized double-blind crossover design. For this study, we only included imaging data collected under placebo. For most participants (i.e., 10 participants with PTSD who completed scanning, 90.1%; and 11 TCs, 73.3%), scalp hair for HCC determination was collected before trial medication administration. We found that HCC did not significantly differ between TCs whose samples were collected prior to versus after trial medication administration, $t(13) = -0.81$, $p = .431$. The average time in days between hair collection and placebo scan did not significantly differ between groups (Table 1). The study was approved by the Institutional Review Board of the Amsterdam University Medical Centers, location Academic Medical Center, Amsterdam, the Netherlands, and was registered in the Netherlands Trial Registry (NTR3516).

Measures

PTSD symptoms. All participants with PTSD were originally diagnosed by a licensed clinician prior to study inclusion. For all participants, current PTSD symptom severity was assessed using the validated Dutch version of the CAPS (Blake et al., 1995; Hovens et al., 1994). The CAPS is the gold standard structured clinical interview for diagnosing PTSD and assessing PTSD symptom severity according to DSM-IV criteria. It assesses the three symptom clusters: reexperiencing (five items, with a possible score range of 0–40), avoidance (seven items, with a possible score range of 0–56), and hyperarousal (five items, with a possible score range of 0–40), and distinguishes between estimated symptom frequency (score range: 0–4) and intensity (score range: 0–4) in the previous month. We calculated a total symptom severity score by summing the intensity and frequency scores for all items. Higher scores indicate a higher level of symptom severity in the past month. The Cronbach's alpha value for internal consistency for all items was high, Cronbach's $\alpha = .98$. As stated, participants met inclusion criteria for the PTSD group if their total score was 45 or higher; this cutoff was used to ensure current symptom severity above the clinical threshold. This cutoff has high sensitivity for PTSD diagnosis (Weathers, Ruscio, & Keane, 1999).

Other Axis I disorders. Dutch versions of the Mini International Neuropsychiatric Interview (M.I.N.I; Sheehan et al., 1998) or the Structured Clinical Interview for DSM-IV (SCID; First, Spitzer, Gibbon, & Williams, 2002) were used to assess other DSM-IV Axis I psychiatric disorders (the SCID was used for patients recruited

through the diagnostic center). Both structured clinical interviews are widely used, valid, and reliable for diagnosing current and lifetime psychiatric disorders (Lobbestael, Leurgans, & Arntz, 2011; Sheehan et al., 1998).

Work-related trauma exposure. The 42-item Dutch Police Life Events Checklist (PLES) was used to assess the number of different police work-related traumatic events participants had encountered (Carlier & Gersons, 1992). A total score was calculated using the first 41 items, each of which inquired about a different event, by summing the number of endorsed items (possible score range: 0–41).

Childhood trauma exposure. The Dutch translation of the short self-report version of the Early Trauma Inventory (ETI-SF) was used to assess trauma exposure during childhood (Bremner, Bolus, & Mayer, 2007; Rademaker, Vermetten, Geuze, Muilwijk, & Kleber, 2008). This is a valid and reliable measure of childhood trauma (Bremner et al., 2007). The questionnaire consists of 21 items that assess whether participants were exposed to different types of physical (nine items), sexual (six items), and emotional abuse (five items) as well as general traumas (11 items) before 18 years of age. We calculated a total score by summing the number of endorsed items (range: 0–21).

Alcohol abuse. The Dutch translation of the validated Alcohol Use Disorder Identification test (AUDIT; Bush, 1998) was used to assess current alcohol use and level of alcohol-related risk. It contains 10 items that assess alcohol consumption and indicators of dependence and harmful drinking. A total score was calculated by summing all item scores (range: 0–40). In the current sample, Cronbach's alpha for all items was questionable, $\alpha = .64$, presumably because all participants except one obtained the lowest possible scores for all seven items inquiring about dependence and harmful drinking whereas scores on the three items regarding consumption quantity varied. Additionally, participants self-reported demographic characteristics, current active police duty (executive function), weight, and height to calculate body mass index, current smoking status, medication and hormonal contraceptive use, and hair characteristics (color, washing frequency, coloration, bleaching, and permanent wave application in past 3 months).

Hair cortisol assessment. Hair was collected from the posterior vertex. Upon collection, samples were taped to paper and stored in closed envelopes at room temperature. We assessed cortisol concentrations in the most proximal 3 cm of scalp hair, covering HCC in the 3 months before sample collection, using a validated protocol (Manenschijn, Koper, Lamberts, and van Rossum, 2011). Samples were cut, weighted, and incubated with 1.0 mL methanol for 16 hr at

52 °C. Then, methanol solutions containing cortisol extracts were transferred to new vials and evaporated under a nitrogen stream. After dissolving dried contents with 250 µl phosphate buffered saline (PBS), HCC were quantified with enzyme-linked immunosorbent assay (ELISA; DRG Instruments GmbH, Marburg, Germany) following the manufacturer's protocol. The previously determined lower-end detection limit for this assay is 1.5 nmol/l. The upper detection limit according to the manufacturer's protocol is 220.69 nmol/l. All measurements were performed in duplicate in one assay. The intra-assay variability for internal controls was on average 1.3% (range: 0.4%–2.3%). As reported by the manufacturer, the assay cross-reactivity with other steroid hormones is corticosterone (29.0%), cortisone (3.0%), 11-deoxycortisol (less than 1.0%), 17-OH progesterone (less than 0.5%), testosterone (less than 0.1%), and estradiol (less than 0.1%). As is standard in steroid hormone hair analysis, HCC were converted to pg/mg, taking the weight of the hair samples into account (*M* 15.1 mg, *SD* 11.7, *Mdn* 12.0, range: 5.77–67.93 mg). Hair weight and final HCC were not significantly correlated, $r = -.06$, $p = .754$. Two samples were excluded from all analyses due to nondetectable (in one participant with PTSD) and extremely high (in one TC; HCC: 180.73 pg/mg, standardized z score = 4.86) HCC.

Functional MRI (fMRI). Structural and functional MRI images were acquired with a 32-channel head coil on a 3T Philips (Andover, MA) Achieva MR system. During the two scanning sessions, we presented two versions of each task, including different stimuli, in randomized counterbalanced order. Scanning sessions were scheduled in the afternoon or early evening (for more details concerning fMRI data acquisition, see Koch et al., 2016b). Amygdala reactivity to negative affective pictures was assessed using a distraction task with three conditions: (a) passive viewing of 20 neutral pictures, (b) passive viewing of 20 negative affective pictures, and (c) working memory performance during presentation of 20 negative affective pictures (McRae et al., 2010). Pictures were presented using an event-related design with pseudorandom order for trial type. All trials were separated by an intertrial interval, which consisted of a fixation cross presented for 2000 ms. In the current study, we used data collected during the two passive viewing conditions. Pictures were selected from the International Affective Picture System (IAPS), based on normative valence and arousal ratings (Lang, Bradley, & Cuthbert, 2008). Pictures in the task versions were matched for normative valence, arousal, complexity, and luminescence. Negative pictures included scenes related to events police officers may encounter in their line of work (e.g., violence, accidents) and more general aversive scenes (e.g., malnourished children, war-related scenes; Koch et al., 2018). Amygdala reactivity to negative affective faces was assessed using an emotional face-matching task that contained three conditions: (a) angry-

fearful faces, (b) neutral-happy faces, and (c) scrambled faces (visuomotor control; Hariri, Tessitore, Mattay, Fera, & Weinberger, 2002). Each trial consisted of three stimuli, with a cue stimulus presented on top and two target stimuli presented below. Participants were instructed to match the emotional expression (emotional condition) or the orientation (visuomotor control) of the cue stimulus with one of the target stimuli. Faces were selected from the NimStim face stimuli set (see Koch et al., 2016b, for more details).

Data Analysis

Functional MRI data were analyzed using SPM8. Preprocessing steps included realignment, slice-time correction, coregistration, normalization to the Montreal Neurological Institute (MNI) template, and smoothing (faces: 5 mm full- width half maximum [FWHM] kernel; pictures: 6 mm FWHM kernel, mirroring primary analyses in the larger study). At first level, the six realignment parameters were included, images were high-pass filtered, and temporal autocorrelation was removed with the AR(1) process (Koch et al., 2016b). One TC was excluded due to a scanner artifact in the temporal cortex. Two participants with PTSD did not complete the placebo scanning session. Additionally, one participant with PTSD did not complete the pictures task.

For the affective pictures, we only used first-level contrast images, which were obtained by subtracting amygdala reactivity to passive viewing of neutral pictures from reactivity to passive viewing of negative pictures (negative > neutral). Individual contrast estimates were extracted from 5 mm spheres surrounding left, xyz = −24, −8, −20, and right, xyz = 20, −6, −14, amygdala peak task activation within the region of interest (ROI) anatomical mask (Harvard–Oxford 50% probabilistic atlas) across participants in the larger study during the placebo session (whole-brain family-wise error corrected, $p_{FWE} < .05$; Koch et al, 2018).

For the affective faces, contrast images were obtained by subtracting amygdala reactivity during the control condition from reactivity to angry-fearful faces (angry-fearful > control) and happy-neutral faces (happy-neutral > control). Individual contrast estimates were extracted from 5 mm spheres surrounding left, xyz = −20, −8, −16, and right, xyz = 24, −10, −14, amygdala peak task activation within the ROI anatomical mask (Harvard–Oxford 50% probabilistic atlas) under placebo across participants and emotion conditions in the larger study (all $p_{FWE} < .05$). For the purpose of comparing results with results from amygdala reactivity to affective pictures, contrast estimates for amygdala reactivity toward neutral-happy faces (vs. control) were subtracted from contrast estimates for amygdala reactivity toward angry-fearful faces (vs. control; angry-fearful > happy-neutral).

Subsequent analyses were performed in SPSS (Version 24). We investigated whether data were normally distributed and contained outliers, standardized *z* score > |3.29|. Aside from the one removed extreme HCC value, no outliers were removed. Questionnaire data other than hair characteristics and medication use were missing for one participant with PTSD. Participants with missing data were excluded from analyses pairwise. Group differences in participant characteristics were assessed with independent sample *t* tests (normally distributed continuous variables); Mann-Whitney *U* tests (nonnormally distributed continuous variables); chi-square tests (categorical variables with cell frequencies of 5 or above), or Fisher's exact tests (categorical variables with cell frequencies less than 5). Group difference in (normally distributed) HCC was first assessed using an independent sample *t* test and Cohen's *d* effect size (representing the standardized difference between group means, with $d = 0.2$, $d = 0.5$, and $d = 0.8$ commonly interpreted as small, medium, and large effects, respectively (Cohen, 1977), followed by analyses of covariance (ANCOVA)s to control for potential confounders. To minimize the influence of included covariates on calculated effect size, generalized eta squared (ηG^2) was calculated, reflecting the amount of variance in HCC explained by PTSD versus TC status. As a benchmark, $\eta G^2 = .01$, $\eta G^2 = .06$, and $\eta G^2 = .14$ can be interpreted as small, medium, and large effects, respectively (Olejnik & Algina, 2003).

Exploratory correlation analyses (Pearson's *r* for normally distributed variables; Spearman's rho for nonnormally distributed variables) were performed to investigate associations between HCC and amygdala reactivity and negative affective stimuli within the PTSD and TC groups separately. To test whether correlation coefficients significantly differed between groups, we applied Fisher's *z* tests to compare correlation coefficients for both groups (Diedenhofen & Musch, 2015). Partial correlations were performed to investigate potential confounding influences of age and daytime of scanning. Additionally, we investigated correlations with PTSD symptom severity in participants with PTSD only due to selected low symptom severity in TCs. We considered p values less than .050 (two-sided) to be statistically significant. Data are expressed as means and standard deviations for continuous variables and absolute frequency and relative percentage for categorical variables.

Results

Group Differences in HCC

Participants with PTSD had significantly lower HCC levels ($M = 15.85$ pg/mg, $SD = 13.23$) than trauma-exposed participants without PTSD ($M = 25.23$ pg/mg, $SD = 8.01$), $t(19.179) = 2.227$, p = . 038, 95% CI [0.57, 18.19] (Figure 1). Cohen's

d was 0.86, indicating a large effect size. This difference remained significant after controlling for work-related traumatic events, $F(1, 24) = 8.00$, $p = .009$, ηG^2 (i.e., explained variance in HCC by PTSD status) = .248, estimated $t(24) = 2.83$, 95% CI [3.20, 20.46]; and current active executive police duty, $F(1, 24) = 5.84$, $p = .024$, $\eta G^2 = .189$, estimated $t(24) = 2.42$, 95% CI [1.44, – 18.22]. Additionally, the difference remained significant after controlling for hair color, $F(1, 25) = 9.15$, $p = .006$, $\eta G^2 = .247$, estimated $t(25) = 3.025$, 95% CI [3.83, 20.18], as well as several characteristics that did not differ between groups but are known to influence HCC, including age, body mass index, frequency of hair washing, hair treatment within the past 3 months, and current use of local glucocorticoids (inhalation), alcohol, nicotine, and hormonal contraceptives, *p*s of group difference = .009–.047. Within PTSD patients, HCC were not significantly correlated with total symptom severity, $r = .16$, $p = .603$, 95% CI [–.73, 55].

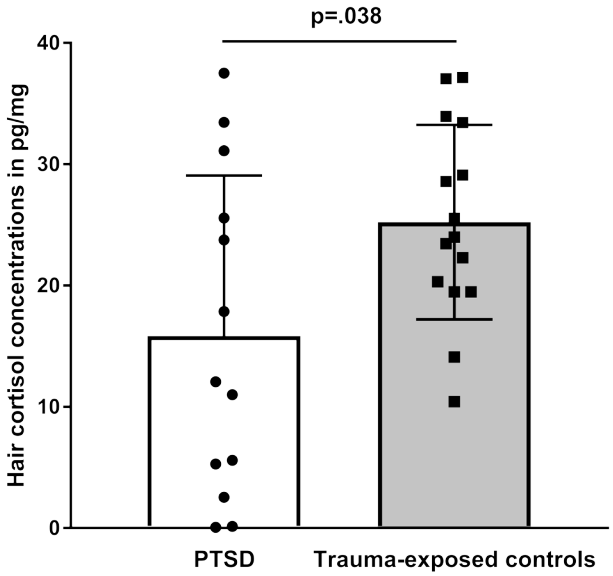

Figure 1: Hair cortisol concentrations (HCC) in 3 cm–long hair strands taken from the scalp of trauma-exposed female police officers with (*n* = 13, left) and without (*n* = 15, right) posttraumatic stress disorder (PTSD).

Points depict HCC values for each individual participant. Descriptive characteristics for each group are depicted as means (bars) and standard deviations (error bars).

Correlations Between Amygdala Reactivity and Negative Affective Stimuli

We investigated whether HCC was correlated with amygdala reactivity to negative affective stimuli in participants with PTSD and TCs and whether the magnitude of the correlation coefficient differed between groups. For right amygdala reactivity to negative affective faces compared to positive/neutral affective faces in the emotional face-matching task, the magnitude of the correlation was significantly different between groups, z = 2.18, p = .030, 95% CI [0.06, 1.27], with a strong positive correlation with participants with PTSD, r = .81, p = .003, 95% CI [.55, .96]; and a nonsignificant correlation in TCs, r = .11, p = .715, 95% CI [–.36, .58] (Figure 2, Panel A). No differential correlation with HCC was observed for left amygdala reactivity to negative faces, z = -0.05, p = .963, 95% CI [–0.74, 0.63]. In both groups, HCC was not significantly associated with amygdala reactivity: ρ = .28, p = .401, 95% CI [–.41, .88] for the PTSD group and r = .50, p = .068, 95% CI [.07, –.78] for the TC group (Figure 2, Panel B). However, after controlling for the time of day when scanning took place, the magnitude of the correlation between HCC and left amygdala reactivity to negative faces was marginally increased for both groups: ρ = .49, p = .131, 95% CI [–.21, .94] for the PTSD group and r = .58, p = .036, 95% CI [.13, .86] for the TC group. Regarding passive viewing of the IAPS negative affective pictures compared to neutral pictures, no differential correlation with HCC was observed for right amygdala reactivity to negative affective pictures and no significant correlations were observed within groups, z = 1.05, p = .292, 95% CI [–0.40, 1.18], and r = .16, p = .658, 95% CI [–.41, .73] for the PTSD group and r = –.33, p = .243, 95% CI [–.84, .14] for the TC group (Figure 2, Panel C). The magnitude of the correlations between groups significantly differed for left amygdala reactivity, z = 2.40, p = .016, 95% CI [0.29, 1.48]. We observed a strong positive correlation within participants with PTSD, r = .66, p = .037, 95% CI [.15, .93], which was absent in the TC group, ρ = –.36, p = .203, 95% CI [–.80, .38] (Figure 2, Panel D). Partial correlations controlling for age and time of day the scanning took place did not alter magnitudes of observed correlations, other than those that have already been described. Amygdala reactivity to both negative faces and negative pictures was not significantly correlated to total symptom severity within participants with PTSD, rs = –.182–.298. Amygdala reactivity to the two types of negative stimuli was not significantly correlated, rs = –.279–.394.

