Prader-Willi Syndrome

New perspectives and effects of growth hormone treatment in children

Roderick F.A. de Lind van Wijngaarden

.

Cover inspired by my view on pediatrics: Accompanying children along their path of life, while supporting and guiding them toward unending possibilities that lie within the scope of the horizon.

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Prader-Willi Syndrome

New perspectives and effects of growth hormone treatment in children

Prader-Willi Syndroom

Nieuwe inzichten en effecten van groeihormoonbehandeling bij kinderen

Proefschrift

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Aan Celine

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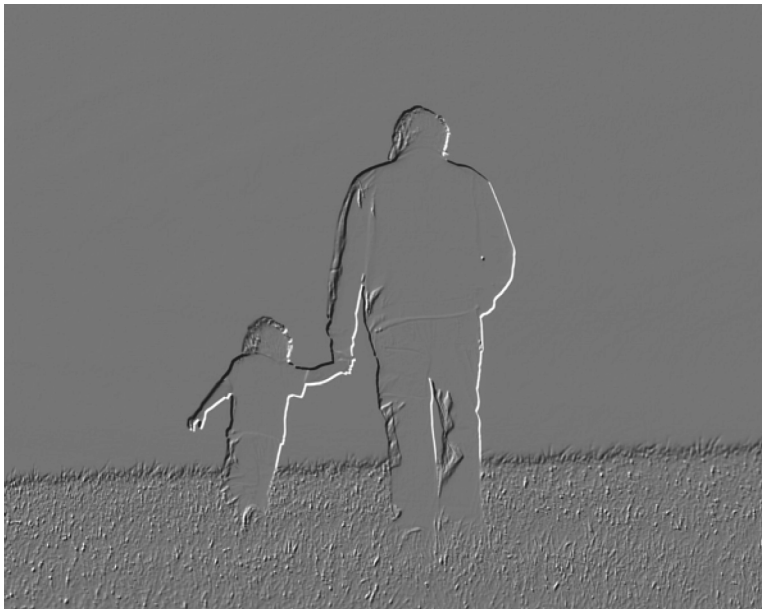

Introduction

This thesis encompasses studies embedded in the Dutch national growth hormone trial for children with Prader-Willi syndrome (PWS). The syndrome was first described by Prader, Labhart, and Willi in 1956, reporting a combination of obesity, short stature, cryptorchidism, oligophrenia, and neonatal hypotonia.[1] To date, our knowledge about this syndrome has grown vastly, although much remains to be elucidated. In concordance with this growing knowledge, new questions and dilemmas are met. This chapter provides an overview of the clinical manifestations of PWS and the chromosomal defects from which it originates. In the scope of this thesis, several symptoms are described in detail and the effects of growth hormone treatment are commented on. Subsequently, the issue of sudden death in children with Prader-Willi syndrome is discussed. Finally, the objectives of the studies described in the following chapters are presented.

1.1 Prader-Willi syndrome

Prader-Willi syndrome is a genetic disorder that originates from lack of expression of the Prader-Willi region of the paternally derived chromosome 15. Clinical characteristics of children with PWS include hypotonia, short stature, psychomotor delay, hypogonadism, behavioral problems, and hyperphagia resulting in obesity when uncontrolled. Symptoms are often the result of hypothalamic dysfunction, to various extents. Based on epidemiological surveys, the birth incidence is estimated to be around 1 in 30,000.[2-5] PWS is not associated with social-economic status and the distribution between both sexes is considered equal. The symptoms of children with PWS vary with age and are usually subdivided into four phases: a fetal and neonatal phase, an infantile hypotonic phase, a childhood obese phase, and an adolescent phase with particular behavioral features.

1.1.1 Prader-Willi syndrome during the fetal and neonatal phase

Although highly variable in nature and severity, a fetal phenotype of PWS can be distinguished, comprising of decreased fetal movements, polyhydramnios and an abnormal posture on an ultrasonogram of hands with flexed wrists and dorsi-extended feet with flexed toes.[6, 7] The incidence of breech delivery and delivery by caesarean section is increased. Breech delivery has been related to hypotonia, but also fetal hypothalamic dysfunction has been suggested to play an important role.[8, 9] Both premature and postterm deliveries are frequently observed, with delivery being more than 3 weeks early or late in about one third of patients.[6, 10] Birth weight is more severely reduced than birth length and is below -2 standard deviation score (SDS) in up to 20%.[2] Neonates with PWS have severe hypotonia

