Long-term cortisol levels measured in scalp hair of obese patients

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ABSTRACT

Objective: In obese subjects a relatively high cortisol output in urine has been observed compared to non-obese individuals. However, cortisol levels in blood, saliva and urine in association with obesity have been inconsistent across studies, possibly due to the high variability of systemic cortisol levels. Cortisol levels measured in scalp hair provide a marker for long-term cortisol exposure, and have been associated with cardiovascular disease in an elderly population and to disease course in Cushing's disease. We aimed to compare hair cortisol levels between obese patients and non-obese controls.

Design and methods: We measured hair cortisol levels of 47 obese patients (median BMI 38.8, range 31.1 – 65.8), 41 overweight and 87 normal-weight subjects using an enzyme-linked immunosorbent assay (ELISA).

Results: Obese patients had higher hair cortisol levels than overweight and normal weight subjects (respectively 30.8 vs 8.5 and 8.4 pg/mg hair, $p<0.001$). No significant difference in hair cortisol levels was found between normal weight and overweight subjects.

Conclusions: Our results suggest a higher long-term cortisol exposure in obese patients, which may contribute to cardiovascular disease risk. Future research will determine whether long-term cortisol levels provide a novel treatment target in the management of cardiovascular disease risk in obesity.

INTRODUCTION

Cortisol, the main glucocorticoid hormone in humans, is produced by the adrenal cortex under the influence of pituitary adrenocorticotropic hormone. An extreme excess of cortisol, as seen in Cushing's syndrome, is often marked by obesity and features of the metabolic syndrome (MetS), including dyslipidemia, hypertension, hyperglycemia and insulin resistance [1, 2]. This observation has given rise to the hypothesis that a modest increase in cortisol exposure may contribute to obesity and MetS in the general population.

In line with this, obesity has been associated with increased cortisol output, as determined by urinary free cortisol. However, these results were inconsistent across studies, and this increased cortisol production does not seem to be reflected by serum and salivary cortisol [3]. These inconsistencies can be partially explained by the high variability in systemic cortisol levels, caused by pulsatile secretion, a diurnal rhythm and day-to-day fluctuations [4]. A relatively novel method to measure cortisol exposure is through scalp hair analysis. Several laboratories have successfully validated hair cortisol concentrations (HCC) as a marker of long-term cortisol exposure for periods of up to several months, thereby avoiding the limitations of time-point measurements [5-7]. With the use of HCC, increased long-term cortisol levels have recently been linked to disease course in Cushing's disease, presence of MetS and cardiovascular disease [8-11].

Previously, a positive correlation has been reported between HCC and body mass index (BMI) and waist circumference [5, 9, 12]. Furthermore, we recently reported increased HCC in obese children [13]. However, in an elderly cohort and a group of healthy adults we found no association between HCC and BMI [5, 10]. Until now, no study has been published which reported long-term cortisol levels in a patient population being evaluated for obesity. Therefore, we devised this study to examine whether obese individuals who visited our obesity center have higher hair cortisol concentrations than non-obese controls.

METHODS AND PROCEDURES

Participants

Obese patients were recruited from an outpatient academic obesity center at the Erasmus MC, Rotterdam, The Netherlands. Inclusion criteria were age above 18 years, BMI above 30 kg/m^2, ability to take part in physical exercise and at least one minor complication related to obesity, such as dyslipidemia, impaired glucose tolerance

or hypertension. Patients were excluded when a secondary cause of the obesity was found, such as Cushing's syndrome, hypothyroidism or syndromal obesity, or if they took glucocorticoid-containing medication. Between October 2011 and September 2013, we approached all patients who fulfilled the inclusion criteria (n=60). In total, 141 healthy normal weight (BMI 18.5 – 24.9) and non-obese overweight (BMI 25.0 – 29.9) subjects from our previous validation study served as controls [5]. This study was approved by the local medical ethics committee. Written informed consent was obtained from all participants. All study procedures were conducted in accordance with the declaration of Helsinki.