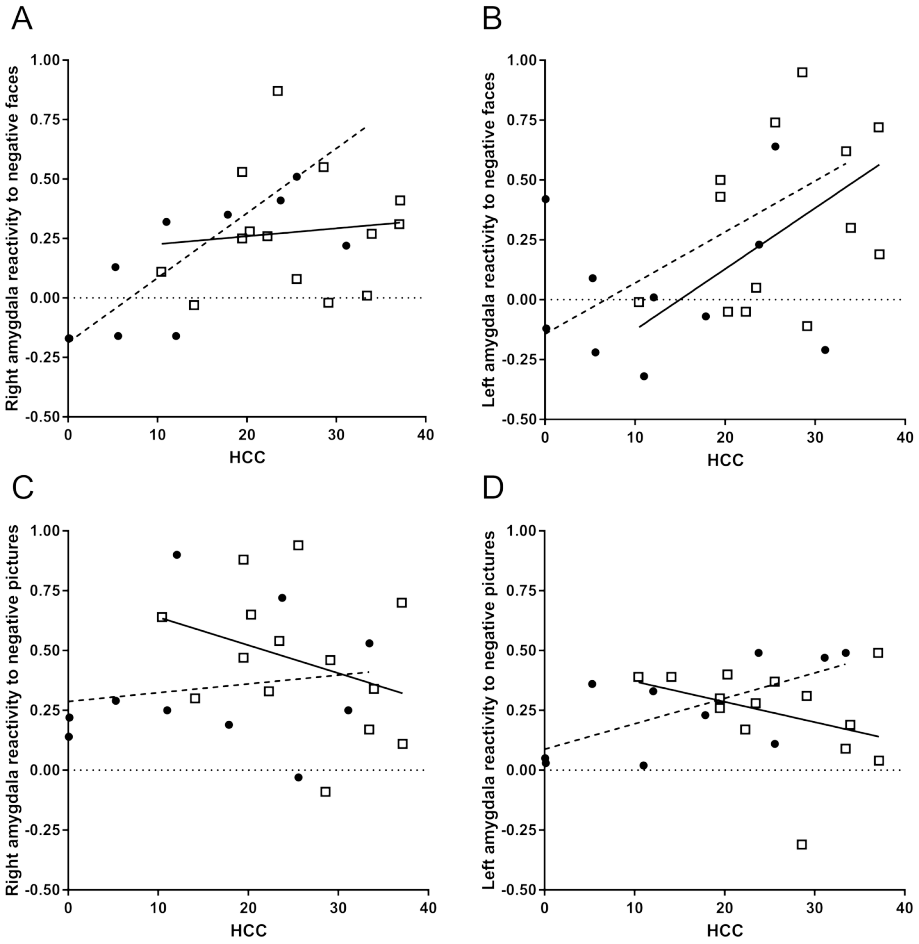

Figure 2: Scatterplots representing correlations between hair cortisol concentrations (HCC, in pg/mg) and contrast estimates of amygdala reactivity (arbitrary units) toward negative emotional faces (A = right; B = left) and negative affective pictures (C = right; D = left) in female trauma-exposed police officers with (circles, dashed line) and without (squares, solid line) posttraumatic stress disorder (PTSD).

Discussion

Compared to female trauma-exposed police officers without PTSD, we observed significantly lower HCC in female police officers with PTSD. This finding is in line with what was reported by Luo and colleagues (2012), who observed lower HCC in individuals with PTSD compared to TCs in a sample of female adolescent earthquake survivors several months after the earthquake; mean HCC in women with PTSD was also comparable to what was found in the current study. Our observed group difference is, however, in apparent contrast to results reported

in two cross-sectional studies, both of which found comparable HCC in trauma-exposed individuals with and without PTSD in two predominantly female samples of individuals for whom a longer period of time had elapsed since trauma exposure (Schalinski et al., 2015; Steudte et al., 2013). Interestingly, however, in the only study to our knowledge that used similar immunoassays to those used in the current study (Russell et al., 2015), the observed mean HCC in individuals with PTSD was highly comparable to our observed mean HCC (Schalinski et al., 2015). Our finding is in apparent contrast with findings of higher HCC in individuals with PTSD compared to TCs in a mixed-gender sample of internally displaced refugees of whom most individuals with PTSD, but not TCs, had pronounced ongoing distress (Steudte et al., 2011).

Steudte-Schmiedgen, Kirschbaum, Alexander, and Stalder (2016) recently proposed a model on the course of trauma-induced changes in cortisol output. It poses that cortisol output changes in a dose- and time-dependent quadratic manner in response to trauma exposure, with initial elevated and subsequent chronically attenuated cortisol, independent of whether exposed individuals develop PTSD. This nonlinear association between trauma exposure and cortisol output may, in part, mediate the repeatedly reported dose-response association between increasing trauma load and increasing PTSD risk (but see also Kessler et al., 2017, for more recent findings on PTSD risk depending on type of previous trauma in combination with lifetime psychiatric history prior to index trauma). Notably, cross-sectional studies that compare trauma-exposed individuals with and without PTSD, including studies that assess HCC, typically report higher trauma exposure in individuals with PTSD. Although this fits with the earlier-mentioned dose-response relationship between trauma load and PTSD risk, it may confound cross-sectional investigations. Interestingly, our trauma-exposed police officers without PTSD reported nominally higher work-related trauma exposure than police officers with PTSD. Nevertheless, we still observed significantly lower HCC in participants with PTSD, which remained significant after controlling for work-related trauma exposure. This suggests that in our noncivilian female sample, PTSD was associated with lower HCC independent of the effects of accumulating trauma exposure. As our female police officers with PTSD reported lower work-related trauma exposure than their matched TCs, it is conceivable that they were more vulnerable to adverse mental health consequences of traumatic stress. Although our study had no longitudinal design, this fits with previously observed low pretrauma HCC (Steudte-Schmiedgen et al., 2015) and high pretrauma glucocorticoid receptor function (van Zuiden et al., 2012) as predictive of PTSD symptom development in male soldiers. However, we only investigated the amount of work-related traumatic event types participants had encountered and not time

since exposure. This is relevant as the model proposed by Steudte-Schmiedgen et al. (2016) describes elevated cortisol output in the acute period after trauma, prior to attenuated output. Additionally, the authors of a recent meta-analysis found significant positive associations between ongoing chronic stress and HCC (Stalder et al., 2017). Thus, an alternative explanation is that our observed group difference reflects relatively elevated HCC in TCs due to ongoing or more recent exposure to work-related traumatic stress rather than of a PTSD-related attenuation. However, as the difference in HCC remained significant after controlling for current active police duty, we deem this alternative explanation less plausible. Additionally, the mean HCC observed in our TC group was comparable to the mean HCC reported in a mixed-gender sample of TCs with little trauma exposure within the last year (Steudte et al., 2011).

Long-term HCC is thought to be a relatively stable and reliable measure of long-term cortisol output of the HPA axis (Staufenbiel et al., 2013). Therefore, the most intuitive interpretation is that our finding supports repeated findings on more acute measures of HPA axis function, indicating PTSD is associated with dysregulation of the HPA axis (Olff & van Zuiden, 2017). As previous studies have reported that HPA axis dysregulation may precede trauma exposure and PTSD development (van Zuiden, Kavelaars, Geuze, Olff, & Heijnen, 2013), chronic HPA axis dysregulation may be causally involved in pathophysiological processes underlying phenotypical expression and maintenance of some PTSD symptoms. Nevertheless, observed associations between HCC and PTSD status could also be influenced by PTSD risk factors associated with altered cortisol output, such as pain (Gaab et al., 2005), or health behaviors commonly associated with PTSD or general psychopathology, such as tobacco use (Olff et al., 2006) and decreased physical activity (Fekedulegn et al., 2018). However, we applied stringent inclusion and exclusion criteria and added several health behavior–related covariates to address potential confounders.

To further investigate whether and how HCC may be associated with the phenotypical expression of PTSD, we performed exploratory analyses to investigate whether HCC was associated with previously observed neural correlates of PTSD. We focused on amygdala reactivity to two types of negative affective stimuli—nontrauma related stimuli (faces) and a mixture of trauma-related and non-trauma-related stimuli (pictures)—as authors of a recent meta-analysis found higher bilateral amygdala reactivity in individuals with PTSD compared to TCs in response specifically to non-trauma-related stimuli (Stark et al., 2015). To facilitate comparison of results for both types of stimuli, we subtracted amygdala reactivity toward the neutral conditions from reactivity toward the negative conditions. Within participants

with PTSD, but not in TCs, we observed a moderate-to-strong positive correlation between HCC and right amygdala reactivity to negative affective faces compared to neutral or positive faces. Furthermore, after correction for the time of day the scanning took place, a significant positive correlation between HCC and left amygdala reactivity to negative faces emerged for TCs, and a moderate positive correlation was observed for participants with PTSD, although this was not significant. We also observed a moderate-to-strong positive correlation between HCC and left amygdala reactivity to negative affective pictures compared to neutral pictures in participants with PTSD. Thus, lower HCC, in itself associated with PTSD status, was associated with diminished differentiation in amygdala reactivity between negative and neutral affective stimuli. This association was most pronounced for participants with PTSD.

If the peripheral findings related to HCC reflect persistent changes in circulating central cortisol, speculatively, the observed association between HCC and diminished differentiation in amygdala reactivity may be influenced by long-term compensatory changes in central GR and/or MR signaling pathways, such as upregulated receptor expression and binding affinity or changes at the signaling route downstream of the receptor. Such changes may have occurred in the amygdala or other brain areas that modulate amygdala reactivity, changing amygdala reactivity to perceived negative stimuli. On the other hand, decreased HCC may result from high signaling in these receptor pathways, leading to reduced cortisol output by the HPA axis (Buckingham, 2006; De Bosscher, Van Craenenbroeck, Meijer, & Haegeman, 2008; de Quervain et al., 2017). Based on the current literature on GR and MR function in PTSD, neither of these two directions can be excluded.

In our larger study, we observed that, in contrast to TCs, police officers with PTSD did not show differentiation in amygdala reactivity to negative versus positive or neutral faces (Koch et al., 2016b). We hypothesized that participants with PTSD may have interpreted neutral faces in the neutral/positive faces condition as ambiguous stimuli signaling potential threat (i.e., increased attributed saliency), resulting in decreased differentiation in amygdala reactivity toward the two affective conditions. Such a lack of differentiation between negative and neutral stimuli may result from deficits in context processing (i.e., a diminished capacity to interpret the environment in a situation-specific manner). A recent model addresses diminished context processing by hippocampal–prefrontal–thalamic brain circuitry modulating amygdala reactivity as key in the pathophysiology of PTSD and specifically intrusive symptomatology (Liberzon & Abelson, 2016). This brain circuitry is critically modulated by GR activation, with GRs mediating effects

on contextual learning and memory consolidation depending on activation of beta-adrenergic receptors in the basolateral amygdala (Quirarte, Roozendaal, & McGaugh, 1997). Clearly, although we observed that activation clusters for the negative pictures task extended posteriorly from the amygdala toward the hippocampus (Koch et al., 2018), the hypothesis that our observed correlation between HCC and decreased amygdala differentiation between negative and neutral emotional stimuli could be associated with context processing should be further investigated.

This study was the first, to our knowledge, to investigate associations between HCC and PTSD in a female noncivilian trauma-exposed sample, but it had some important limitations. First, the study had a cross-sectional design, and therefore, we cannot address questions of causality and directionality. In theory, relatively long hair strands provide the possibility to retrospectively assess HCC and therefore investigate HCC changes over time in relation to trauma and PTSD development. However, this seems more relevant when the demarcation of a single traumatic event in time is more apparent than it was in our sample. Also, the small sample size resulted in modest statistical power. Based on Bender and Lange's (2001) recommendation that multiple comparison corrections should not be applied for studies of an exploratory nature, we opted not to apply corrections for multiple testing in the correlational analyses. It should however be stressed that hypotheses derived from our exploratory correlational analyses warrant future confirmatory studies with adequate statistical power. Also, although affective faces are generally designated as non-trauma-related, the pictures task contained a mixture of non-trauma-related and likely trauma-related stimuli that we could not further subdivide into separate conditions. Therefore, it remains to be investigated whether observed associations are specific to non- trauma-related stimuli or also hold for trauma-related stimuli. Additionally, as we only included female police officers willing to participate in our pharmacological neuroimaging study, we cannot be certain that our findings generalize to the larger population of female police officers. Additionally, it remains to be investigated whether observed findings also extend to men. Furthermore, as mentioned, frequency and time since trauma exposure were not investigated nor was non-work-related trauma exposure during adulthood. We were also not able to include a trauma-naive control group with similar demographic and work-related characteristics as the included trauma-exposed participants, as active police service for several years is generally associated with exposure to traumatic events in the line of duty. Together, these limitations precluded more detailed analyses of associations and directionality of causation.

In summary, we observed lower long-term hair cortisol levels in female police officers with PTSD compared to trauma-exposed female police officers without PTSD. Exploratory analyses indicated that lower HCC was associated with lower differentiation of amygdala reactivity between negative and neutral affective stimuli, which was more pronounced in participants with PTSD. Future studies should further investigate the associations between HCC, trauma, and PTSD, as well as associated neurobiological mechanisms.

References

Ashburner, J., Barnes, G., Chen, C., Daunizeau, J., Flandin, G., Friston, K., . . . Philips, C. (2013). *SPM8 Manual*. London, UK: Welcome Trust Centre for Neuroimaging.

Bender, R., & Lange, S. (2001). Adjusting for multiple testing–when and how? *Journal of Clinical Epidemiology*, 54, 343–349.

Blake, D. D., Weathers, F. W., Nagy, L. M., Kaloupek, D. G., Gusman, F. D., Charney, D. S., & Keane, T. M. (1995). The development of a Clinician-Administered PTSD Scale. *Journal of Traumatic Stress*, 8, 75.

Bremner, J. D., Bolus, R., & Mayer, E. A. (2007). Psychometric properties of the Early Trauma Inventory-Self Report. *The Journal of Nervous and Mental Disease*, 195, 211–218.

Buckingham, J. C. (2006). Glucocorticoids: Exemplars of multi-tasking. *British Journal of Pharmacology*, 147, S258–268.

Bush, K. (1998). The AUDIT Alcohol Consumption Questions (AUDIT-C): An effective brief screening test for problem drinking. *Archives of Internal Medicine*, 158, 1789.

Carlier, I. V., & Gersons, B. P. (1992). Development of a scale for traumatic incidents in police officers. *Psychiatria Fennica*, 23, 59.

Cohen, J. (1977). *Statistical power analysis for the behavioral sciences*. New York City, NY: Routledge.

De Bosscher, K., Van Craenenbroeck, K., Meijer, O. C., & Haegeman, G. (2008). Selective transrepression versus transactivation mechanisms by glucocorticoid receptor modulators in stress and immune systems. *European* Journal Pharmacology, 583, 290–302.

de Quervain, D., Schwabe, L., & Roozendaal, B. (2017). Stress, glucocorticoids and memory: Implications for treating fear-related disorders. Nature reviews.Neuroscience, 18, 7–19.

Diedenhofen, B., & Musch, J. (2015). Cocor: A comprehensive solution for the statistical comparison of correlations. *PloS one*, 10, e0121945.

Fekedulegn, D., Innes, K., Andrew, M. E., Tinney-Zara, C., Charles, L. E., Allison, P., . . . Knox, S. S. (2018). Sleep quality and the cortisol awakening response (CAR) among law enforcement officers: The moderating role of leisure time physical activity. *Psychoneuroendocrinology*, 95, 158–169.

First, M. B., Spitzer, R. L., Gibbon, M., & Williams, J. B. (2002). *Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), Clinician version, aministration booklet*. Washington, DC: American Psychiatric Publishing Inc.

Gaab, J., Baumann, S., Budnoik, A., Gmunder, H., Hottinger, N., & Ehlert, U. (2005). Reduced reactivity and enhanced negative feedback sensitivity of the hypothalamus-pituitary-adrenal axis in chronic whiplash-associated disorder. *Pain*, 119, 219–224.

Hariri, A. R., Tessitore, A., Mattay, V. S., Fera, F., & Weinberger, D. R. (2002). The amygdala response to emotional stimuli: A comparison of faces and scenes. *Neuroimage*, 17, 317–323.

Hayes, J. P., Hayes, S. M., & Mikedis, A. M. (2012). Quantitative meta-analysis of neural activity in posttraumatic stress disorder. *Biology of Mood & Anxiety Disorders*, 2, 9.

Hovens, J. E., van der Ploeg, H. M., Klaarenbeek, M. T., Bramsen, I., Schreuder, J. N., & Rivero, V. V. (1994). The assessment of posttraumatic stress disorder with the Clinician Administered PTSD Scale: Dutch results. *Journal of Clinical Psychology*, 50, 325–340.

Kessler, R. C., Aguilar-Gaxiola, S., Alonso, J., Bromet, E. J., Gureje, O., Karam, E. G., . . . Zaslavsky, A. M. (2017). The associations of earlier trauma exposures and history of mental disorders with PTSD after subsequent traumas. *Molecular Psychiatry*, 23(9), 1–8.

Koch, S. B., van Zuiden, M., Nawijn, L., Frijling, J. L., Veltman, D. J., & Olff, M. (2016a). Aberrant resting-state brain activity in posttraumatic stress disorder: A meta-analysis and systematic review. *Depression & Anxiety*, 33, 592–605.

Koch, S. B., van Zuiden, M., Nawijn, L., Frijling, J. L., Veltman, D. J., & Olff, M. (2016b). Intranasal oxytocin administration dampens amygdala reactivity towards emotional faces in male and female PTSD patients. *Neuropsychopharmacology*, 41, 1495–1504.

Koch, S. B., van Zuiden, M., Nawijn, L., Frijling, J. L., Veltman, D. J., & Olff, M. (2018). Effects of intranasal oxytocin on distraction as emotion regulation strategy in patients with posttraumatic stress disorder (Advance online publication). *European Neuropsychopharmacology*.

Lang, P. J., Bradley, M. M., & Cuthbert, B. N. (2008). *International affective picture system (IAPS): Affective ratings of pictures and instruction manual (A-8)*. Gainesville University of Florida.

Liberzon, I., & Abelson, J. L. (2016). Context processing and the neurobiology of posttraumatic stress disorder. *Neuron*, 92, 14.

Lobbestael, J., Leurgans, M., & Arntz, A. (2011). Inter-rater reliability of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID I) and Axis II Disorders (SCID II). *Clinical Psychology and Psychotherapy*, 18, 75–79.

Luo, H., Hu, X., Liu, X., Ma, X., Guo, W., Qiu, C., . . . Li, T. (2012). Hair cortisol level as a biomarker for altered hypothalamic-pituitary-adrenal activity in female adolescents with posttraumatic stress disorder after the 2008 wenchuan earthquake. *Biological Psychiatry*, 72, 65–69.

Manenschijn, L., Koper, J. W., Lamberts, S. W., & van Rossum, E. F. (2011). Evaluation of a method to measure long term cortisol levels. *Steroids*, 76, 1032–1-36.

McRae, K., Hughes, B., Chopra, S., Gabrieli, J. D., Gross, J. J., & Ochsner, K. N. (2010). The neural bases of distraction and reappraisal. *Journal of Cognitive Neuroscience*, 22, 248–262.

Meewisse, M. L., Reitsma, J. B., de Vries, G. J., Gersons, B. P., & Olff, M. (2007). Cortisol and posttraumatic stress disorder in adults: Systematic review and meta-analysis. *The British Journal of Psychiatry*, 191, 387–392.

Mewes, R., Reich, H., Skoluda, N., Seele, F., & Nater, U. M. (2017). Elevated hair cortisol concentrations in recently fled asylum seekers in comparison to permanently settled immigrants and non-immigrants. *Transl Psychiatry*, 7, e1051.

Olejnik, S., & Algina, J. (2003). Generalized eta and omega squared statistics: Measures of effect size for some common research designs. *Psychological Methods*, 8, 434–447.