mainly affecting the neck and have a weak or absent cry (Figure 1).[11, 12] Due to the severe hypotonia and poor sucking reflex, feeding via a nasogastric canula is usually required to prevent failure to thrive, defined as descent across the centiles of weight or body mass index (BMI). Hypoplasia of the external genitalia is common in both sexes. Nearly all boys have cryptorchidism, a hypoplastic scrotum and a decreased penile length. Girls may have hypoplastic labia, but this is less obvious and easily missed. The saliva is usually sticky and rich in proteins. Additionally, the salivary flow is 20% of that in healthy children, severely increasing the risk for caries later in life.[13] Neonates with PWS often have temperature instability.[14] With the availability of rapid genetic testing and recommendations to exclude the diagnosis of PWS in any neonate with hypotonia, feeding difficulties, low reflexes, and/or hypoplasia of the external genitalia, the diagnosis is now usually confirmed in the neonatal period.[2, 5, 15-17]

1.1.2 Prader-Willi syndrome in the infant phase

After the neonatal phase, hypotonia becomes gradually less marked, although infants with PWS persist to have feeding difficulties, and failure to thrive (Figure 1). Psychomotor development is delayed, in particular gross motor development and speech development. Body composition is abnormal, with a low lean body mass and a high body fat percentage, even in infants who are underweight and have failure to thrive.[18, 19] Although also present in the neonatal phase, typical dysmorphic features become more pronounced in the infant and childhood phase, showing a narrow bifrontal diameter, almond-shaped eyes, a thin downturned upper lip, and a narrow nose.[14] Many children with PWS have hypopigmentation, fair hair, and blue eyes, although these characteristics are primarily seen in children with a deletion.[20] Sleep-related breathing disorders (SRBD) are a common feature in PWS and are seen in almost 100% of children, even at a very early age.[21-23]

1.1.3 Prader-Willi syndrome in childhood

In childhood (Figure 1), the hypotonia improves, although reduced physical activity remains present. The original feeding difficulties improve and excessive appetite with hyperphagia may develop, which may result in extreme obesity, when uncontrolled by dietary measures. Abnormal body composition persists in childhood, contributing to exercise intolerance. Furthermore, children may have genu valgum and often develop scoliosis and kyphosis.[24, 25] They have poor growth, resulting in short stature and small hands and feet, and have behavioral problems, such as temper tantrums and obsessive compulsive behavior, often in relation to food. Skin picking may be present. Many have mild to moderate learning disability, but some children are able to attend regular schools.[26]

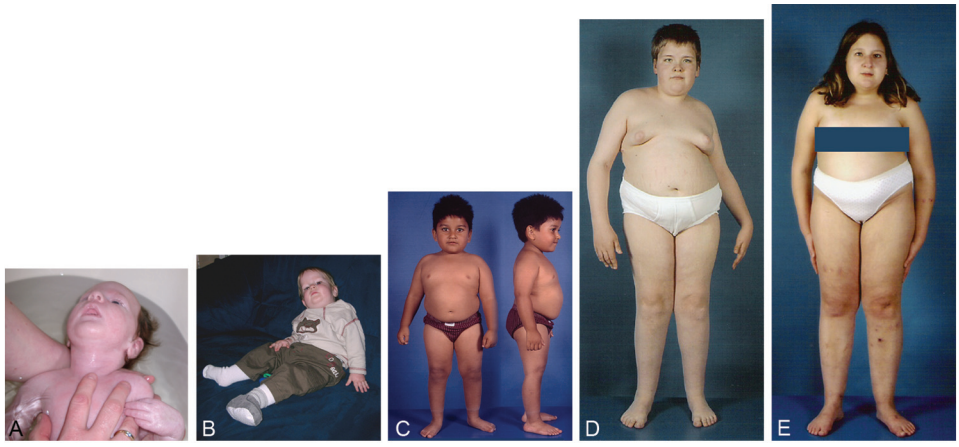

Figure 1. Different phases of PWS.
PWS during the neonatal and infant phase in the same boy at 6 weeks (A) and 10 months of age (B). PWS in childhood in a boy of 3 years and 11 months (C). PWS in adolescence in a boy of 11 years and 7 months (D) and a girl of 16 years (E). None of the children had received growth hormone treatment. Photos are depicted with permission of parents and children.