Hair sample collection and analysis

From all subjects, a lock of approximately 100 hairs was collected from the posterior vertex prior to treatment for obesity, cut as close to the scalp as possible. Preparation and analysis of hair samples was performed as described previously [5]. In brief, approximately 15 mg of the proximal 3 cm of hair was weighed and finely cut. Extraction took place in 1 ml of methanol at 52 °C for 16 hours. After extraction, methanol was transferred into glass tubes, and evaporated under constant nitrogen stream. Next, 250 µl of phosphate buffered saline (PBS, pH 8.0) were added. Samples were vortexed prior to analysis, which was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit for cortisol in saliva (DRG Instruments GmbH, Marburg, Germany).

Laboratory diagnostics

In obese patients, blood was drawn after an overnight fast. HbA1c, glucose, triglycerides and total, LDL and HDL cholesterol were measured using routine laboratory procedures. Patients were coded as having MetS according to ATPIII criteria [2].

Statistical analysis

IBM SPSS version 21 and GraphPad Prism version 5.01 were used for analysis. Differences in characteristics were tested using Chi-square and Kruskall-Wallis tests. HCC values were logarithmically transformed to attain normal distribution. HCC differences between groups of subjects were analyzed using analyses of (co)variance. Correlations between HCC and metabolic parameters were tested using Spearman's rho. Statistical significance was defined as a *P*-value <0.05.

RESULTS

Sixty obese patients were approached. Of these, 53 had sufficient scalp hair to obtain a sample. One patient was excluded, because she was diagnosed with an obesity-related syndrome. We successfully measured HCC in 47 out of 52 obese patients, and in 128 out of 141 non-obese controls. Baseline characteristics of all subjects are summarized in Table 1. Compared to controls, obese patients were on average older and comprised more women. The majority of obese patients (n=39, 75%) fulfilled ATPIII criteria for diagnosis of MetS, while all of the 13 remaining patients had at least one component of MetS.

Table 1. Baseline and hair characteristics and hair cortisol levels for normal weight, overweight and obese subjects.

	Normal weight	Overweight	Obese	*P* value of difference
	N=87	N=41	N=47	
Baseline characteristics				
Male	37 (43%)	25 (61%)	11 (23%)	0.002
Age	33 (20 – 61)	34 (19 – 63)	47 (18-68)	0.024
BMI (kg/m^2)	22.5 (19.3 – 24.8)	27.2 (25.0 – 29.7)	38.8 (31.1 – 65.8)	<0.001
Hair characteristics				
Hair washings >3 week	65 (76%)	30 (73%)	31 (66%)	0.491
Use of hair products [a]	41 (48%)	20 (49%)	19 (40%)	0.666
Hair coloring [b]	17 (20%)	6 (15%)	14 (30%)	0.170
Hair bleaching [b]	6 (7%)	0 (0%)	5 (11%)	0.116
Hair cortisol levels				
Hair cortisol (pg/mg hair)	8.4 (6.5 – 10.9)	8.5 (5.9 – 12.4)	30.8 (21.8 – 43.5)	<0.001

Values of baseline and hair characteristics represent median (range) or number (percentage). Hair cortisol values represent mean (95% CI). [a]Hair products concern hairspray, mousse, gel and wax used on the day of sample collection. [b]Concerns coloring or bleaching in the 3 months before hair sample collection.

Obese patients had significantly higher HCC than normal-weight and overweight controls ($F(2, 172)=19.788$, $P<0.001$, see Figure 1). However, HCC did not significantly differ between normal weight and overweight controls (post hoc comparison, $P=0.96$; Figure 1, Table 1). Additional stepwise adjustment for age, sex, hair treatment and use of hair products did not significantly change our results. Within obese patients, HCC did not correlate significantly with BMI, waist circumference, HbA1c, LDL cholesterol, HDL cholesterol, triglycerides and glucose (data not shown).