Olff, M., Meewisse, M. L., Kleber, R. J., van der Velden, P. G., Drogendijk, A. N., van Amsterdam, J. G., . . . Gersons, B. P. (2006). Tobacco usage interacts with postdisaster psychopathology on circadian salivary cortisol. *International Journal of Psychophysiology*, 59, 251–258.

Olff, M., & van Zuiden, M. (2017). Neuroendocrine and neuroimmune markers in PTSD: Pre, peri- and posttrauma glucocorticoid and in- flammatory dysregulation. *Current Opinion in Psychology*, 14, 132–137.

Pacella, M. L., Hruska, B., Steudte-Schmiedgen, S., George, R. L., & Delahanty, D. L. (2017). The utility of hair cortisol concentrations in the prediction of PTSD symptoms following traumatic physical injury. *Social Science & Medicine*, 175, 228–234.

Patel, R., Spreng, R. N., Shin, L. M., & Girard, T. A. (2012). Neurocircuitry models of posttraumatic stress disorder and beyond: A meta-analysis of functional neuroimaging studies. *Neuroscience and Biobehavioral Reviews*, 36, 2130–2142.

Quirarte, G. L., Roozendaal, B., & McGaugh, J. L. (1997). Glucocorticoid enhancement of memory storage involves noradrenergic activation in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, 94, 14048.

Rademaker, A. R., Vermetten, E., Geuze, E., Muilwijk, A., & Kleber, R. J. (2008). Self-reported early trauma as a predictor of adult personality: A study in a military sample. *Journal of Clinical Psychology*, 64, 863–875.

Russell, E., Kirschbaum, C., Laudenslager, M. L., Stalder, T., de Rijke, Y., van Rossum, E. F., . . . Koren, G. (2015). Toward standardization of hair cortisol measurement: Results of the first international interlaboratory round robin. *Therapeutic Drug Monitoring*, 37, 71–75.

Sartory, G., Cwik, J., Knuppertz, H., Schurholt, B., Lebens, M., Seitz, R. J., & Schulze, R. (2013). In search of the trauma memory: A meta-analysis of functional neuroimaging studies of symptom provocation in posttraumatic stress disorder (PTSD). *PloS one*, 8, e58150.

Schalinski, I., Elbert, T., Steudte-Schmiedgen, S., & Kirschbaum, C. (2015). The cortisol paradox of trauma-related disorders: Lower phasic responses but higher tonic levels of cortisol are associated with sexual abuse in childhood. *PloS one*, 10, e0136921.

Sheehan, D. V., Lecrubier, Y., Sheehan, K. H., Amorim, P., Janavs, J., Weiller, E., . . . Dunbar, G. C. (1998). The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *The Journal of Clinical Psychiatry*, 59(Suppl 20) 22.

Stalder, T., Steudte-Schmiedgen, S., Alexander, N., Klucken, T., Vater, A., Wichmann, S., . . . Miller, R. (2017). Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. *Psychoneuroendocrinology*, 77, 261–274.

Stark, E. A., Parsons, C. E., Van Hartevelt, T. J., Charquero-Ballester, M., McManners, H., Ehlers, A., . . . Kringelbach, M. L. (2015). Posttraumatic stress influences the brain even in the absence of symptoms: A systematic, quantitative meta-analysis of neuroimaging studies. *Neuroscience and Biobehavioral Reviews*, 56, 207–221.

Staufenbiel, S. M., Penninx, B. W., Spijker, A. T., Elzinga, B. M., & van Rossum, E. F. (2013). Hair cortisol, stress exposure, and mental health in humans: A systematic review. *Psychoneuroendocrinology*, 38, 1220–1235.

Steudte, S., Kirschbaum, C., Gao, W., Alexander, N., Schonfeld, S., Hoyer, J., & Stalder, T. (2013). Hair cortisol as a biomarker of traumatization in healthy individuals and posttraumatic stress disorder patients. *Biological Psychiatry*, 74, 639–646.

Steudte, S., Kolassa, I. T., Stalder, T., Pfeiffer, A., Kirschbaum, C., & Elbert, T. (2011). Increased cortisol concentrations in hair of severely traumatized Ugandan individuals with PTSD. *Psychoneuroendocrinology*, 36, 1193– 1200.

Steudte-Schmiedgen, S., Kirschbaum, C., Alexander, N., & Stalder, T. (2016). An integrative model linking traumatization, cortisol dysregulation and posttraumatic stress disorder: Insight from recent hair cortisol findings. *Neuroscience and Biobehavioral Reviews*, 69, 124–135.

Steudte-Schmiedgen, S., Stalder, T., Schonfeld, S., Wittchen, H. U., Trautmann, S., Alexander, N., . . . Kirschbaum, C. (2015). Hair corti- sol concentrations and cortisol stress reactivity predict PTSD symptom increase after trauma exposure during military deployment. *Psychoneuroendocrinology*, 59, 123–133.

van Zuiden, M., Geuze, E., Willemen, H. L., Vermetten, E., Maas, M., Amarouchi, K., . . . Heijnen, C. J. (2012). Glucocorticoid receptor pathway components predict posttraumatic stress disorder symptom development: A prospective study. *Biological Psychiatry*, 71, 309–316.

van Zuiden, M., Kavelaars, A., Geuze, E., Olff, M., & Heijnen, C. J. (2013). Predicting PTSD: Pre-existing vulnerabilities in glucocorticoid-signaling and implications for preventive interventions. *Brain Behavior and Immunity*, 30, 12–21.

Weathers, F. W., Ruscio, A. M., & Keane, T. M. (1999). Psychometric properties of nine scoring rules for the clinician-administered posttraumatic stress disorder scale. *Psychological Assessment*, 11, 124–133.

Chapter 14

General Discussion

General Discussion

This thesis contains our work in which we have focused on the role of endogenous and exogenous glucocorticoids in obesity and stress-related diseases. In this chapter, we summarize our findings and discuss these in the context of existing literature and their clinical and societal relevance. We will conclude with future perspectives regarding screening and treatment of obesity especially in light of glucocorticoid use and elevated glucocorticoid exposure.

1. Obesity

The impact of obesity on health, society, and economics is becoming increasingly evident with the rising number of patients over time. More than a third of the adult population in the United States is currently obese [(1)] which is accompanied by an estimated annual cost of over 200 billion dollars [(2)]. The prevalence rate in the Netherlands is more favorable with one in six adult individuals being obese, however, numbers have been nearly doubled within one generation time [(3)]. Unhealthy dietary habits and less physical activity are generally considered as the culprits. Tackling obesity should, in theory, be relatively simple by reversing these factors, but unfortunately, the results of many attempts are not in keeping with what would be expected. The complexity of obesity originates in, among others, its multifactorial etiology and biological resistance to weight loss. Scientific observations have established various obesogenic factors other than lifestyle which are assumed to induce weight gain and/or impede weight loss. Glucocorticoids, with the stress hormone cortisol as the predominant hormone, are potentially important determinants in this context.

2. Glucocorticoids and obesity

Stress and obesity are generally linked to adverse health consequences, however, the link between these entities is somewhat less clear. This is in part due to differential effects of acute and chronic stress. Short-term overactivation of the hypothalamus-pituitary-adrenal (HPA) axis and thus temporarily increased cortisol secretion is in fact beneficial. Acute stress with for instance public speaking or exercising increases alertness, suppresses appetite, and tightens the muscle to prepare for a fight or flight reaction. The adverse effects of stress become evident in case of ongoing stressors in which the HPA axis is chronically overactive and the stress hormone cortisol is constantly flooding into the circulation. There is no certain cutoff regarding the duration in terms of chronic stress, but it would be anticipated in certain conditions as low socioeconomic state, chronic diseases and pain, and ongoing psychological distress. Chronic stress negatively impacts the architecture and function of the brain, muscles, bone, reproduction organs, and so on. With respect to metabolism, cortisol induces insulin resistance, glycolysis, and

gluconeogenesis which eventually leads to impaired glucose tolerance. Elevated long-term cortisol exposure additionally leads to an increased appetite [4] with a special preference for high-caloric foods. Moreover, it stimulates accumulation of fat cells in the visceral area and inhibits the favorable thermogenic activity of brown adipose tissue [5]. This may eventually lead to central adiposity which, independent of other glucocorticoid-related metabolic events, is a harbinger of metabolic syndrome. The latter constitutes a combination of several risk factors (i.e. visceral obesity, high blood pressure, high triglycerides, low HDL-cholesterol, and glucose intolerance) which increases the risk of developing diabetes mellitus and serious cardiovascular conditions like stroke and myocardial infarction.

Since many individuals with a hypercortisolistic state develop (centripetal) obesity and other cardiometabolic conditions as frequently observed in common obesity, it was soon suggested that the latter might be a consequence of too much cortisol. As mentioned in the general introduction, it was indeed found that individuals with obesity had higher cortisol concentrations in comparison to non-obese subjects. In **chapter 2** we investigated in a population of 408 individuals with obesity the prevalence of a variety of obesogenic factors. We found that nearly half of the study population was using a potentially weight-inducing drug with corticosteroids as the most commonly used agent. Over one in four was using any type of corticosteroids at time of visit clinic. Interestingly, among subjects who had experienced a period of marked weight gain drug use and especially use of corticosteroids was reported as the most common trigger. In **chapter 3** we focused on corticosteroid use in the past three months and assessed the differences in use between our outpatient population with obesity and non-obese controls from two other Dutch cohorts. Here, we also found that corticosteroids were significantly more often used in obesity with nearly doubled prevalence in comparison to non-obese subjects. The most striking was the relatively higher use of local corticosteroids and especially of inhaled administration forms with obesity (13.9% versus 3.0% and 3.8% in non-obese cohorts).

Glucocorticoid use during weight loss attempt

A variety of strategies are available that individuals can try to lose weight, however, lifestyle adaptations including a healthy diet and sufficient physical activity are essential for sustainable weight loss. Before commencing a weight-loss intervention, a thorough evaluation of obesogenic factors as described in **chapter 2** is necessary to recognize and, if possible, to tackle those which could hamper weight loss. Participants with obesity who participated in our 75-week combined lifestyle intervention with cognitive behavioral therapy were hence at start and during each evaluation moment evaluated for among others use of corticosteroids.

Nearly one in every fifth subject had however used systemic corticosteroids during the intervention despite specific instructions to both the participants and their general practitioners to avoid these unless absolutely necessary. There were overall great improvements in anthropometrics which were also sustained till the end of the intervention, however, weight change was less beneficial in users of systemic corticosteroids **(chapter 8**). This emphasizes the need for careful consideration of corticosteroid use in individuals with obesity and especially in those who are engaging in a weight loss intervention. Unfortunately, there is currently a lack of high-quality randomized controlled trials (RCTs) that have investigated interventions to remedy weight gain in patients in whom systemic corticosteroid use is inevitable. A recent systematic review focused on this topic and could identify only three RCTs with overall poor quality, small sample size, and just one study with adult participants [6]. This concerned a trial in which 23 overweight female patients with systemic lupus erythematosus and on treatment with systemic corticosteroids were assigned to a six-week diet-intervention aimed at a low glycaemic index or calorie restriction [7]. Both interventions led to significant and comparable improvements in weight, waist- and hip circumferences. Given the short-term intervention and no follow-up assessments, it is of concern whether patients can sustain these beneficial changes in the long-term if guidance is no longer offered and due to compensatory biological mechanisms (e.g. increased appetite, decreased resting energy expenditure) as frequently observed after (intense) dietary interventions [8,9].

Consequences of local corticosteroid use

It is often acknowledged that serious adverse events with corticosteroid use are limited to systemic corticosteroids as prednisolone or dexamethasone. Broersen *et al.* showed in an extensive meta-analysis that use of systemic forms was associated with around 50% risk of developing adrenal insufficiency (10), a consequence of the negative feedback system which occurs in case of prolonged supraphysiological exposure to glucocorticoids. In the same study, the authors also showed that local corticosteroids as topical, nasal, and inhaled types can also induce adrenal insufficiency. This risk was especially prominent in users of inhaled corticosteroids (absolute risk of 7.8% [95% CI, 4.2 – 13.9]) with differing percentages based on duration and dosage of corticosteroid use [10]. These inhaled types are usually prescribed for pulmonary conditions like asthma and chronic obstructive pulmonary disease. The prevalence of adrenal insufficiency in asthmatic patients using only inhaled corticosteroids ranged for example from 1.5% with low doses to 18.5% with high doses, and 1.3% in case of short-term duration to 20.3% in long-term users (10). Within our cohort of individuals with obesity, asthma was also the most prominent indication for corticosteroid use (**chapter 3**).

There are nowadays several inhaled corticosteroids available with various pharmacokinetic and pharmacodynamic properties. These agents can enter the systemic circulation by at least two different pathways (Figure 1). In general, depending on the type of inhaled corticosteroid, a large proportion of the inhaled agent is deposited in the oropharyngeal area. This bulk is transported to the gastrointestinal tract and absorbed from there to subsequently be exposed to the first-pass metabolism by the liver. On the other hand, a fraction of the inhaled dose will be deposited in the lung and completely absorbed into the circulation. The oral bioavailability after liver passage and the pulmonary bioavailability make up the portion of the inhaled agent what eventually gets absorbed in the systemic circulation and thus could potentially induce serious glucocorticoid-related adverse event (Figure 1).

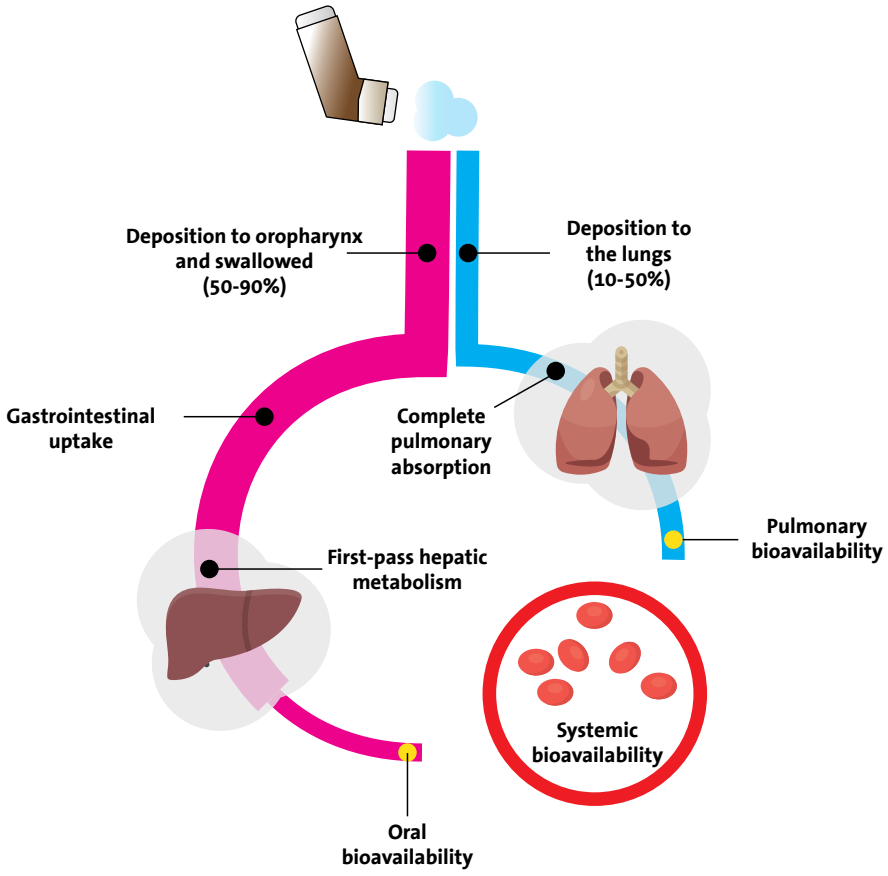

Figure 1: Pathways to systemic uptake of inhaled corticosteroids.

Adapted from Derendorf et al. [11].

This of course raises the question of whether the use of inhaled corticosteroids does indeed lead to systemic adverse events. A convenient way to assess this is by reviewing the incidence of adverse events similar to the clinical phenotype as seen in patients with endogenous Cushing's syndrome. A panel of experts from three medical associations (American College of Chest Physicians, American Academy of Allergy, Asthma, and Immunology, and American College of Allergy, Asthma, and Immunology) reviewed literature up to the year 2000 to assess evidence for five serious glucocorticoid-related adverse events with inhaled corticosteroid use in patients with asthma [12] (Table 01). They found that various studies indeed observed evident adverse outcomes with use of inhaled corticosteroids, but results were not consistent for the different complications which could be due to differences in for instance study design, assessment of specific outcomes, or other methodological flaws. It is not clear why the panel chose to investigate these specific complications especially giving the fact that lipodystrophy and weight gain have repeatedly been reported as one of the most common adverse events with systemic corticosteroid use [13,14]. There is currently a paucity of studies investigating the link between adiposity and cardiometabolic outcomes with inhaled corticosteroids or with any of the other frequently prescribed local administration forms.

Table 1: Evidence for glucocorticoid-related complications in asthmatic patients using inhaled corticosteroids.

#	Serious adverse event	Level of evidence	Conclusions
1	Growth retardation	A	Therapy with ICSs is associated with a decrease in short-term growth rates in children, but the overall effect is small and may not be sustained with long-term therapy.
2	Skin thinning / ease of bruising	B	The risk of skin thinning and easy bruising is elevated in patients receiving ICS. Dose, duration of use, and patient gender are important variables affecting overall risk.
3	Reduced bone mineral density	C	Adult asthma patients generally do not sustain a significant reduction in BMD in response to ICS treatment, although the effect may become clinically important in patients receiving high-dose ICSs for many years.
4	Cataracts	C	The risk of subcapsular and nuclear cataracts associated with ICS use is negligible in young asthma patients, however, it may be elevated in older patients.
5	Glaucoma	F	The risk of glaucoma associated with ICS use is likely to be small, however, further study is warranted.