1.1.4 Prader-Willi syndrome in adolescence

Due to hypogonadotropic hypogonadism, puberty in adolescents with PWS may be delayed, incomplete, or even absent (Figure 1).[27] Pubertal delay may be caused by a combination of both hypothalamic dysfunction and primary hypogonadism. In these patients pubertal signs should be pharmacologically induced. In contrast, some children have precocious puberty.[28] Isolated premature pubarche has been reported in 14% and is probably related to early maturation of the zona reticularis of the adrenal gland.[29, 30] Spontaneous growth velocity is impaired and the pubertal growth spurt may be lacking, which both contribute to a decreased adult height. In adolescence, behavioral abnormalities may become worse, in particular in relation to daily life routines.[31] Most patients with PWS have mental disability, although there is a wide variation in severity. Psychosis may occur, in particular in children with maternal uniparental disomy (UPD).[32, 33]

1.1.5 Prader-Willi syndrome in adulthood

The transition from childhood to adulthood is complex. In patients with PWS, this transition is even more complicated due to behavioral and psychiatric problems.[26, 34, 35] Pervasive food seeking behavior warrants vigorous control, but ethical concerns should be appreciated, especially with regard to autonomy of the patient. The clinical manifestation of PWS results

in adults being incapable of living independently.[26, 35] Although the concept of group homes or living alone under continuous supervision may at first be rejected by the patient, the structure provided by these housing concepts improves quality of live and is later often preferred. The change in health care settings and providers also produces challenges, but disruption of comprehensive multidisciplinary care should be avoided.

Median adult height is 145 to 150 cm for women and 155 to 162 cm for men.[35-37] In adults with compromised pubertal development and absence of pubertal induction, secondary sex characteristics are often absent or incomplete.[26, 35] In contrast, there are few case reports of pregnancy in females with PWS, although paternity in PWS has never been reported.[2, 38, 39]

The current adult population was usually diagnosed relatively late. The diagnosis was based on the number of symptoms listed in the consensus diagnostic criteria, often when obesity was already present.[26, 40, 41] To date, the majority of patients are diagnosed during the first months of life by genetic testing.[5, 42] An earlier diagnosis should allow earlier introduction of care aiming to reduce morbidity and improve quality of life. There are, however, no reports available on the clinical picture of adult patients with PWS who have been diagnosed early in life and have been treated from infancy onwards with diet, exercise, and hormonal substitution.

1.2 Genetics

Until 1981, the diagnosis of PWS was solely based on the appearance of a combination of symptoms listed in the consensus diagnostic criteria.[14] In 1981, the chromosome affected in patients with PWS was first identified.[43] This report showed that the majority of patients with PWS suffered a deletion in chromosome 15. In 1982, it was discovered that this deletion only affected the paternally inherited chromosome.[44] To date, Prader-Willi syndrome is defined as the lack of expression of paternally inherited genes sited on chromosome 15 locus q11-q13. Expression of these genes is lost due to either a deletion, a maternal uniparental disomy, an imprinting center defect, or a Robertsonian translocation.[2, 20, 45-50]

1.2.1 Chromosomal defects

Deoxyribonucleic acid (DNA) contains the genetic instructions used in the development and functioning of all known living organisms and some viruses. The main role of DNA molecules is the long-term storage of information. The DNA segments that carry the genetic information are called genes. A gene consists of two strands of amino acids, which are entwined and form the shape of a double helix (Figure 2). DNA is folded several times and densely packed into chromosomes. Thus, a chromosome is a single piece of DNA that contains many genes, regulatory elements and other nucleotide sequences.

Chromosome 15 contains a region q11-q13, which is called the Prader-Willi region. As implied by its name, this region is critical for the Prader-Willi syndrome. In healthy subjects, the q11-q13 region of the maternally inherited chromosome 15 is silenced by a process called imprinting, whereas this region of the paternally derived chromosome is not (Figure 3). Loss of the signal in this region of the paternally inherited chromosome causes Prader-Willi syndrome.