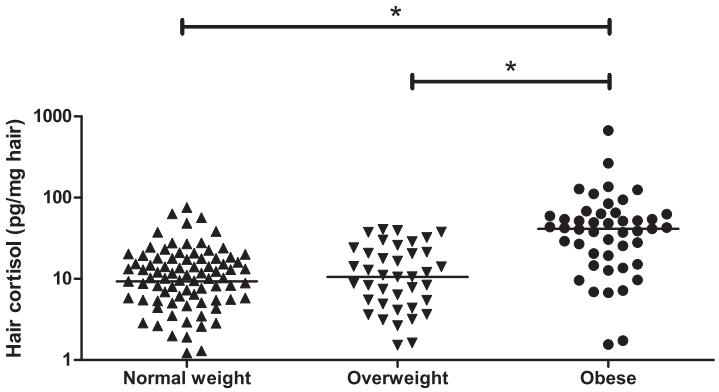

Figure 1. Hair cortisol levels (pg/mg hair, logarithmic scale) in normal weight and overweight controls, and in obese patients.
Horizontal lines represent the group median. *P<0.001

DISCUSSION

In this case-control study, we found that obese patients had higher cortisol levels in scalp hair compared to non-obese controls. Our results are in agreement with results from Stalder *et al*. and our own results in a population of shift work employees, as both studies reported a positive correlation between HCC and BMI [9, 12]. In our previous studies in healthy adults and in a group of elderly subjects, we did not find this correlation, however we observed a correlation with waist/hip ratio in healthy adults [5, 10].

Notably, obese individuals had higher HCC than overweight and normal weight individuals, but HCC was similar between normal weight and overweight subjects. Possibly, the association between increased weight and long-term cortisol only reveals itself in a more extreme phenotype of adiposity. BMI does not discriminate between muscle and fat mass. Although it stands to reason that our obese group (with a median BMI of 38.8) has a higher fat mass than non-obese controls, the same may not be true for overweight versus normal-weight individuals. This may also explain why we did not find a correlation between BMI and HCC in our previous study, which comprised individuals mainly of the normal to overweight BMI group [5].

It would be of interest to study the correlation with features of MetS, however, metabolic data were only available for obese patients, and we found no significant correlation between HCC and cardiometabolic risk factors in this group. Previously, Stalder *et al*., reported significant associations between HCC and all separate components of MetS[9]. However, obese patients in our study represent a relatively metabolically unhealthy

population, with a 75% prevalence of MetS. Presumably, a wider range of risk profiles is needed to reveal associations.

Due to the cross-sectional design of this study, our results do not provide evidence for a causal relationship between high cortisol exposure and obesity. Further research is warranted to investigate whether relatively high cortisol exposure precedes weight gain and other cardiometabolic risk factors. We recently reported an increased HCC in obese children, indicating that long-term cortisol exposure may influence weight early in life [13]. An explanation for the increase in long-term cortisol in obese patients may be that obesity has been associated with psychosocial stress and mental health disorders [14, 15]. This may increase long-term cortisol levels [16]. Furthermore, the increase in HCC we previously reported in shift workers suggests that changes in the diurnal rhythm may be causative for an increased long-term cortisol exposure [12]. Alternatively, one might speculate that liver steatosis associated with obesity affects cortisol breakdown, inducing a vicious circle with elevated long-term systemic cortisol levels, which may in turn promote adiposity [17].

In follow up studies of these patients we will investigate whether therapies for weight loss are associated with a decrease in long-term cortisol levels. The associations between HCC and cardiovascular disease and MetS that recently have been reported provide a first indication that strategies that lower long-term cortisol levels may reduce cardiovascular risk [9-11].

In conclusion, we found that obese patients who visit an outpatient obesity clinic have significantly higher cortisol levels in scalp hair than normal-weight and overweight subjects. Future research will show whether long-term cortisol provides a novel treatment target in the reduction of cardiovascular disease risk in obese patients.

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