Data derived from Leone *et al.* [12]. The grading of evidence (level A - F) was designed for the specific study with grade A-C indicating sufficient data for evaluation of the relationship between inhaled corticosteroids and complications. Grade D indicates insufficient or conflicting data and grade F denotes failed evidence review. Abbreviation: *BMD*, bone mineral density; *ICS*, inhaled corticosteroids

In a population-based study including 140.879 adult individuals, we investigated the association between inhaled corticosteroids and other administration forms with various cardiometabolic traits including body mass index and waist circumference (**chapter 4**). We additionally studied the relationship between local and systemic corticosteroid use with metabolic syndrome which is deemed present in case of several predefined cardiometabolic risk factors (i.e. abdominal obesity, hypertension, dyslipidemia, and hyperglycemia). The relevance of metabolic syndrome in this case is that glucocorticoids are being linked to all of the listed risk conditions [15,16]. Moreover, the health consequences of metabolic syndrome are far-reaching as patients have an increased risk of developing type 2 diabetes mellitus and cardiovascular diseases, including coronary heart diseases and stroke [17-19]. We found that use of locally applied corticosteroids was associated with an increased waist circumference in male and female users whereas women also had significantly higher body mass index. These associations were mainly with use of inhaled corticosteroids. Users of inhaled agents had significantly higher waist circumference as well as a higher body mass index in comparison to nonusers in both sexes. Metabolic syndrome was also more common in corticosteroid users when compared to nonusers. Interestingly, the relative difference in prevalence in metabolic syndrome between nonusers and overall corticosteroid users was nearly doubled in women in comparison to men (+5.3% vs. +2.7%). After full adjustments for potential covariates, only female corticosteroid users were more likely to have metabolic syndrome and especially in users of systemic and inhaled corticosteroids. A number of hypotheses could be proposed for this sexual dimorphism, with the most obvious that women are more adherent to medication than men. Sundberg *et al.* observed that among asthmatic patients women indeed reported higher compliance and had a more positive attitude concerning drug use in comparison to men [20]. Female patients were also more likely to report anxiety and insomnia which are, interestingly, known adverse events of systemic glucocorticoids and thus possibly related to increased systemic glucocorticoid exposure. Other possible explanations to be considered are the differences in pharmacokinetics between sexes [21] and reduction in sex hormones with corticosteroid use which could yield different cardiometabolic effects in men and women [22,23]. Giving the fact that abdominal adiposity is a major driver of adverse metabolic changes [24], it could also be suggested that glucocorticoid-induced visceral fat accumulation becomes more prominent in female users since men naturally tend to have a higher waist circumference than women. Nevertheless, other studies investigating adverse events of corticosteroids also reported sex differences with women being more prone to lipodystrophy [25], reduced bone mineral density [26], and skin bruising [27].

The relevance of genetics and glucocorticoid receptor sensitivity

A crucial link in the trail of glucocorticoid action is the glucocorticoid receptor. When in the inactive state, the glucocorticoid receptor remains in the cytoplasm where it is being coupled to chaperone proteins. Binding with glucocorticoids activates the receptor upon which it releases from the protein-complex and translocates to the nucleus. Activated glucocorticoid receptors can eventually by a variety of pathways positively and negatively alter gene expression (28). It is generally assumed that an increased transactivational activity (i.e. upregulation of transcription) leads to the cardiometabolic traits as seen in patients with endogenous and exogenous Cushing's syndrome [29]. Downregulation of the transcription of certain genes like interleukin-2, interleukin-6, and tumor necrosis factor-α is known as transrepression and is the mode of action as preferred with therapeutic corticosteroid use [30,31]. Earlier studies have identified several functional polymorphisms of the glucocorticoid receptor and found these to be associated with an altered glucocorticoid sensitivity and accompanying clinical and biochemical features [32].

In continuation of our findings in chapter 4, we further focused on the association between corticosteroid use with cardiometabolic outcomes and metabolic syndrome by glucocorticoid receptor genotype (**chapter 5**). We performed a genome-wide association study in over 10.000 individuals of the same study population and extracted genotypes of four functional glucocorticoid receptor variants. Overall corticosteroid use was significantly associated with a higher body mass index, waist circumference, and metabolic syndrome in users with a wild-type or glucocorticoid hypersensitive genotype (Bcl*I* and/or N363S) but not in users harboring glucocorticoid resistant variants (ER22/23EK and/or 9β). These findings were especially evident in users of inhaled corticosteroids. With respect to waist circumference, however, inhaled type users of all three genotypes had a significantly worse outcome in comparison to nonusers. The findings in users of systemic corticosteroids were less consistent which is probably partly due to the relatively small numbers (i.e. 5.3% of the total group of users). Earlier studies investigating the link between systemic corticosteroid use and adverse events found however that carriers of hypersensitive variants indeed more often developed glucocorticoid-related adverse events. Children with acute lymphoblastic leukemia for example were more likely to develop hepatotoxicity, glucose abnormalities, and Cushingoid-like symptoms such as adiposity and hypertension [33,34] when treated with systemic corticosteroids. On the other hand, patients with hypersensitive polymorphisms also had a better therapeutic response to inhaled corticosteroids in asthma [35] or treatment with systemic agents in different clinical conditions [33,36-38]. In contrast, systemic corticosteroids seemed less effective in

children with glucocorticoid resistant variants treated for inflammatory bowel disease [39] or nephrotic syndrome [38]. These and our observations may indicate that glucocorticoid receptor genotype partially influences the effectiveness as well as the occurrence of adverse events such as adiposity, cardiometabolic traits, and metabolic syndrome with corticosteroid use. It remains however difficult to fully separate the individual effect of these functional variants since they are to different extents associated with changes in transactivation or transrepression [40,41] and given more recent clues about both pathways to be somewhat dependent on each other [42].

3. Complex interplay between glucocorticoids and obesity

Although the relationship between glucocorticoids and obesity seems straightforward, the truth is that not each individual with high glucocorticoid exposure will develop obesity and vice versa. Circulating free cortisol concentrations, whether endogenously secreted or exogenously administered, are of utmost importance in the extent of glucocorticoid action. With respect to biological response, synthetic glucocorticoids generally possess a much higher glucocorticoid activity than endogenous cortisol. In comparison to cortisol, prednisone and prednisolone have for instance four times higher glucocorticoid activity whereas dexamethasone is relatively 25 times more potent [43]. As to inhaled corticosteroids, we described in **chapter 4** that the synthetic compounds budesonide and fluticasone were predominantly used in our study population. The relative binding affinity to the glucocorticoid receptor of these corticosteroids is respectively 94 and 180 greater than cortisol [11]. As discussed previously, considerable research has been conducted on the link between functional glucocorticoid receptor polymorphisms and anthropometric features and body composition and found this indeed to be interrelated to varying degree. There are however also other factors that could influence glucocorticoid availability and sensitivity such as the concentration of corticosteroid-binding globulins, activity of 11β-hydroxysteroid dehydrogenases, number of glucocorticoid receptors, post-translational modification of the glucocorticoid receptor, activity of multidrug efflux pumps, and so on [44]**.** The significance of alterations in any of these elements in obesity is currently still matter for future research.

In the schematic overview of the relationships between glucocorticoids and obesity (Figure 2), it is important to also consider the presence of environmental and behavioral factors that could increase the secretion of glucocorticoids such as sleep deprivation [45], alcohol intake [46], and shift work [47]. Unfortunately, obesity per se is also often accompanied by various individual characteristics leading to a vicious circle of increased stress response, increased glucocorticoid action,

and eventually weight gain or difficulties in weight-loss attempts. Jackson *et al.* found for example that perceived weight stigma in obesity was associated with higher long-term cortisol concentrations than individuals with obesity who did not experience weight-discrimination [48]. Moreover, obesity is a considerable risk factor for the development of a variety of physical disorders (e.g. asthma, arthrosis, hypertension, and diabetes mellitus) which often require pharmacological interventions with potentially weight-inducing drugs like corticosteroids, beta blockers, and insulins.

4. Glucocorticoids and cerebral effects

In addition to anthropometric and cardiometabolic alterations, corticosteroids are also notorious for affecting the central nervous system. Glucocorticoid receptors are diffusely present in the brain and overexposure to glucocorticoids is associated with cognitive and neuropsychiatric disorders. Glucocorticoids are known for inducing neurotoxic effects and it was Varney and colleagues who invented the term "*steroid dementia*" after witnessing cognitive changes as with dementia in patients treated with high-dose corticosteroids [49]. Patients showed impairments in several cognitive processes including memory retention, attention, concentration, and mental speed and efficiency [49]. Studies in patients with endogenous Cushing's syndrome also revealed impaired memory and concentration [50] as well as lower IQ scores and worse performance in terms of processing of visual and spatial information, reasoning, and verbal fluency [51]. Imaging studies even showed loss of brain volume [52,53] which was partly reversible after correction of excess cortisol levels [53].

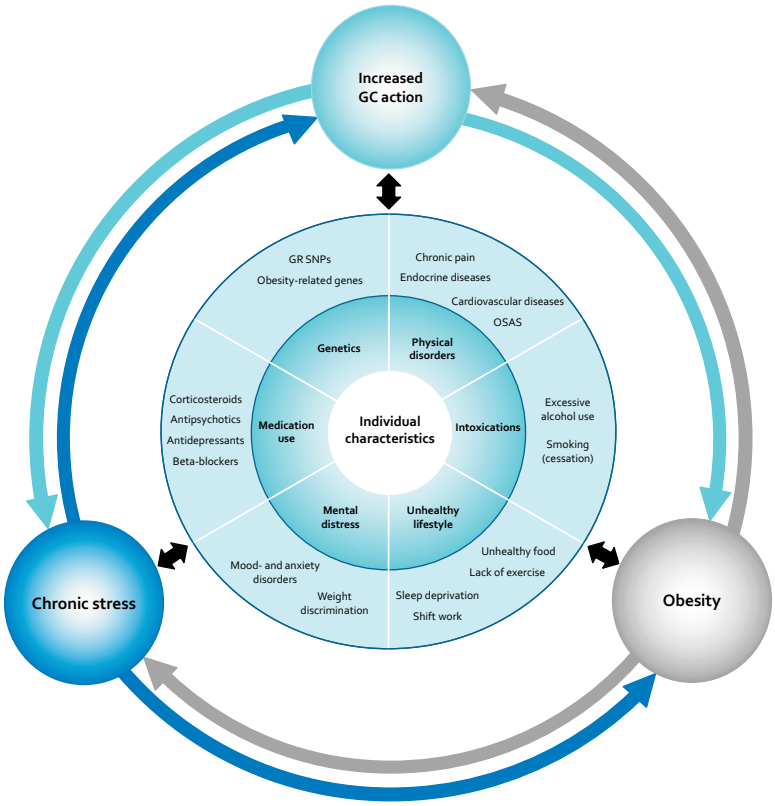

Figure 2: The interaction between the stress system and obesity.

Adapted from van der Valk, Savas, and van Rossum [54]. *GC*, glucocorticoids; *GR*, glucocorticoid receptor; *OSAS*, obstructive sleep apnea syndrome; *SNPs*, single-nucleotide polymorphisms.

Behavioral changes and psychological symptoms are unfortunately also often encountered with supraphysiological exposure to glucocorticoids. Insomnia, restlessness, euphoria, (hypo-)mania, depression, anxiety, and even psychoses are in the spectrum of steroid-related neuropsychiatric conditions [55]. Numbers in literature about the occurrence of these mental alterations differ. Fardet *et al.* prospectively followed a cohort of systemic corticosteroid naïve patients who were put on long-term prednisone [13]. Three months after initiation of therapy, just over half of the subjects reported insomnia and neuropsychiatric conditions (e.g. irritability, anxiety, depression, and euphoria) with some even being admitted to hospital due to mental disorder. In **chapter 6** we showed that in the general adult population overall use of corticosteroids was associated with reduced executive cognitive functioning as well as a higher likelihood of mood and anxiety disorder. Impairments in nonverbal fluency, which is part of executive cognitive functioning, were especially evident in systemic corticosteroid users. Significantly lower scores

were however also observed in users of inhaled types. Moreover, mood and anxiety disorders were also more likely to occur in users of local corticosteroids and again in especially inhaled corticosteroid users which altogether suggest systemic availability of these formulations. Mental distress and neuropsychiatric conditions like mood- and anxiety disorders are also more frequent in obesity, especially with abdominal adiposity [56], and are linked to increased chronic stress and cortisol levels. In short, the bidirectional relationship between the stress system and obesity is complex and depends on a wide range of individual characteristics. This emphasizes the importance of a personalized approach with a thorough evaluation of potential weight- and stress-inducing factors in individuals with obesity who are willing to lose weight.

5. Magnitude of corticosteroid use

In our cross-sectional analysis in chapter 4, we showed that nearly one in every nine individuals of the adult general population was using one or more corticosteroid agents. The main administration routes were local types (>95%) and in particular the inhaled, nasal, and topical formulations. The Dutch National Health Care institute keeps record of the annual consumer data of prescribed drugs in the Netherlands and makes it online available (www.gipdatabank.nl). According to their Drug Information System (Dutch: *Genees- en hulpmiddelen Informatie Project (GIP) databank*), there were at least 13 million corticosteroids prescribed in 2018 (most actual data). Interestingly, corticosteroids were mentioned 17 times among the top 100 drugs with most users in the same year in the Netherlands (Figure 3). In terms of total number of prescriptions, dermal corticosteroids were most often prescribed (~3.9 million times) followed by inhaled corticosteroids (~2.9 million prescriptions), nasal corticosteroids (~2.7 million prescriptions), and systemic corticosteroids (~2.7 million prescriptions).

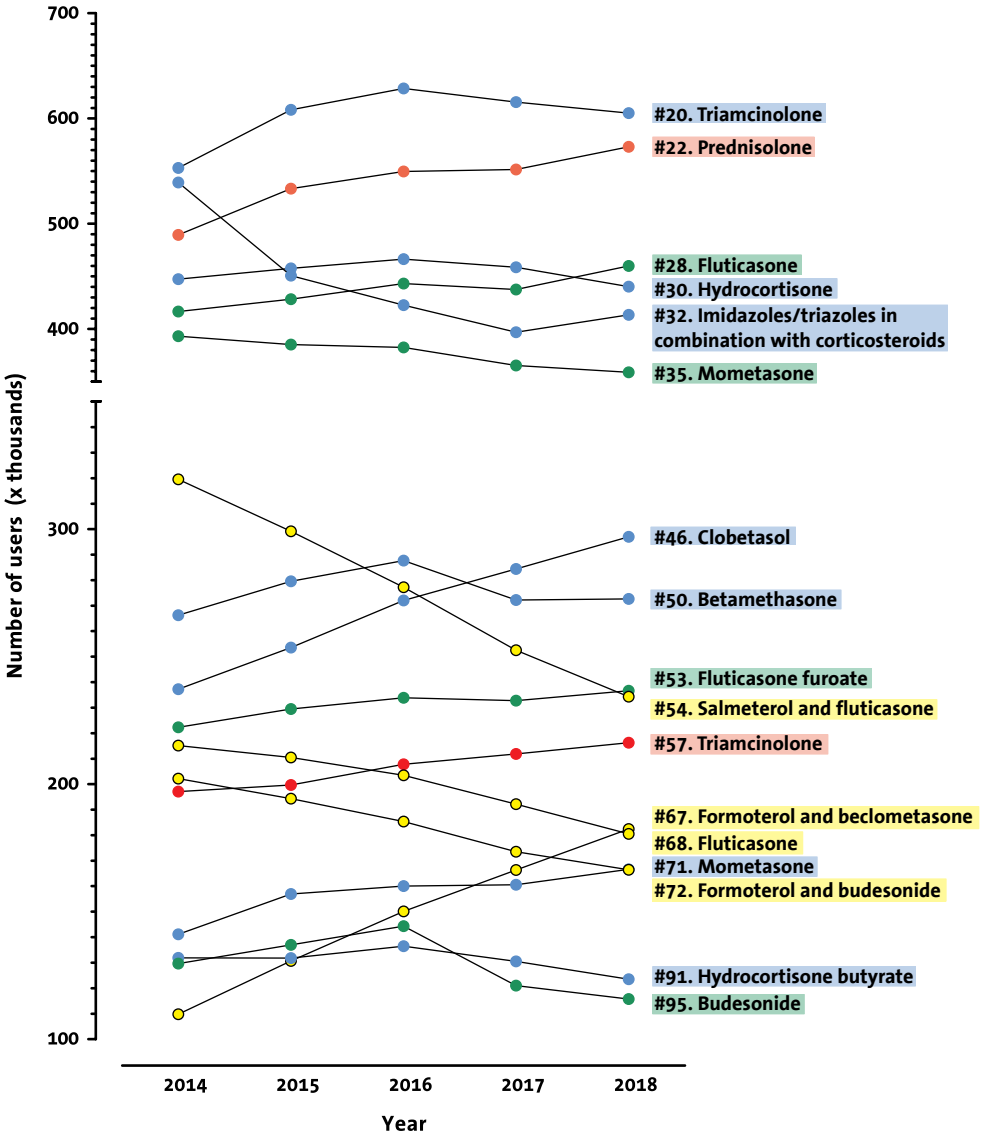

Figure 3: Corticosteroids with the most number of users in 2018 in the Netherlands with corresponding numbers in the past years.

Numbers before corticosteroid formulations depict their place in the top 100 drugs with most users in 2018. Colors illustrate the type of corticosteroid: *blue*, dermal; *red*, systemic; *green*, nasal; *yellow*, inhaled. Data derived from www.gipdatabank.nl.

6. Scalp hair glucocorticoids

Scalp hair glucocorticoids as screening tool

Most of the serious adverse events of too much glucocorticoids manifest after an extended period of time. The currently employed tests for assessment of cortisol

levels make use of blood, saliva, and urine. These matrices are only convenient in capturing short-term cortisol exposure ranging from seconds to minutes with serum and saliva and hours to days with urinary samples. Measurement of long-term cortisol exposure is however possible with scalp hair analysis. As hair growths with nearly one centimeter per month, it can be used to assess cumulative cortisol concentrations over the past months to years depending on the length of the collected sample. There has been a lot of development in recent decade towards the analytical methods and usefulness of hair scalp glucocorticoids. We are now able to quantify with high sensitivity and specificity cortisol and the inactive variant cortisone as well as several other steroid hormones in a single hair sample by using liquid chromatography-tandem mass spectrometry (LC-MS/MS) (57). Concerning endogenous Cushing's syndrome, current diagnostic guideline recommends screening with traditional matrices (58). In **chapter 7** we demonstrated in a multicenter, multinational, prospective, case-control study that hair cortisol and hair cortisone possess high diagnostic performances in screening for Cushing's syndrome. In terms of diagnostic efficacy, our findings were quite comparable to midnight salivary cortisol and urinary free cortisol as shown in pooled data from literature [59]. Interestingly, we found that hair cortisone was superior to hair cortisol in differentiating Cushing patients from controls. Further research is needed to determine whether scalp hair cortisone could serve as a better marker of accumulated cortisol exposure over time. In line with this hypothesis, however, a previous animal study in which guinea pigs were injected with radiolabeled cortisol showed a radioactive peak in hair analysis coinciding with the compound cortisone [60]. Moreover, in another study by Kapoor and colleagues it was also observed that a considerable amount of administered radiolabeled cortisol was taken up as cortisone in primate hair [61]. The authors suggested that perhaps the lower polarity of cortisone in comparison to cortisol could favor incorporation in hair matrix. Another explanation could perhaps lie in the conversion of cortisol to cortisone by the enzyme 11 beta-hydroxysteroid dehydrogenase type 2 which is among others located in hair follicles but also in salivary glands. This could play a role in our observation and also explain the relatively high concentration of cortisone found with hair and salivary assessments.