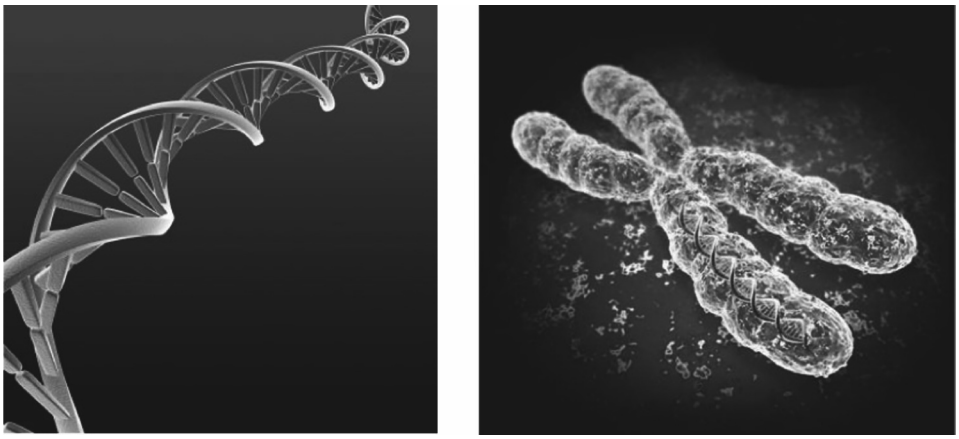

Figure 2. Schematic overview of the DNA double helix and a chromosome during duplication.

In patients with a deletion, the q11-q15 region is absent. According to literature, a deletion is the most common chromosomal defect, present in 70% of patients.[2, 20, 45] The defect occurs either as a large deletion (type I) or as a smaller deletion with a more distal breakpoint (type II). In children with maternal uniparental disomy (UPD), the paternally inherited chromosome 15 is absent, while two copies of the maternally inherited chromosome 15 are present. UPD has been reported in 20 to 25% of patients.[46, 47] In case of an imprinting center defect (ICD), occurring in 5% of patients, the PWS region of the paternally inherited chromosome 15 is silenced (imprinted).[48, 49] In less than 1% of patients, part of the paternally derived chromosome 15 is situated on another chromosome, called a Robertsonian translocation.[20, 50, 51] It should be underlined that prevalences of specific chromosomal defects are based on studies which included patients diagnosed according to clinical criteria. Therefore, patients with less evident forms of PWS were not included and these reported prevalences may be biased.

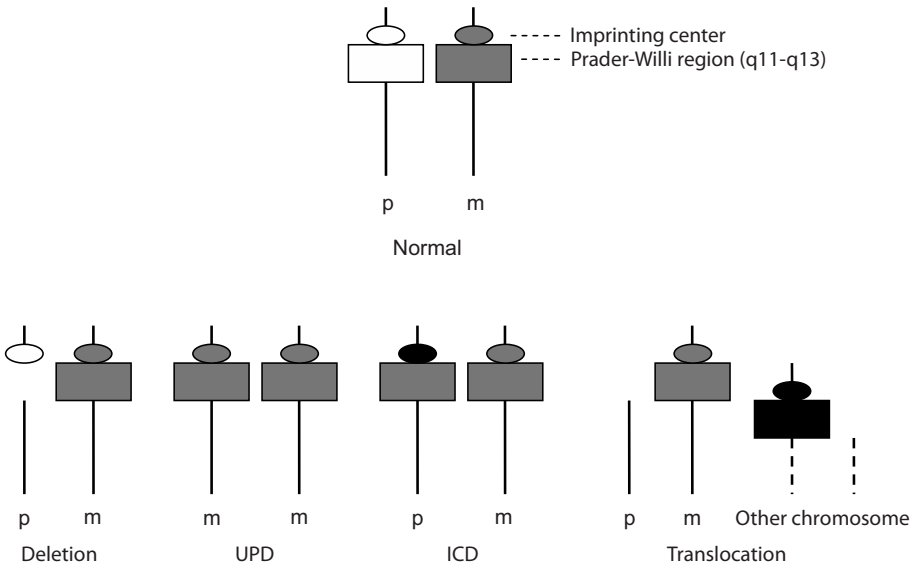

Figure 3. Simplified schematic overview of the chromosomal defects described in Prader-Willi syndrome.
P, paternally derived chromosome; m, maternally derived chromosome; UPD, maternal uniparental disomy; ICD, imprinting center defect causing imprinting of the paternal locus; Translocation, Robertsonian translocation to another chromosome. Grey squares represent silenced loci. Black represents a defect.