Besides the fact that scalp hair analysis allows evaluation of long-term glucocorticoid exposure, it also has advantages over other assessments in terms of collection, storage, and ease for patients. In the case of screening for Cushing's syndrome, hair analysis should just like the other cortisol tests be limited to individuals with a high a priori probability given the rarity of endogenous Cushing's syndrome and high prevalence of conditions associated with a (mild) hypercortisolistic state like obesity and diabetes mellitus. Another important

notion with the current scalp hair analysis is that quantification with LC-MS/MS requires expensive machinery and highly-skilled personnel limiting assessments to certain centers with sufficient capability. Furthermore, caution is required with assessments of timelines since concentrations seem to decrease across hair shaft [57] probably due to increased exposure to damaging factors (e.g. ultraviolet radiation, chemical agents) potentially leading to wash-out and/or cross-linking between hair matrix and glucocorticoids [62].

Scalp hair glucocorticoids and obesity

Since assessment of scalp hair glucocorticoids provides insight into relatively chronic glucocorticoid exposure, it has become an interesting tool to study whether common obesity is indeed associated with long-term increased cortisol levels. In our earlier study we investigated scalp hair cortisol in subjects with different weight classes and found that individuals with obesity had significantly higher levels (30.8 pg/mg) in comparison to others who were overweight (8.5 pg/mg) or normal weight (8.4 pg/mg) [63]. We additionally showed a positive association between hair cortisol concentrations and body mass index in a specific study population consisting of patients with erythropoietic protoporphyria (**chapter 10**). Concurring with our observations, a recent comprehensive meta-analysis demonstrated that an increase of 2.5 body mass index points was associated with 9.8% higher cortisol concentrations [64]. The same study also confirmed a positive association between scalp hair cortisol and other glucocorticoid-related features as higher waist-to-hip ratio and systolic blood pressure. Although cortisol and adiposity are mutually reinforcing, it is often unknown which of these factors precede. The causal role of cortisol in the development of visceral adiposity and adverse cardiometabolic features is clear giving the findings in patients with pathological concentrations like with Cushing's syndrome. On the other hand, there are also indications that weight gain per se can induce HPA axis activation. A small group of lean men for instance deliberately gained an average of 20% of their body weight by excessive intake of calories and were found to have an increased cortisol production rate and excretion of cortisol metabolites [65]. In **chapter 8** we further hooked on this by investigating whether weight loss in individuals with obesity altered long-term glucocorticoid concentrations in hair. Participants with obesity who completed our 75-week combined lifestyle intervention with cognitive behavioral therapy showed sustained weight loss as well as beneficial changes in waist circumference and body mass index and also lower cortisol concentrations at end of the intervention. Regarding the latter, alterations in HPA axis activity and/or cortisol metabolism including 11β-hydroxysteroid dehydrogenases would be expected. Although we did not assess cortisol metabolites, the peculiar course of cortisone during intervention with an initial rise and eventual decrease to baseline concentrations could suggest

alterations at the level of 11β-hydroxysteroid dehydrogenases especially giving the decrease in cortisol/cortisone-ratio over time. In line with this, earlier findings in bariatric patients losing weight after surgery showed indeed a lowered ratio of cortisol/cortisone-metabolites in urine [66] as well as a reduced expression of 11β-hydroxysteroid dehydrogenase type 1 in adipocytes [67,68]. Interestingly, in the same chapter, we did not observe a significant association between changes in anthropometrics (i.e. weight, body mass index, and waist circumference) and hair glucocorticoids. This could perhaps be explained by changes in other HPA-axis related factors which are additionally influenced by the intervention. It would be expected that the intervention consisting of guidance in diets and exercising with psychotherapy not only leads to weight loss but also less psychological distress, improvements in sleep pattern, and a healthier dietary composition which could additionally influence glucocorticoid dynamics. Moreover, individuals with obesity are considered to have a low-grade inflammatory state [69] and losing body weight and especially visceral adiposity might alter inflammatory markers which are associated with HPA axis activation. Askarpour and colleagues recently conducted a meta-analysis regarding the changes in pro-inflammatory factors after weight loss in patients with obesity who had undergone bariatric surgery (70). They found among others a reduction in the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor-α, both known to stimulate HPA-axis activity [71]. These and other factors related to both obesity and HPA-axis activity are currently being investigated in participants engaging in our combined lifestyle intervention with cognitive behavioral therapy.

Scalp hair glucocorticoids and stress-related features

Glucocorticoid-related adverse events other than weight gain and cardiometabolic alterations would also be expected in subjects exposed to supraphysiological cortisol concentrations. In **chapter 9** and **chapter 10** we evaluated scalp hair cortisol concentrations in patients with Turner syndrome (TS) and erythropoietic protoporphyria (EPP). These patient populations generally have clinical features that are known to be associated with hypercortisolism. We showed that both groups indeed have significantly higher long-term cortisol concentrations in comparison to age- and sex-matched controls. Despite the fact that TS and EPP are genetic disorders, it seems implausible to assume that the increased cortisol levels are the result of genetic alterations at HPA axis level. Psychological distress could potentially have induced higher biological stress levels since both groups experience various mental stressors in daily life from a young age. Several explanations could be proposed for this such as exclusion or teasing because of short stature [72] or low self-esteem and depression due to infertility in TS [73] to pain and fear of sunlight-induced skin lesions causing limitations in personal life in EPP [74]. The observed association between high hair cortisol concentrations with

short stature and lower quality of life in respectively patients with TS and EPP fits in with this. Moreover, van Uum *et al.* showed that chronic pain was associated with higher perceived stress as well as increased hair cortisol concentrations [75]. There was however no significant association between perceived stress as measured with the Perceived Stress Scale (PSS) questionnaire and cortisol concentrations [75], something we also observed in our patient populations (**chapters 9, 10, and 11**). This could perhaps be due to differences in the period of interest covered with the assessments (i.e. months with scalp hair and past four weeks with PSS), stability of the outcomes over time, and/or dependence on other specific factors. The relevance of mental stress on scalp hair glucocorticoids was also assessed in **chapter 11** in which Year 1 medical students before and after raising academic performance standards were investigated. This measure indeed led to higher passing rates and especially in male students. Hair cortisol concentrations however did not differ between both cohorts, whereas perceived psychological stress was higher in the group with raised performance standards and particularly in female students. Combining both stress outcomes revealed that only those with above-median biological and psychological stress levels were at risk for poor academic performance.

The possibility to assess cortisol levels over different time points with one sample is unique to scalp hair analysis compared to the traditional matrices. This enables evaluation of cortisol concentrations before and after certain events or interventions. We previously showed that cortisol concentrations in scalp hair of four-year-old children increased after entering elementary school [76]. In **chapter 12** we investigated the same population after entering third grade two years later and found again an increase in cortisol concentrations. The degree of cortisol increase after school entry was however not the same for everyone. In the previous study, we found that a high social fearfulness was linked to a larger cortisol increase, whereas in the current observation higher cortisol levels after school entry were found to be associated with less inhibitory control. Different circumstances could have played a role hereby giving the fact that these children now had to make a transition to formal learning which could be more challenging in those with a difficult temperament. Alternatively, high cortisol levels or stress reactivity might have affected prefrontal regulatory systems and thereby impaired executive abilities [77] leading to altered behavioral inhibition.

Regarding the link between long-term cortisol concentrations and neural correlates we measured scalp hair cortisol and performed neuroimaging with functional magnetic resonance in trauma-exposed police officers **(chapter 13)**. Participants with posttraumatic stress disorder had lower hair cortisol

concentrations which correspond to the long-term findings in survivors of a natural disaster who subsequently developed such disorder [78] which might be the result of HPA axis dysregulation [79]. Interestingly, differentiation in amygdala reactivity between negative affective stimuli and neutral affective stimuli was lowered in participants with low scalp hair cortisol and especially in those with posttraumatic stress disorder. The exact mechanisms behind these findings need to be elucidated, possible explanations could perhaps lie in the prominent availability of glucocorticoid receptors in the amygdala (80) and dynamic changes in cortisol secretion with posttraumatic stress disorder over time [81].

7. Methodological considerations and limitations

The studies included here possess a variety of strengths such as relatively large number of participants, in-depth phenotyping, and the availability of unique and high-quality data in most. However, some general methodological considerations and limitations must be mentioned. With respect to the analyses regarding corticosteroid use, we unfortunately had no data on the cumulative exposure of these agents. The most profound associations were observed with inhaled corticosteroid use and since these types are generally used in a chronic fashion (e.g. asthma and chronic obstructive pulmonary disease) it would be expected that many of the agents were prescribed for an extended period of time. Despite previous research that has hinted at systemic availability and adverse effects of local and especially inhaled corticosteroids, the observational design of our studies does not prove causality and thus further randomized controlled trials are needed to address temporality. Potential confounding by indication could also be considered as an important limitation, however, we have performed various sensitivity analyses in which many of the main findings persisted. Finally, future studies are needed to reveal whether the findings in our study population with obesity can be extrapolated to individuals with obesity in the general population.

8. Concluding remarks and future perspectives

Glucocorticoids and obesity are linked to each other in a complex interaction. This complexity necessitates a thorough evaluation of potential obesogenic factors in individuals with obesity since adiposity can directly and indirectly increase glucocorticoid concentrations and vice versa. Our studies suggest that the use of systemic but also local exogenous glucocorticoids, and especially of the often overlooked inhaled corticosteroids, should be considered as an important obesogenic factor in obesity. This statement is further emphasized by the fact that these agents are one of the most commonly used drugs, with over ten million being prescribed annually in the Netherlands only. We would recommend to ascertain whether the indication for corticosteroids is still present in users

and to assess whether alternatives are available when dealing with obesity, increased cardiovascular risk and/or mental problems. Evidence is accumulating about various asthmatic phenotypes in obesity with differential insensitivity for glucocorticoids (82) and thus perhaps avoidable use of inhaled corticosteroids in specific subpopulations. It was also shown that nearly half of asthmatic patients with obesity did not have asthma after pulmonary testing of which a part was still on inhaled corticosteroids (83). More awareness is needed for the potential serious cardiometabolic and mental adverse events in case that use of (inhaled) corticosteroids is necessary and unavoidable.

Interesting developments are ongoing in the pursuit of selective glucocorticoid receptor ligands which are designed to minimize the transactivational activity and thus unwanted adverse events while increasing the desired transrepressional and anti-inflammatory effects. Schäcke *et al.* identified a novel nonsteroidal selective glucocorticoid receptor agonist (compound ZK 216348) which was proven to show anti-inflammatory effects comparable to prednisone with less adverse events as induced by transactivation in rodent models [(84)]. *In vivo* experiments with rats showed no significant changes in blood glucose whereas levels increased in a dose-dependent manner with prednisone [(84)]. Similar dissociated effects with preferential transrepressional activity were also demonstrated for the glucocorticoid receptor ligand AL-438 [(85)]. These compounds would presumably have no benefit regarding HPA axis suppression and adrenal insufficiency in comparison to current glucocorticoids since the regulation of corticotropin-releasing hormone and adrenocorticotropic hormone (ACTH) seems to be via transpressional activity [(86)]. Compound ZK 216438 and prednisone were indeed shown to suppress ACTH levels to similar extents (84). On the other hand, newer inhaled corticosteroids are also (becoming) available which possess better pharmacokinetic profiles compared to older agents. A number of conditions for an ideal inhaled corticosteroid with regard to the agent as well as the inhaler device have been proposed earlier. As suggested by Kemp, the formulations should have *"a high affinity for and potency at the glucocorticoid receptor; prolonged retention in the lung; minimal or no oral bioavailability; and rapid, complete systemic inactivation.*" [(87)]. Inhaled corticosteroids that meet these requirements should in theory have maximum efficacy with minimal systemic adverse events. Ciclesonide, a relatively novel inhaled corticosteroid agent, is interesting in this context giving its excellent pharmacokinetic properties which largely meets the proposed conditions. This formulation is inactive upon inhalation and is converted on-site into the biologically active desisobutyryl-ciclesonide (des-cic) by esterases in bronchial epithelial cells [(88)]. The active compound possess a 12 times higher receptor affinity compared to dexamethasone and has been shown to have a low oral bioavailability of less than

1% with nearly complete protein-binding (88). Moreover, des-cic forms conjugates with fatty acids in the lungs in a reversible fashion which ensures a prolonged retention in the lung given the slow-release of the compound over time [88]. A high systemic clearance by the liver (88,89) further contributes to its favourable properties. Previous trials have demonstrated a high efficacy of ciclesonide and showed it to be superior to other inhaled corticosteroids in terms of local adverse events (e.g. oral candidiasis, hoarseness, pharyngitis) and HPA axis suppression [90]. Several trials with different treatment durations showed that ciclesonide, even in high doses, did not induce significant cortisol suppression in comparison to placebo [91-93], which was also confirmed in a meta-analysis investigating urinary cortisol suppression for several inhaled corticosteroids [94]. Future research should reveal whether use of ciclesonide as inhaled corticosteroid also goes with less potential systemic adverse events such as weight gain and cardiometabolic alterations.

Apart from exogenous glucocorticoids, individuals with obesity are more likely to have an increased endogenous cortisol secretion. Since the regulation of cortisol is complex and regulated at different levels, future studies will also have to show which central and/or peripheral factors play an important role in the hypercortisolistic state in obesity and whether these can be influenced with beneficial effects regarding adiposity and cardiometabolic profile. We have at least demonstrated that a combined lifestyle intervention with cognitive behavioral therapy leads to sustained improvements in anthropometry and body composition and also lowers long-term endogenous cortisol levels. Our work and efforts have even resulted in the implementation of comparable interventions in the basic health insurance for Dutch citizens enabling novel options in combating overweight and obesity in individuals who were not eligible or were not willing to undergo bariatric surgery. Other works that are ongoing regarding lowering endogenous cortisol levels involve drug treatments aimed at inhibiting 11β-hydroxysteroid dehydrogenase type 1 (and thus lowering conversion of cortisone into active cortisol) [95] and blocking glucocorticoid receptor with its antagonist mifepristone [96,97].

With respect to quantifying endogenous glucocorticoids, scalp hair cortisol seems a promising biomarker for monitoring long-term cortisol and cortisone exposure. The significance and meaning of higher cortisone concentrations in Cushing's syndrome need further research which could answer whether it resembles accumulative exposure to cortisol and/or is the result of conversion by 11β-hydroxysteroid dehydrogenases in cutaneous appendages. Finally, international collaboration is needed to standardize protocol for hair analysis and to harmonize fixed cutoff values with LC-MS/MS.

References

1. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity in the United States, 2009-2010. NCHS Data Brief 2012:1-8
2. Hammond RA, Levine R. The economic impact of obesity in the United States. Diabetes Metab Syndr Obes 2010; 3:285-295
3. Volksgezondheidenzorg.info (2020): www.volksgezondheidenzorg.info/onderwerp/overgewicht/cijfers-context/trends#node-trend-obesitas-volwassenen, RIVM: Bilthoven, 2020.
4. Dallman MF, Pecoraro N, Akana SF, La Fleur SE, Gomez F, Houshyar H, Bell ME, Bhatnagar S, Laugero KD, Manalo S. Chronic stress and obesity: a new view of "comfort food". Proc Natl Acad Sci U S A 2003; 100:11696-11701
5. Soumano K, Desbiens S, Rabelo R, Bakopanos E, Camirand A, Silva JE. Glucocorticoids inhibit the transcriptional response of the uncoupling protein-1 gene to adrenergic stimulation in a brown adipose cell line. Mol Cell Endocrinol 2000; 165:7-15
6. Conklin AI, Hong J. Obesity prevention in corticosteroid-treated patients: Use and effectiveness of strategies for weight management. Clin Obes 2019; 9:e12312
7. Davies RJ, Lomer MC, Yeo SI, Avloniti K, Sangle SR, D'Cruz DP. Weight loss and improvements in fatigue in systemic lupus erythematosus: a controlled trial of a low glycaemic index diet versus a calorie restricted diet in patients treated with corticosteroids. Lupus 2012; 21:649-655
8. Doucet E, Imbeault P, St-Pierre S, Almeras N, Mauriege P, Richard D, Tremblay A. Appetite after weight loss by energy restriction and a low-fat diet-exercise follow-up. Int J Obes Relat Metab Disord 2000; 24:906-914
9. Redman LM, Heilbronn LK, Martin CK, de Jonge L, Williamson DA, Delany JP, Ravussin E, Pennington CT. Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. PLoS One 2009; 4:e4377
10. Broersen LH, Pereira AM, Jorgensen JO, Dekkers OM. Adrenal Insufficiency in Corticosteroids Use: Systematic Review and Meta-Analysis. J Clin Endocrinol Metab 2015; 100:2171-2180
11. Derendorf H, Nave R, Drollmann A, Cerasoli F, Wurst W. Relevance of pharmacokinetics and pharmacodynamics of inhaled corticosteroids to asthma. Eur Respir J 2006; 28:1042-1050
12. Leone FT, Fish JE, Szefler SJ, West SL. Systematic review of the evidence regarding potential complications of inhaled corticosteroid use in asthma: collaboration of American College of Chest Physicians, American Academy of Allergy, Asthma, and Immunology, and American College of Allergy, Asthma, and Immunology. Chest 2003; 124:2329-2340
13. Fardet L, Flahault A, Kettaneh A, Tiev KP, Genereau T, Toledano C, Lebbe C, Cabane J. Corticosteroid-induced clinical adverse events: frequency, risk factors and patient's opinion. Br J Dermatol 2007; 157:142-148