As the signal on the paternal chromosome 15q11-q13 is lost, genes only demonstrate a maternal imprint.[50, 52] The diagnosis of PWS can therefore be confirmed or rejected by DNA methylation analysis, most commonly performed by using DNA methylation-specific techniques at the SNURF-SNRPN locus.[2, 20, 53] If DNA methylation analysis shows only a maternal pattern, then PWS is confirmed. Further tests may then be performed to determine the genetic subtype. Genetic counseling is required, in particular with regard to the recurrence risk. In case of an imprinting center defect, the recurrence risk is up to 50% if the father is a carrier for the imprinting center deletion. In case of chromosomal translocations, the recurrence risk may be up to 10%. For the remaining genetic subtypes, the recurrence risk is the same as in the general population (less than 1%).[49, 54]

1.2.2 Genotype-phenotype correlations

The different chromosomal defects causing PWS result in an underexpression of paternally derived genes and/or overexpression of maternally derived genes. These differences in

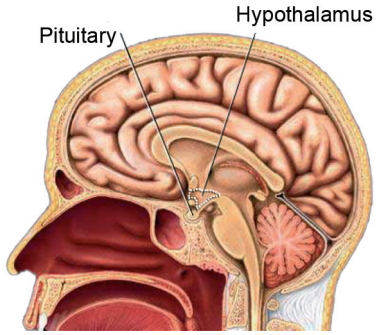

Figure 4. The role of the hypothalamus is critical in the pathophysiology of PWS.

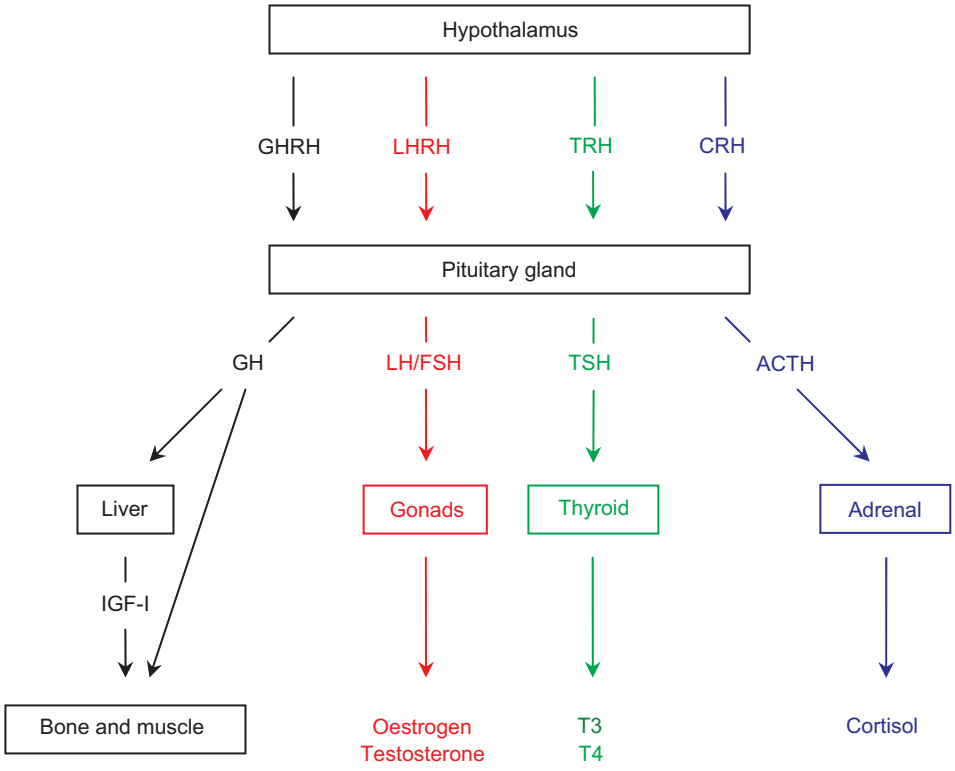

Figure 5. Simplified schematic overview of hormonal secretory control by the hypothalamus. GHRH, growth hormone releasing hormone; GH, growth hormone; IGF-I, insulin-like growth factor I; LHRH, luteinizing hormone releasing hormone; LH, luteinizing hormone; FSH, follicle stimulating hormone; TRH, thyrotropin releasing hormone; TSH, thyroid stimulating hormone; CRH, corticotropin releasing hormone; ACTH, adrenocorticotropin releasing hormone.