14. Curtis JR, Westfall AO, Allison J, Bijlsma JW, Freeman A, George V, Kovac SH, Spettell CM, Saag KG. Population-based assessment of adverse events associated with long-term glucocorticoid use. Arthritis Rheum 2006; 55:420-426
15. Fardet L, Kassar A, Cabane J, Flahault A. Corticosteroid-induced adverse events in adults: frequency, screening and prevention. Drug Saf 2007; 30:861-881
16. Fardet L, Feve B. Systemic glucocorticoid therapy: a review of its metabolic and cardiovascular adverse events. Drugs 2014; 74:1731-1745
17. Galassi A, Reynolds K, He J. Metabolic syndrome and risk of cardiovascular disease: a meta-analysis. Am J Med 2006; 119:812-819
18. Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, Taskinen MR, Groop L. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001; 24:683-689
19. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation 2005; 112:3066-3072
20. Sundberg R, Toren K, Franklin KA, Gislason T, Omenaas E, Svanes C, Janson C. Asthma in men and women: treatment adherence, anxiety, and quality of sleep. Respir Med 2010; 104:337-344
21. Soldin OP, Mattison DR. Sex differences in pharmacokinetics and pharmacodynamics. Clin Pharmacokinet 2009; 48:143-157
22. Da Silva JA. Sex hormones and glucocorticoids: interactions with the immune system. Ann N Y Acad Sci 1999; 876:102-117; discussion 117-108
23. Quinn M, Ramamoorthy S, Cidlowski JA. Sexually dimorphic actions of glucocorticoids: beyond chromosomes and sex hormones. Ann N Y Acad Sci 2014; 1317:1-6
24. Wajchenberg BL. Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. Endocr Rev 2000; 21:697-738
25. Fardet L, Cabane J, Lebbe C, Morel P, Flahault A. Incidence and risk factors for corticosteroid-induced lipodystrophy: a prospective study. J Am Acad Dermatol 2007; 57:604-609
26. Marystone JF, Barrett-Connor EL, Morton DJ. Inhaled and oral corticosteroids: their effects on bone mineral density in older adults. Am J Public Health 1995; 85:1693-1695
27. Mak VH, Melchor R, Spiro SG. Easy bruising as a side-effect of inhaled corticosteroids. Eur Respir J 1992; 5:1068-1074
28. Petta I, Dejager L, Ballegeer M, Lievens S, Tavernier J, De Bosscher K, Libert C. The Interactome of the Glucocorticoid Receptor and Its Influence on the Actions of Glucocorticoids in Combatting Inflammatory and Infectious Diseases. Microbiol Mol Biol Rev 2016; 80:495-522
29. Stahn C, Lowenberg M, Hommes DW, Buttgereit F. Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. Mol Cell Endocrinol 2007; 275:71-78

30. Barnes PJ. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. Clin Sci (Lond) 1998; 94:557-572
31. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med 2005; 353:1711-1723
32. Manenschijn L, van den Akker EL, Lamberts SW, van Rossum EF. Clinical features associated with glucocorticoid receptor polymorphisms. An overview. Ann N Y Acad Sci 2009; 1179:179-198
33. Eipel OT, Nemeth K, Torok D, Csordas K, Hegyi M, Ponyi A, Ferenczy A, Erdelyi DJ, Csoka M, Kovacs GT. The glucocorticoid receptor gene polymorphism N363S predisposes to more severe toxic side effects during pediatric acute lymphoblastic leukemia (ALL) therapy. Int J Hematol 2013; 97:216-222
34. Kaymak Cihan M, Karabulut HG, Yurur Kutlay N, Ilgin Ruhi H, Tukun A, Olcay L. Association Between N363S and BclI Polymorphisms of the Glucocorticoid Receptor Gene (NR3C1) and Glucocorticoid Side Effects During Childhood Acute Lymphoblastic Leukemia Treatment. Turk J Haematol 2017; 34:151-158
35. Keskin O, Farzan N, Birben E, Akel H, Karaaslan C, Maitland-van der Zee AH, Wechsler ME, Vijverberg SJ, Kalayci O. Genetic associations of the response to inhaled corticosteroids in asthma: a systematic review. Clin Transl Allergy 2019; 9:2
36. Xue L, Li C, Wang Y, Sun W, Ma C, He Y, Yu Y, Cai L, Wang L. Single nucleotide polymorphisms in non-coding region of the glucocorticoid receptor gene and prednisone response in childhood acute lymphoblastic leukemia. Leuk Lymphoma 2015; 56:1704-1709
37. De Iudicibus S, Stocco G, Martelossi S, Drigo I, Norbedo S, Lionetti P, Pozzi E, Barabino A, Decorti G, Bartoli F, Ventura A. Association of BclI polymorphism of the glucocorticoid receptor gene locus with response to glucocorticoids in inflammatory bowel disease. Gut 2007; 56:1319-1320
38. Teeninga N, Kist-van Holthe JE, van den Akker EL, Kersten MC, Boersma E, Krabbe HG, Knoers NV, van der Heijden AJ, Koper JW, Nauta J. Genetic and in vivo determinants of glucocorticoid sensitivity in relation to clinical outcome of childhood nephrotic syndrome. Kidney Int 2014; 85:1444-1453
39. Krupoves A, Mack D, Deslandres C, Seidman E, Amre DK. Variation in the glucocorticoid receptor gene (NR3C1) may be associated with corticosteroid dependency and resistance in children with Crohn's disease. Pharmacogenet Genomics 2011; 21:454-460
40. Russcher H, Smit P, van den Akker EL, van Rossum EF, Brinkmann AO, de Jong FH, Lamberts SW, Koper JW. Two polymorphisms in the glucocorticoid receptor gene directly affect glucocorticoid-regulated gene expression. J Clin Endocrinol Metab 2005; 90:5804-5810
41. van den Akker EL, Russcher H, van Rossum EF, Brinkmann AO, de Jong FH, Hokken A, Pols HA, Koper JW, Lamberts SW. Glucocorticoid receptor polymorphism affects transrepression but not transactivation. J Clin Endocrinol Metab 2006; 91:2800-2803

42. King EM, Chivers JE, Rider CF, Minnich A, Giembycz MA, Newton R. Glucocorticoid repression of inflammatory gene expression shows differential responsiveness by transactivation- and transrepression-dependent mechanisms. PLoS One 2013; 8:e53936
43. Heming N, Sivanandamoorthy S, Meng P, Bounab R, Annane D. Immune Effects of Corticosteroids in Sepsis. Front Immunol 2018; 9:1736
44. Quax RA, Manenschijn L, Koper JW, Hazes JM, Lamberts SW, van Rossum EF, Feelders RA. Glucocorticoid sensitivity in health and disease. Nat Rev Endocrinol 2013; 9:670-686
45. Minkel J, Moreta M, Muto J, Htaik O, Jones C, Basner M, Dinges D. Sleep deprivation potentiates HPA axis stress reactivity in healthy adults. Health Psychol 2014; 33:1430-1434
46. Badrick E, Bobak M, Britton A, Kirschbaum C, Marmot M, Kumari M. The relationship between alcohol consumption and cortisol secretion in an aging cohort. J Clin Endocrinol Metab 2008; 93:750-757
47. Manenschijn L, van Kruysbergen RG, de Jong FH, Koper JW, van Rossum EF. Shift work at young age is associated with elevated long-term cortisol levels and body mass index. J Clin Endocrinol Metab 2011; 96:E1862-1865
48. Jackson SE, Kirschbaum C, Steptoe A. Perceived weight discrimination and chronic biochemical stress: A population-based study using cortisol in scalp hair. Obesity (Silver Spring) 2016; 24:2515-2521
49. Varney NR, Alexander B, MacIndoe JH. Reversible steroid dementia in patients without steroid psychosis. Am J Psychiatry 1984; 141:369-372
50. Starkman MN, Schteingart DE, Schork MA. Depressed mood and other psychiatric manifestations of Cushing's syndrome: relationship to hormone levels. Psychosom Med 1981; 43:3-18
51. Forget H, Lacroix A, Somma M, Cohen H. Cognitive decline in patients with Cushing's syndrome. J Int Neuropsychol Soc 2000; 6:20-29
52. Simmons NE, Do HM, Lipper MH, Laws ER, Jr. Cerebral atrophy in Cushing's disease. Surg Neurol 2000; 53:72-76
53. Bourdeau I, Bard C, Noel B, Leclerc I, Cordeau MP, Belair M, Lesage J, Lafontaine L, Lacroix A. Loss of brain volume in endogenous Cushing's syndrome and its reversibility after correction of hypercortisolism. J Clin Endocrinol Metab 2002; 87:1949-1954
54. van der Valk ES, Savas M, van Rossum EFC. Stress and Obesity: Are There More Susceptible Individuals? Curr Obes Rep 2018; 7:193-203
55. Brown ES, Chandler PA. Mood and Cognitive Changes During Systemic Corticosteroid Therapy. Prim Care Companion J Clin Psychiatry 2001; 3:17-21
56. Rivenes AC, Harvey SB, Mykletun A. The relationship between abdominal fat, obesity, and common mental disorders: results from the HUNT study. J Psychosom Res 2009; 66:269-275
57. Noppe G, de Rijke YB, Dorst K, van den Akker EL, van Rossum EF. LC-MS/MS-based method for long-term steroid profiling in human scalp hair. Clin Endocrinol (Oxf) 2015; 83:162-166

58. Nieman LK, Biller BM, Findling JW, Newell-Price J, Savage MO, Stewart PM, Montori VM. The diagnosis of Cushing's syndrome: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2008; 93:1526-1540
59. Elamin MB, Murad MH, Mullan R, Erickson D, Harris K, Nadeem S, Ennis R, Erwin PJ, Montori VM. Accuracy of diagnostic tests for Cushing's syndrome: a systematic review and metaanalyses. J Clin Endocrinol Metab 2008; 93:1553-1562
60. Keckeis K, Lepschy M, Schopper H, Moser L, Troxler J, Palme R. Hair cortisol: a parameter of chronic stress? Insights from a radiometabolism study in guinea pigs. J Comp Physiol B 2012; 182:985-996
61. Kapoor A, Schultz-Darken N, Ziegler TE. Radiolabel validation of cortisol in the hair of rhesus monkeys. Psychoneuroendocrinology 2018; 97:190-195
62. Wester VL, van der Wulp NR, Koper JW, de Rijke YB, van Rossum EF. Hair cortisol and cortisone are decreased by natural sunlight. Psychoneuroendocrinology 2016; 72:94-96
63. Wester VL, Staufenbiel SM, Veldhorst MA, Visser JA, Manenschijn L, Koper JW, Klessens-Godfroy FJ, van den Akker EL, van Rossum EF. Long-term cortisol levels measured in scalp hair of obese patients. Obesity (Silver Spring) 2014; 22:1956-1958
64. Stalder T, Steudte-Schmiedgen S, Alexander N, Klucken T, Vater A, Wichmann S, Kirschbaum C, Miller R. Stress-related and basic determinants of hair cortisol in humans: A meta-analysis. Psychoneuroendocrinology 2017; 77:261-274
65. O'Connell M, Danforth E, Jr., Horton ES, Salans L, Sims EA. Experimental obesity in man. 3. Adrenocortical function. J Clin Endocrinol Metab 1973; 36:323-329
66. Rask E, Simonyte K, Lonn L, Axelson M. Cortisol metabolism after weight loss: associations with 11 beta-HSD type 1 and markers of obesity in women. Clin Endocrinol (Oxf) 2013; 78:700-705
67. Simonyte K, Olsson T, Naslund I, Angelhed JE, Lonn L, Mattsson C, Rask E. Weight loss after gastric bypass surgery in women is followed by a metabolically favorable decrease in 11beta-hydroxysteroid dehydrogenase 1 expression in subcutaneous adipose tissue. J Clin Endocrinol Metab 2010; 95:3527-3531
68. Woods CP, Corrigan M, Gathercole L, Taylor A, Hughes B, Gaoatswe G, Manolopoulos K, Hogan AE, O'Connell J, Stewart PM, Tomlinson JW, O'Shea D, Sherlock M. Tissue specific regulation of glucocorticoids in severe obesity and the response to significant weight loss following bariatric surgery (BARICORT). J Clin Endocrinol Metab 2015; 100:1434-1444
69. Das UN. Is obesity an inflammatory condition? Nutrition 2001; 17:953-966
70. Askarpour M, Khani D, Sheikhi A, Ghaedi E, Alizadeh S. Effect of Bariatric Surgery on Serum Inflammatory Factors of Obese Patients: a Systematic Review and Meta-Analysis. Obes Surg 2019; 29:2631-2647
71. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. N Engl J Med 1995; 332:1351-1362

72. Rickert VI, Hassed SJ, Hendon AE, Cunniff C. The effects of peer ridicule on depression and self-image among adolescent females with Turner syndrome. J Adolesc Health 1996; 19:34-38
73. Schmidt PJ, Cardoso GM, Ross JL, Haq N, Rubinow DR, Bondy CA. Shyness, social anxiety, and impaired self-esteem in Turner syndrome and premature ovarian failure. JAMA 2006; 295:1374-1376
74. Langendonk JG, Balwani M, Anderson KE, Bonkovsky HL, Anstey AV, Bissell DM, Bloomer J, Edwards C, Neumann NJ, Parker C, Phillips JD, Lim HW, Hamzavi I, Deybach JC, Kauppinen R, Rhodes LE, Frank J, Murphy GM, Karstens FPJ, Sijbrands EJG, de Rooij FWM, Lebwohl M, Naik H, Goding CR, Wilson JHP, Desnick RJ. Afamelanotide for Erythropoietic Protoporphyria. N Engl J Med 2015; 373:48-59
75. Van Uum SH, Sauve B, Fraser LA, Morley-Forster P, Paul TL, Koren G. Elevated content of cortisol in hair of patients with severe chronic pain: a novel biomarker for stress. Stress 2008; 11:483-488
76. Groeneveld MG, Vermeer HJ, Linting M, Noppe G, van Rossum EF, van IMH. Children's hair cortisol as a biomarker of stress at school entry. Stress 2013; 16:711-715
77. Loman MM, Gunnar MR, Early Experience S, Neurobehavioral Development C. Early experience and the development of stress reactivity and regulation in children. Neurosci Biobehav Rev 2010; 34:867-876
78. Luo H, Hu X, Liu X, Ma X, Guo W, Qiu C, Wang Y, Wang Q, Zhang X, Zhang W, Hannum G, Zhang K, Liu X, Li T. Hair cortisol level as a biomarker for altered hypothalamic-pituitary-adrenal activity in female adolescents with posttraumatic stress disorder after the 2008 Wenchuan earthquake. Biol Psychiatry 2012; 72:65-69
79. Olff M, van Zuiden M. Neuroendocrine and neuroimmune markers in PTSD: pre-, peri- and post-trauma glucocorticoid and inflammatory dysregulation. Curr Opin Psychol 2017; 14:132-137
80. Wang Q, Verweij EW, Krugers HJ, Joels M, Swaab DF, Lucassen PJ. Distribution of the glucocorticoid receptor in the human amygdala; changes in mood disorder patients. Brain Struct Funct 2014; 219:1615-1626
81. Steudte-Schmiedgen S, Kirschbaum C, Alexander N, Stalder T. An integrative model linking traumatization, cortisol dysregulation and posttraumatic stress disorder: Insight from recent hair cortisol findings. Neurosci Biobehav Rev 2016; 69:124-135
82. Sutherland ER, Goleva E, King TS, Lehman E, Stevens AD, Jackson LP, Stream AR, Fahy JV, Leung DY, Asthma Clinical Research N. Cluster analysis of obesity and asthma phenotypes. PLoS One 2012; 7:e36631
83. van Huisstede A, Castro Cabezas M, van de Geijn GJ, Mannaerts GH, Njo TL, Taube C, Hiemstra PS, Braunstahl GJ. Underdiagnosis and overdiagnosis of asthma in the morbidly obese. Respir Med 2013; 107:1356-1364
84. Schacke H, Schottelius A, Docke WD, Strehlke P, Jaroch S, Schmees N, Rehwinkel H, Hennekes H, Asadullah K. Dissociation of transactivation from transrepression by a

selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. Proc Natl Acad Sci U S A 2004; 101:227-232

85. Coghlan MJ, Jacobson PB, Lane B, Nakane M, Lin CW, Elmore SW, Kym PR, Luly JR, Carter GW, Turner R, Tyree CM, Hu J, Elgort M, Rosen J, Miner JN. A novel antiinflammatory maintains glucocorticoid efficacy with reduced side effects. Mol Endocrinol 2003; 17:860-869
86. Reichardt HM, Schutz G. Glucocorticoid signalling--multiple variations of a common theme. Mol Cell Endocrinol 1998; 146:1-6
87. Kemp JE. Expected characteristics of an ideal, all-purpose inhaled corticosteroid for the treatment of asthma. Clin Ther 2003; 25 Suppl C:C15-27
88. Derendorf H. Pharmacokinetic and pharmacodynamic properties of inhaled ciclesonide. J Clin Pharmacol 2007; 47:782-789
89. Mutch E, Nave R, McCracken N, Zech K, Williams FM. The role of esterases in the metabolism of ciclesonide to desisobutyryl-ciclesonide in human tissue. Biochem Pharmacol 2007; 73:1657-1664
90. Humbert M. Ciclesonide: a novel inhaled corticosteroid. Expert Opin Investig Drugs 2004; 13:1349-1360
91. Derom E, Van De Velde V, Marissens S, Engelstatter R, Vincken W, Pauwels R. Effects of inhaled ciclesonide and fluticasone propionate on cortisol secretion and airway responsiveness to adenosine 5'monophosphate in asthmatic patients. Pulm Pharmacol Ther 2005; 18:328-336
92. Szefler S, Rohatagi S, Williams J, Lloyd M, Kundu S, Banerji D. Ciclesonide, a novel inhaled steroid, does not affect hypothalamic-pituitary-adrenal axis function in patients with moderate-to-severe persistent asthma. Chest 2005; 128:1104-1114
93. Hansel TT, Benezet O, Kafe H, Ponitz HH, Cheung D, Engelstatter R, Barnes PJ. A multinational, 12-week, randomized study comparing the efficacy and tolerability of ciclesonide and budesonide in patients with asthma. Clin Ther 2006; 28:906-920
94. Kowalski ML, Wojciechowski P, Dziewonska M, Rys P. Adrenal suppression by inhaled corticosteroids in patients with asthma: A systematic review and quantitative analysis. Allergy Asthma Proc 2016; 37:9-17
95. Anagnostis P, Katsiki N, Adamidou F, Athyros VG, Karagiannis A, Kita M, Mikhailidis DP. 11beta-Hydroxysteroid dehydrogenase type 1 inhibitors: novel agents for the treatment of metabolic syndrome and obesity-related disorders? Metabolism 2013; 62:21-33
96. Okada S, York DA, Bray GA. Mifepristone (RU 486), a blocker of type II glucocorticoid and progestin receptors, reverses a dietary form of obesity. Am J Physiol 1992; 262:R1106-1110
97. Gross C, Blasey CM, Roe RL, Allen K, Block TS, Belanoff JK. Mifepristone treatment of olanzapine-induced weight gain in healthy men. Adv Ther 2009; 26:959-969

Chapter 15

Summary / Samenvatting

Summary

Glucocorticoids, in particular the main effector hormone cortisol, are important endocrine regulators essential for a myriad of physiological and mental functions. The concentration and action of glucocorticoids, also known as stress hormones, is delicately controlled by the hypothalamus-pituitary-adrenal axis as well as through other means at different levels. The importance of this becomes clear giving that too much glucocorticoid action can have detrimental effects with respect to essentially every aspect of human functioning. There are various ways by which this can occur such as increased secretion of endogenous glucocorticoids as well as due to administration of corticosteroids, i.e. drugs which contain synthetic glucocorticoids. The metabolic effects, including the development of (abdominal) obesity, are of special interest since they are frequently observed with high glucocorticoid action and can increase the risk of comorbidities as type 2 diabetes mellitus, cardiovascular diseases, depression, and stroke. This becomes even more relevant giving the fact that the prevalence of obesity has taken pandemic proportions. In this thesis, we describe the findings of our scientific work in which we focus on endogenous and exogenous glucocorticoids and their role in obesity and stress-related diseases.

In **chapter 1** we provide a general background into the field of endogenous and exogenous glucocorticoids. The synthesis of glucocorticoids, methods of quantification, the magnitude of corticosteroid use, the diverse adverse effects of glucocorticoids as well as the link with obesity are among the topics that are discussed.

Chapter 2 describes our findings of a comprehensive evaluation of potential obesogenic factors besides diet and physical activity in an adult cohort of 408 individuals with obesity. Interestingly, genetic analysis in participants suspected of a monogenic cause or syndromic obesity yielded a definitive diagnosis in 2.0% whereas 5.6% was found to carry likely pathogenic contributing genetic variants. Furthermore, nearly half of the complete cohort were using one or more drugs which are associated with weight gain and this especially included the use of corticosteroids. One in six individuals reported to have experienced period(s) of marked weight gain preceded by the use of obesogenic drugs among which corticosteroids were mentioned most frequently.

In **chapter 3** we compared the recent use of corticosteroids in persons with obesity compared to individuals without obesity from two different cohorts. We found that overall corticosteroid use was twice more likely in the group with obesity. The

largest differences were found for the use of local corticosteroids and especially for the inhaled types.

We subsequently investigated the associations between different corticosteroid forms with metabolic syndrome and body mass index in over 140.000 adults from the general population. In **chapter 4** we describe our findings concerning significantly higher body mass index and waist circumference in users of inhaled corticosteroids from both sexes. Moreover, users of systemic as well as local corticosteroids, more specifically for the inhaled and nasal types, were more likely to have metabolic syndrome but only in female users. These findings were largely persistent in users of systemic and inhaled corticosteroids when stratified by menopausal status, inflammatory status, and obesity.

In **chapter 5** we further elucidated the previous findings by investigating the role of glucocorticoid receptor polymorphisms related to a relative glucocorticoid resistance (ER22/23EK and 9β variants) or glucocorticoid hypersensitivity (*Bcl*I and N363S variants) in over 10.000 individuals from the adult general population. Overall, corticosteroid users harboring wild-type or glucocorticoid hypersensitive variants were more likely to have adverse anthropometric features in terms of increased body mass index, waist circumference, and increased presence of metabolic syndrome in comparison to nonusers with strongest associations found for users of inhaled types. The differences in users with glucocorticoid resistant variants were less pronounced and only reached statistical significance for waist circumference in users of inhaled corticosteroids.

In **chapter 6** we extended the scope of potential adverse effects of exogenous glucocorticoids by investigating the link with executive cognitive functioning and neuropsychiatric disorders. In 83,592 adults from the same population-based cohort study, we analyzed outcomes on the Ruff Figural Fluency test (i.e. cognitive test for measuring nonverbal fluency as part of executive cognitive functioning) and the Mini-International Neuropsychiatric Interview survey. We found that corticosteroid use, especially of systemic and inhaled administration forms, was associated with lower executive cognitive functioning and was independent of inflammation as proxied by high-sensitive CRP. Overall corticosteroid use was also associated with a higher likelihood of mood and anxiety disorders. These associations were especially present in users of inhaled types and were independent of physical quality-of-life as assessed with the RAND-36 questionnaire.

With respect to quantifying endogenous glucocorticoid concentrations, we assessed the diagnostic efficacy of scalp hair glucocorticoids in the screening

of Cushing's syndrome in **chapter 7.** For this purpose, we analyzed hair samples of 295 controls from the general population and 89 patients with endogenous Cushing's syndrome from two different centers by using a state-of-the-art LC-MS/MS technique. High diagnostic performances were found for both hair cortisol and cortisone concentrations in screening of Cushing's syndrome. Interestingly, hair cortisone was more accurate than hair cortisol and sum of both in differentiating patients from controls.

In **chapter 8** we investigated the effects of combined lifestyle intervention with cognitive behavioral therapy in obesity. Participants showed significant improvements in body weight, waist circumference, body mass index, and body composition after the first evaluation at 10 weeks and maintained these at the end of the intervention at 75 weeks. The weight course was different in systemic corticosteroid users compared to nonusers. No significant weight change at end of the intervention was observed in users of systemic corticosteroids. There were no significant changes in hair cortisone between the start and end of the program whereas hair cortisol concentrations dropped significantly. The changes in anthropometrics and hair glucocorticoids were however not associated with each other. This suggests that other factors than weight loss per se could have been influencing long-term cortisol levels during the intervention.

In **chapter 9** we investigated hair cortisol concentrations in patients with Turner syndrome and age-and-sex matched controls from the general population. This syndrome originates from a (partial) loss and/or abnormalities of the X-chromosomes and is amongst the most prevalent genetic syndromes. Patients are often encountered with a variety of physical and psychological difficulties including stress-related features as diabetes mellitus, hypertension, and obesity. We found that patients with Turner syndrome have a worse cardiometabolic profile in comparison to controls with regard to fasting glucose and triglycerides. Long-term cortisol was also significantly higher in patients and was associated with higher total cholesterol and seemed to be inversely related with height. No significant associations were found with psychological measures regarding perceived stress (PSS-14), fatigue (CIS-20), and health-related quality of life (RAND-36).

In another study (**chapter 10**) we focused on patients with erythropoietic protoporphyria, which is a rare metabolic disorder caused by an enzyme deficiency in the haem biosynthetic pathway. As with Turner syndrome, patients with erythropoietic protoporphyria frequently suffer from a multitude of (disease-related) stressors. In this study, we analyzed long-term cortisol concentrations in scalp hair of 15 patients and 45 age-and-sex matched controls. We found that

patients with erythropoietic protoporphyria had significantly higher cortisol concentrations in comparison to controls. Importantly, we showed that higher hair cortisol concentrations were associated with a higher body mass index as well as a lower quality-of-life in these patients.

In chapter 11 we report the findings of our prospective comparative cohort study investigating the effects of raising performance standards in medical students. We focused on psychological well-being, biological stress levels, and academic performance in student cohorts before (n=410) and after (n=413) raising academic performance standards. First-year students, especially male individuals, were more likely to pass whereas perceived psychological stress (PSS-14) was increased in case of raised standards. In contrast, no differences were observed in biological stress as measured with hair cortisol concentrations. The combination of high perceived psychological and biological stress was found to be associated with a lower passing rate.

In **chapter 12** we investigated scalp hair cortisol concentrations in school children before and after entering third grade. This study was a follow-up to our previous research concerning first school entry which showed that hair cortisol concentrations increased after entering elementary school and in particular for fearful children. We found that entering third grade was also associated with increasing hair cortisol concentrations. However, this was only associated with increasing cortisol levels in children with low inhibitory control and was not linked to social fearfulness or academic achievement. This suggests a crucial role of controlling impulsive responsivity in stress regulation in children who make the transition to formal learning.

In **chapter 13** we studied long-term cortisol level and its neural correlates as measured with functional magnetic resonance imaging in trauma-exposed female police officers. We found that hair cortisol concentrations were lowered in participants who had developed a post-traumatic stress disorder. Furthermore, hair cortisol levels showed to be associated with important post-traumatic stress disorder-related neurobiological correlates as reduced differentiation between negative affective and neutral stimuli in the amygdala was observed with lower cortisol levels.

In the final **chapter 14**, we discuss our findings in the context of current literature and extent on their clinical and societal relevance and potential future implications.

Samenvatting

Glucocorticoïden, met name het belangrijkste hormoon cortisol, behoren tot de meest essentiële hormonale regulatoren en zijn noodzakelijk voor een groot aantal fysiologische en mentale functies. De concentratie en het uiteindelijke effect van glucocorticoïden, die ook wel bekend staan als stresshormonen, wordt gereguleerd door de hypothalamus-hypofyse-bijnier-as en verschillende regelmechanismen op allerlei niveaus. Dit is van wezenlijk belang aangezien te veel glucocorticoïden werking vrijwel elk aspect van het menselijk functioneren nadelig kan beïnvloeden. Een verhoogd glucocorticoïd effect kan op verschillende manieren plaatsvinden, zoals door een verhoogde secretie van endogene glucocorticoïden maar ook door het gebruik van geneesmiddelen die synthetische glucocorticoïden bevatten. De metabole effecten, waaronder de ontwikkeling van (abdominale) obesitas, zijn van bijzonder belang omdat ze vaak worden waargenomen bij een verhoogde glucocorticoïd blootstelling. Hierbij wordt het risico op comorbiditeiten zoals diabetes mellitus type 2, hart- en vaatziekten, depressie en beroerte verhoogd. Dit wordt des te relevanter omdat obesitas pandemische proporties heeft aangenomen. In dit proefschrift beschrijven we de bevindingen van ons wetenschappelijke werk waarin we ons richten op endogene en exogene glucocorticoïden en hun rol bij obesitas en stress gerelateerde ziekten.

In **hoofdstuk 1** geven we een algemene achtergrond op het gebied van endogene en exogene glucocorticoïden. De synthese van glucocorticoïden, kwantificatiemethoden, omvang van het gebruik van corticosteroïden, de diverse nadelige effecten van glucocorticoïden en het verband met obesitas passeren de revue.

Hoofdstuk 2 beschrijft onze bevindingen van een uitgebreide evaluatie van mogelijke obesogene ('dikmakende') factoren naast voeding en fysieke activiteit in een volwassen cohort van 408 personen met obesitas. Interessant is dat genetische analyse bij deelnemers die verdacht werden van een monogenetische oorzaak of syndromale obesitas een definitieve genetische diagnose opleverde bij 2,0%, terwijl 5.6% drager bleek te zijn van mogelijk pathogene bijdragende genetische varianten. Bovendien bleek dat bijna de helft van de personen van de volledige onderzoekspopulatie één of meerdere mogelijke gewichtsverhogende geneesmiddelen gebruikt waaronder met name de corticosteroïden. Eén op de zes personen meldde een periode van opmerkelijke gewichtstoename te hebben gehad welke voorafgegaan werd door het gebruik van obesogene geneesmiddelen waarbij corticosteroïden het vaakst werden genoemd.

In **hoofdstuk 3** vergeleken we het recente gebruik van corticosteroïden bij personen met obesitas met personen zonder obesitas uit twee verschillende cohorten. We ontdekten dat het gebruik van corticosteroïden in het algemeen bijna verdubbeld was in individuen met obesitas ten opzichte van de groepen zonder obesitas. De grootste verschillen werden gevonden voor het gebruik van lokaal toegediende corticosteroïden en vooral van de inhalatoren.

Vervolgens hebben we in **hoofdstuk 4** de associatie onderzocht tussen het gebruik van verschillende typen corticosteroïden met het metabool syndroom en de body mass index bij meer dan 140.000 volwassenen uit de algemene bevolking. Zowel mannelijke als vrouwelijke gebruikers van inhalatiecorticosteroïden bleken een significant hogere body mass index en buikomtrek te hebben ten opzichte van niet-gebruikers van corticosteroïden. Vrouwelijke gebruikers van zowel systemische als lokale corticosteroïden, met name de inhalatie en nasale typen, bleken vaker het metabool syndroom te hebben dan vrouwen die geen corticosteroïden gebruikten. Deze bevindingen bleven grotendeels onveranderd bij stratificatie voor menopauze, inflammatoire status en obesitas.

In **hoofdstuk 5** hebben we in het verlengde van de eerdere bevindingen ook de rol van genetische polymorfismen van het glucocorticoïd receptor onderzocht, waarbij we onderscheid hebben gemaakt tussen varianten die geassocieerd zijn met een relatieve glucocorticoïdresistentie (ER22/23EK en 9β) of overgevoeligheid voor glucocorticoïden (*Bcl*I en N363S). In dit onderzoek met meer dan 10.000 individuen uit de volwassen algemene bevolking vonden we dat gebruikers van corticosteroïden met een wildtype of hypersensitieve variant meer nadelige cardiometabole en antropometrische kenmerken hadden wat betreft body mass index, buikomvang en metabool syndroom in vergelijking met niet-gebruikers. De sterkste associaties werden gevonden voor gebruikers van inhalatiecorticosteroïden. De verschillen bij gebruikers met glucocorticoïd-resistente varianten waren minder uitgesproken, waarbij slechts gebruikers van inhalatiecorticosteroïden een statistisch significant toegenomen buikomvang hadden ten opzichte van niet-gebruikers.

In **hoofdstuk 6** hebben we ons gefocust op andere mogelijke nadelige effecten van exogene glucocorticoïden door het verband tussen corticosteroïdgebruik, executief cognitief functioneren en neuropsychiatrische aandoeningen te onderzoeken. Bij 83.592 volwassenen uit dezelfde populatie-gebaseerde cohortstudie analyseerden wij de uitkomsten van de Ruff Figural Fluency-test (i.e. een cognitieve test voor het meten van non-verbale fluency als onderdeel van executief cognitief functioneren) en de Mini-International Neuropsychiatric Interview vragenlijst. We ontdekten dat het gebruik van corticosteroïden, vooral

van systemische en geïnhaleerde toedieningsvormen, gepaard ging met een verminderd executieve cognitief functioneren welke onafhankelijk bleek te zijn van de mate van ontsteking (gemeten met high-sensitive CRP). Het gebruik van corticosteroïden ging in het algemeen ook gepaard met een grotere kans op stemmings- en angststoornissen. Deze associaties werden vooral waargenomen bij gebruikers van inhalatiecorticosteroïden en waren onafhankelijk van de fysieke kwaliteit van leven (gemeten met de RAND-36 vragenlijst)

Met betrekking tot het kwantificeren van endogene glucocorticoïden, hebben we in **hoofdstuk 7** de diagnostische kwaliteiten van glucocorticoïden in hoofdhaar bij de screening van het syndroom van Cushing onderzocht. Hiervoor hebben we haarmonsters van 295 controles uit de algemene bevolking en 89 patiënten met endogene syndroom van Cushing uit twee verschillende centra onderzocht met behulp van de modernste LC-MS/MS techniek. We vonden dat zowel haar cortisol als haar cortison veelbelovende biomarkers zijn bij de screening op het syndroom van Cushing. Interessant is dat haar cortison nauwkeuriger was dan haar cortisol en de som van beide hormonen bij het onderscheiden van patiënten van controles.

In **hoofdstuk 8** hebben we de effecten van een gecombineerde leefstijlinterventie met cognitieve gedragstherapie bij individuen met obesitas onderzocht. Deelnemers toonden bij de eerste evaluatie na 10 weken significante verbeteringen in lichaamsgewicht, buikomvang, body mass index en lichaamssamenstelling welke ook na 75 weken (einde van de interventie) behouden bleven. Het gewichtsbeloop was verschillend naargelang het gebruik van systemische corticosteroïden. De gebruikers bleken aan het eind van het programma geen significante gewichtsverandering te hebben in tegenstelling tot niet-gebruikers. Tussen begin en einde van het programma werden geen significante veranderingen in haar cortison waargenomen, terwijl de haar cortisol concentraties aanzienlijk daalden. De veranderingen in antropometrie en haar glucocorticoïden waren echter niet met elkaar geassocieerd. Dit suggereert dat andere factoren dan gewichtsverlies de lange-termijn cortisolspiegels zouden kunnen beïnvloeden tijdens de interventie.

In **hoofdstuk 9** onderzochten we haar cortisol concentraties van patiënten met het syndroom van Turner en van op leeftijd en geslacht gematchte controles uit de algemene bevolking. Het syndroom van Turner komt voort uit een (gedeeltelijk) verlies en/of afwijkingen van één van de X-chromosomen en behoort tot één van de meest voorkomende genetische syndromen. Patiënten worden vaak geconfronteerd met een verscheidenheid aan fysieke en psychologische problemen, waaronder stressgerelateerde aandoeningen zoals diabetes mellitus, hypertensie en obesitas. We ontdekten dat patiënten met het syndroom van

Turner een ongunstiger cardiometabool profiel hebben vergeleken met de controles ten aanzien van nuchter glucose en triglyceridenwaarden in het bloed. Cortisol in hoofdhaar was tevens significant hoger bij patiënten en ging gepaard met een hoger totaal cholesterol en had een omgekeerd evenredig verband met lichaamslengte. Er werden geen significante associaties gevonden met psychologische uitkomsten met betrekking tot ervaren stress (PSS-14), vermoeidheid (CIS-20) en gezondheidsgerelateerde kwaliteit van leven (RAND-36).

In een ander onderzoek (**hoofdstuk** 10) richtten we ons op patiënten met erytropoëtische protoporfyrie, een zeldzame stofwisselingsziekte die wordt veroorzaakt door een enzymdeficiëntie in de biosynthese van heem. Net als bij het syndroom van Turner hebben patiënten met erytropoëtische protoporfyrie vaak last van een veelvoud aan (ziektegerelateerde) stressfactoren. In deze studie analyseerden we lange-termijn cortisol in hoofdhaar van 15 patiënten en 45 op leeftijd en geslacht gematchte controles. We ontdekten dat patiënten met erytropoëtische protoporfyrie significant hogere cortisol concentraties hadden in vergelijking met controles. Hogere cortisol concentraties in hoofdhaar waren tevens geassocieerd met een hogere body mass index en een lagere kwaliteit van leven bij deze patiënten.

In **hoofdstuk 11** rapporteren we de bevindingen van ons prospectieve vergelijkende cohortonderzoek naar de effecten van het verhogen van prestatienormen bij medische studenten. Hierbij hebben we ons gericht op psychologisch welbevinden, biologische stressniveaus en academische prestaties in studentencohorten vóór (N=410) en na (N=413) het verhogen van de academische prestatienorm. Eerstejaarsstudenten, vooral mannelijke individuen, slaagden vaker bij een verhoogde prestatienorm. Deze studenten bleken echter ook meer psychologische stress (PSS-14) te ervaren. Daarentegen werden geen verschillen waargenomen in biologische stress zoals gemeten met haar cortisol concentraties. De combinatie van hoog ervaren psychologische én biologische stress ging gepaard met een lager slagingspercentage.

In **hoofdstuk 12** hebben we cortisol concentraties in hoofdhaar van schoolkinderen vóór en na het start van het derde leerjaar onderzocht. Deze studie was een vervolg op ons eerdere onderzoek ten tijde van het eerste schooljaar, waaruit bleek dat haar cortisol concentraties toenamen bij start van de basisschool en in het bijzonder bij angstige kinderen. We ontdekten nu dat de overgang naar groep drie ook geassocieerd was met een toename in haar cortisol concentraties. Dit bleek echter alleen het geval te zijn bij kinderen met een lage ‘inhibitory control’ en was niet gekoppeld aan sociale angst of academische prestaties. Dit suggereert

een cruciale rol van het beheersen van impulsieve responsiviteit bij stressregulatie bij kinderen die de overgang maken naar formeel leren.

In **hoofdstuk 13** hebben we lange termijn cortisolspiegels en de relatie hiervan met neuronale correlaten, gemeten met functionele magnetische resonantiebeeldvorming, bij aan trauma blootgestelde vrouwelijke politieagenten onderzocht. We vonden lagere haar cortisol concentraties bij deelnemers die een posttraumatische stressstoornis hadden ontwikkeld. Bovendien bleek dat haar cortisolspiegels geassocieerd zijn met belangrijke posttraumatische stressstoornis-gerelateerde neurobiologische correlaten, zoals een verminderd onderscheid tussen negatieve affectieve en neutrale stimuli in de amygdala, hetgeen werd waargenomen bij lagere cortisolspiegels.

In het laatste **hoofdstuk 14** bespreken we onze bevindingen in de context van de huidige literatuur en gaan we in op de klinische en maatschappelijke relevantie alsmede de mogelijke toekomstige implicaties hiervan.

Appendices

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List of Publications

1. **Lokale corticosteroïden en de relatie met obesitas [Local Corticosteroids in Relation to obesity].** van Rossum E.F.C., Savas M., Wester V.L. *Nederlands Tijdschrift voor Dermatologie en Venereologie. 2016;26(4):206-210.*

2. **Systematic Evaluation of Corticosteroid Use in Obese and Non-obese Individuals: A Multi-cohort Study.** Savas M., Wester V.L., Staufenbiel S.M., Koper J.W., van den Akker E.L.T., Visser J.A., van der Lely A.J., Penninx B., van Rossum E.F.C. *Int. J. Med. Sci. 2017; 14(7): 615-621.*

3. **Hair Analysis Reveals Subtle HPA Axis Suppression Associated with Use of Local Corticosteroids: The Lifelines Cohort Study.** Wester V.L., Noppe G., Savas M., van den Akker E.L.T., de Rijke Y.B., van Rossum E.F.C. *Psychoneuroendocrinology. 2017;80:1-6.*

4. **Associations Between Systemic and Local Corticosteroid Use with Metabolic Syndrome and Body Mass Index.** Savas M., Muka T., Wester V.L., van den Akker E.L.T., Visser J.A., Braunstahl G.J., Slagter S.N., Wolffenbuttel B.H.R., Franco O.H., van Rossum E.F.C. *J Clin Endocrinol Metab. 2017;102(10):3765-3774.*

5. **Obesitas in de spreekkamer [Obesity in the Clinic Room: Diagnostics First, Followed by Effective Treatment].** van der Valk E.S., Savas M., Burgerhart J.S., de Vries M., van den Akker E.L.T., van Rossum E.F.C. *Ned Tijdschr Geneeskd 2017; 161:D2310.*

6. **Long-Term Cortisol Levels Are Elevated in Erythropoietic Protoporphyria Patients and Correlate With Body Mass Index and Quality of Life.** Suijker I., Savas M., van Rossum E.F.C., Langendonk J.G. *Br J Dermatol. 2018;178(5):1209-1210.*

7. **Stress and Obesity: Are There More Susceptible Individuals?** van der Valk E.S., Savas M., van Rossum E.F.C. *Curr Obes Rep. 2018;7(2):193-203.*

8. **Genetic Obesity: Next-Generation Sequencing Results of 1230 Patients With Obesity.** Kleinendorst L., Massink M.P.G., Cooiman M.I., Savas M., van der Baan-Slootweg O.H., Roelants R.J., Janssen I.C.M., Meijers-Heijboer H.J., Knoers N., Ploos van Amstel H.K., van Rossum E.F.C., van den Akker E.L.T., van Haaften G., van der Zwaag B., van Haelst M.M. *J Med Genet. 2018;55(9):578-586.*

9. **Beter Een Vetarm of Koolhydraatarm Dieet: Is Dat Te Voorspellen?** Savas M., van Rossum E.F.C. *Ned Tijdschr Geneeskd 2018; 162:D2924.*

10. **Impact of Glucocorticoid Receptor Polymorphisms on Glucocorticoid Action.** Savas M., van Rossum E.F.C. *Encyclopedia of Endocrine Diseases, Second Edition, vol. 3, pp. 147–156. Oxford: Academic Press; 2019.*

11. **Associations Among Hair Cortisol Concentrations, Posttraumatic Stress Disorder Status, and Amygdala Reactivity to Negative Affective Stimuli in Female Police Officers**. van Zuiden M., Savas M., Koch S.B.J., Nawijn L., Staufenbiel S.M., Frijling J.L., Veltman D.J., van Rossum E.F.C., Olff M. *J Trauma Stress. 2019;32(2):238-248.*

12. **A Comprehensive Diagnostic Approach to Detect Underlying Causes of Obesity in Adults.** van der Valk E.S., van den Akker E.L.T., Savas M., Kleinendorst L., Visser J.A., Van Haelst M.M., Sharma A.M., van Rossum E.F.C. *Obes Rev. 2019;20(6):795-804.*

13. **Hair Glucocorticoids as Biomarker for Endogenous Cushing's Syndrome: Validation in Two Independent Cohorts.** Savas M., Wester V.L., de Rijke Y.B., Rubinstein G., Zopp S., Dorst K., van den Berg S.A.A., Beuschlein F., Feelders R.A., Reincke M., van Rossum E.F.C. *Neuroendocrinology. 2019;109(2):171-178.*

14. **Long-Term Cortisol Exposure and Associations With Height and Comorbidities in Turner Syndrome.** Savas M., Wester V.L., Dykgraaf R.H.M., van den Akker E.L.T., Roos-Hesselink J.W., Dessens A.B., de Graaff L.C.G., de Rijke Y.B., van Rossum E.F.C. *J Clin Endocrinol Metab. 2019;104(9):3859-3867.*

15. **Extensive Phenotyping for Potential Weight-Inducing Factors in an Outpatient Population With Obesity.** Savas M., Wester V.L., Visser J.A., Kleinendorst L., van der Zwaag B., van Haelst M.M., van den Akker E.L.T., van Rossum E.F.C. *Obes Facts. 2019;12(4):369-384.*

16. **Systemic and Local Corticosteroid Use is Associated With Reduced Cognition and Mood and Anxiety Disorders.** Savas M., Vinkers C.H., Rosmalen J.G.M., Hartman C.A., Wester V.L., van den Akker E.L.T., Iyer A.M., McEwen B.S., van Rossum E.F.C. *Neuroendocrinology. 2020;110(3-4):282-291.*

17. **Hair Cortisol Concentrations in Chronic Central Serous Chorioretinopathy.** van Haalen F.M., van Dijk E.H.C., Savas M., Brinks J., Dekkers O.M., Dijkman G.,

van Rossum E.F.C., Biermasz N.R., Boon C.J.F., Pereira A.M. *Acta Ophthalmol. 2020 Jun;98(4):390-95.*

18. **Gender-Specific Effects of Raising Year 1 Standards on Medical Student's Performance and Stress Levels.** Stegers-Jager K.M., Savas M., van der Waal J., van Rossum E.F.C., Woltman A.M. *Med Educ. 2020 Jun;54(6):538-46.*

19. **Children's Hair Cortisol as a Biomarker of Stress at School: A Follow-Up Study.** Groeneveld M.G., Savas M., van Rossum E.F.C., Vermeer H.J. *Stress. 2020 Sep;23(5):590-96.*

20. **In Adults With Obesity, Copeptin Is Linked With BMI but Is Not Associated With Long-Term Exposure to Cortisol and Cortisone.** van der Valk E.S., van der Voorn B., Iyer A.M., van den Berg S.A.A., Savas M.**,** de Rijke Y.B., van den Akker E.L.T., Melander O., van Rossum E.F.C. *Eur J Endocrinol. 2020 Dec;183(6):669-76.*

21. **Anthropometrics And Metabolic Syndrome In Relation To Glucocorticoid Receptor Polymorphisms In Corticosteroid Users.** Savas M., Wester V.L., van der Voorn B., Iyer A.M., Koper J.W., van den Akker E.L.T., van Rossum E.F.C. *Neuroendocrinology. 2020.*

22. **Coping With Stress Before and After Mild Traumatic Brain Injury: A Pilot Hair Cortisol Study.** Spikman J.M., van der Horn H.J., Scheenen M.E., de Koning M.E., Savas M., Langerak T., van Rossum E.F.C., van der Naalt J. *Brain Injury. 2021.*

23. **Anthropometrics in Relation to Endogenous and Exogenous Glucocorticoids During Combined Lifestyle Intervention With Cognitive Behavioral Therapy.** Savas M., van der Voorn B., Janmaat S.C., van der Valk E.S., Wester V.L., Jiskoot G., Iyer A.M., de Rijke Y.B., van den Akker E.L.T., van Rossum E.F.C. *Manuscript submitted.*

24. **Hair Glucocorticoids in Adults With Intellectual Disabilities and Depressive Symptoms Pre and Post Light Therapy: First Explorations.** Hamers P.C.M., Savas M., van Rossum E.F.C., de Rijke Y.B., Bindels P.J.E., Festen D.A.M., Hermans H. *Manuscript submitted.*

25. **Bone Mineral Density Associations With Hair Glucocorticoids and Changes During Combined Lifestyle Intervention With Cognitive Behavioral Therapy in Obesity.** Savas M., Mustafa Z., Zillikens M.C., van Rossum E.F.C. *Manuscript in preparation.*

26. **Long-Term Glucocorticoids Are Associated With Increased Odds of Metabolic Syndrome After an Intensive Combined Lifestyle Intervention.** Mohseni M., van der Valk E.S., Lengton R., Savas M., de Rijke Y.B., van den Berg S.A.A., van der Voorn B., van Rossum E.F.C. *Manuscript in preparation.*

PhD Portfolio

Summary of PhD Training and Teaching

Name PhD student	Mesut Savas
Erasmus MC department	Internal Medicine, division of Endocrinology
Research School	Molecular Medicine
PhD Period	2015 – 2020
Promotor	Prof. Dr. Elisabeth F.C. van Rossum
Co-promotor	Dr. Erica L.T. van den Akker

1. PhD training

General academic skills	Year	Work load (ECTS)
Basic Introduction Course on SPSS	2013	1.0
Advanced Immunology: a short course	2015	0.3
Biostatistical Methods	2015	5.7
Scientific Integrity Course	2016	0.3
Basic Course on R	2016	1.8
Microbiomics I	2017	0.6
Microsoft Excel 2010: Basic	2017	0.3
Microsoft Excel 2010: Advanced	2017	0.3
Introduction in GraphPad Prism version 6	2017	0.3
Bayesian Statistics and JASP	2017	0.3
Workshop Supervising Master and PhD students	2018	0.3
Survival Analysis	2020	0.6

Congress and Meeting Visits – Oral presentations	Venue	Year	Work load (ECTS)
5th Dutch Endocrine Meeting	Noordwijkerhout (NL)	2015	1.0
Science Days Internal Medicine	Antwerp (BE)	2016	1.0
Science Days Internal Medicine	Antwerp (BE)	2016	1.0
6th Dutch Endocrine Meeting	Noordwijkerhout (NL)	2016	1.0
Science Days Internal Medicine	Antwerp (BE)	2017	1.0
7th Dutch Endocrine Meeting	Noordwijkerhout (NL)	2017	1.0
ENDO 2017	Orlando (FL, USA)	2017	1.0
20th European Congress of Endocrinology	Barcelona (ESP)	2018	1.0
25th European Congress on Obesity	Vienna (AU)	2018	1.0
Science Days Internal Medicine	Sint-Michielgestel (NL)	2019	1.0
9th Dutch Endocrine Meeting	Noordwijkerhout (NL)	2019	1.0
Annual Dutch Diabetes Research Meeting	Wageningen (NL)	2019	1.0
ECOICO 2020	Virtual event (online)	2020	1.0
ENEA 2020	Virtual event (online)	2020	1.0

Congress and Meeting Visits – Poster presentations	Venue	Year	Work load (ECTS)
Science Days Internal Medicine	Antwerp (BE)	2015	0.5
ENDO 2016	Boston (MA, USA)	2016	0.5
Science Days Internal Medicine	Antwerp (BE)	2018	0.5
8th Dutch Endocrine Meeting	Noordwijkerhout (NL)	2018	0.5
IMPROCUSH-3	Munich (GE)	2018	0.5

2. Teaching

Supervising students	Year	Work load (ECTS)
Anne Verlinde (BSc student, *Biology*, Wageningen University & Research)	2016	2.0
Simone Janmaat (MSc student, *Medicine*, Erasmus MC)	2017-2018	6.0
Vera Rotee (MSc student, *Medicine*, Erasmus MC)	2018	2.5
Zeynab Mustafa (BSc student, Liberal Arts & Sciences (Pre-Med), Erasmus University College)	2018-current	4.0

Education	Year	Work load (ECTS)
Lecturer skills training medicine students on endocrine topics	2015-2019	2.0

Other	Year	Work load (ECTS)
Physician at academic center of excellence in obesity CGG (Centrum Gezond Gewicht)	2015-2019	5.0
Erasmus MC PhD committee member (representative of all PhD students from the Postgraduate School Molecular Medicine)	2016-2019	1.0
Organizer monthly interdisciplinary research seminars for obesity center CGG affiliated research departments and collaborators	2016-2019	4.0
Peer-reviewer scientific journals	2015-current	3.0
Organizer PhD day Erasmus MC: "*A healthy PhD!*"	2018	0.5

3. Other

Lectures	Venue	Year	Work load (ECTS)
MOSA Conference	Maastricht (NL)	2015	1.0
International Obesity Genetics Collaborations Meeting	Amsterdam (NL)	2016	1.0
Studiedag Paramedici	Apeldoorn (NL)	2017	1.0
International Obesity Genetics Collaborations Meeting	Amsterdam (NL)	2017	1.0

Nederlandse Lipoedeemdag	Maastricht (NL)	2017	1.0
Junior Med School	Rotterdam (NL)	2017	1.0
International Obesity Genetics Collaborations Meeting	Amsterdam (NL)	2018	1.0
Molecular Medicine Course Lecture	Rotterdam (NL)	2018	1.0
The Netherlands Association for the Study of Obesity Spring Meeting	Utrecht (NL)	2018	1.0
Jaarcongres ClaudicatioNet	Apeldoorn (NL	2019	1.0
Students Experienced in Lifestyle and Food (SELF)	Virtual event (online)	2020	1.0

Media performances	Medium	Information	Year
Katja's Bodyscan	TV (KRO-NCRV)	Item about our research in stress and stress-related diseases	2015
Zorg.nu	TV (AVROTROS)	Item about stress in general and measurement of scalp hair cortisol	2016
Livestream press conference	Online (AD.nl, NU.nl and other websites)	Press release about our study on the relationship between use of inhaled corticosteroids and obesity/metabolic syndrome	2017
Studio Erasmus	Show	Talk show about science and current affairs; item about meaning of stress and how to measure its effects	2018
Brard & Jekel: VetGelukkig?!	TV (NTR)	Item about obesity and future obesity treatments	2018

4. Awards and Prizes

Type	Funder	Year
Conference Participation Grant	Stichting Erasmus Trustfonds	2016
Goodlife Healthcare Travel Grant	Dutch Society for Endocrinology	2016
Early Career Award	Endocrine Society	2016
Goodlife Healthcare Travel Grant	Dutch Society for Endocrinology	2017
Best Poster Prize	Erasmus Medical Center (dept. of Internal Medicine)	2018
EASO Travel Grant	European Association for the Study of Obesity	2018
ESE Meeting Grant	European Society of Endocrinology	2018
ESE Journal of Endocrinology Travel Grant	European Society of Endocrinology	2018
Conference Participation Grant	Stichting Erasmus Trustfonds	2018
NASO Travel Grant	Netherlands Association for the Study of Obesity	2018
Goodlife Healthcare Travel Grant	Dutch Society for Endocrinology	2018

About the Author

Mesut Savas was born on September 25th 1990 in Voorburg, The Netherlands. After completing higher education he studied Medicine at the Erasmus University Medical Center in Rotterdam, The Netherlands. He was selected for an extracurricular master's programme and started with his research master Molecular Medicine at the same university. During his education, he published his book "Leidraad Fysische Diagnostiek" about the interpretation of findings with physical examination and was the co-founder of SURE, the student union for national and international students following research master at Erasmus Medical Center. As a junior scientist, he studied the genotype-phenotype correlation of alpha-glucosidase gene mutations in Pompe's disease at the department of Clinical Genetics (Erasmus Medical Center, Rotterdam, The Netherlands) under supervision of prof. dr. Arnold Reuser. For his master thesis, he performed studies related to in vitro glucocorticoid sensitivity and scalp hair glucocorticoids in patients with Turner syndrome at the department of Internal Medicine, division of Endocrinology. After obtaining his master's degrees in Medicine and Molecular Medicine, he continued his scientific career and started his Ph.D. trajectory in the same research group under supervision of prof. dr. Elisabeth van Rossum. He additionally consulted patients at the academic obesity center CGG (Centrum Gezond Gewicht), organized monthly scientific meetings for researchers conducting obesity- and stress-related studies, actively participated in various committees, and was selected for the young talent network as well as for an independent advisory group of experts in healthcare and social domain of the Dutch governmental Council for Public Health and Society (*Raad voor Volksgezondheid* en *Samenleving*). His scientific work has resulted in numerous (inter)national publications and presentations for which he has received multiple awards and prizes. As of May 2019, he started with his residency in Internal Medicine at Reinier de Graaf Gasthuis, Delft, The Netherlands under supervision of dr. Henk Boom and dr. Adrienne Zandbergen (Erasmus Medical Center, Rotterdam).