expression may explain the increasingly recognized genotype-phenotype correlations. For example, hypopigmentation is primarily seen in those with a deletion. The characteristic facial phenotype is less pronounced in patients with UPD, but have a higher risk of psychosis. Fruthermore, patients with UPD have higher verbal intelligence scores and less maladaptive behavior, compared to those with a deletion.[20, 32, 33, 55-57] Intellectual ability, academic achievement, and behavioral and psychological problems are more severely infected in patients with a type I deletion than in those with a smaller type II deletion or UPD.[58-62]

1.3 Hypothalamic dysregulation

The hypothalamus is an “organic control center” situated in the brain. As most symptoms in PWS are related to hypothalamic dysregulation, knowledge of the anatomy and function of the hypothalamus is critical for understanding the pathophysiology of PWS.[9]

1.3.1 Anatomy, function and dysregulation of the hypothalamus

The hypothalamus is an almond-sized control center situated in the middle of the brain, just above the brain stem and under the thalamus (Figure 4). The hypothalamus is a portion of the brain that contains a number of small nuclei with a variety of functions. It is connected to the pituitary gland via the infundibulum (pituitary stalk). One of the most important functions of the hypothalamus is to link the nervous system to the endocrine system via the pituitary gland. The hypothalamus synthesizes and secretes neurohormones, often called hypothalamic releasing hormones, and these in turn stimulate or inhibit the secretion of hormones from the anterior lobe of the pituitary gland. The pituitary hormones include growth hormone (GH), the gonadotropins luteinizing and follicle stimulating hormones (LH/FSH), thyroid stimulating hormone (TSH), and adrenocorticotropic hormone (ACTH) (Figure 5).

The hypothalamus is responsible for numerous metabolic processes and activities of the autonomic nervous system, including the control of body temperature, activity level, appetite, anger, breathing, and circadian cycles. Symptoms in PWS that have been related to hypothalamic dysregulation include abnormal temperature control, excessive daytime sleepiness, sleep-related breathing disorders, abnormalities of sleep architecture, insatiable hunger, decreased activity level and energy expenditure, temper tantrums, hypogonadotropic hypogonadism, cryptorchidism, and growth hormone deficiency or insufficiency.[2, 9, 27, 63-68] Studies using magnetic resonance imaging (MRI) techniques, have demonstrated neuroradiologic alterations in patients with PWS, such as absence of the pituitary bright spot as a marker of hypothalamic-pituitary axis integrity and reduced pituitary height possibly reflecting hypothalamic insufficiency.[69, 70]

1.3.2 Growth hormone and insulin-like growth factor I

The hypothalamus plays a central role in the control of the growth hormone and insulin-like growth factor I axis (GH/IGF-I axis). Growth hormone releasing hormone (GHRH) is produced in the arcuate nucleus of the hypothalamus and is carried by the hypothalamo-hypophyseal portal circulation to the anterior pituitary gland where it induces GH secretion by stimulating the GHRH receptor. GHRH is released in a pulsatile manner, stimulating similar pulsatile release of GH. Peaks of GH secretion occur at 3- to 5-hour intervals lasting 10 to 30 minutes. Most GH is secreted during sleep: nearly 50% of GH secretion occurs during the third en fourth cycle of REM sleep stages.[71, 72]

Much of the GH in the circulation is bound to a protein (growth hormone binding protein, GHBP) which is derived from the growth hormone receptor. GH exerts its anabolic function both directly and indirectly. It directly stimulates bone growth and increases muscle mass. GH also stimulates production of insulin-like growth factor I (IGF-I), primarily in the liver. IGF binding protein 3 (IGFBP-3) is the major carrier protein of IGF-I and binds 70-95% of IGF-I as a binary complex or as a ternary complex together with the acid labile subunit (ALS). IGF-I has growth-stimulating effects on a wide variety of tissues. IGF-I is also independently generated within various tissues. Thus, IGF-I is considered both an endocrine and an autocrine/paracrine hormone. IGF-I also has stimulatory effects on osteoblast and chondrocyte activity to promote bone growth. Reduced production and secretion of GH is called GH deficiency.

Depending on the cut-off levels used, 60-80% of children with PWS might be considered GH deficient. Spontaneous 24-hour GH secretion and the response to GH provocation tests using arginine, clonidine, or insulin-induced hypoglycaemia are reduced.[73-77] Low GH levels are also found in children with non-syndromal obesity, then coinciding with normal IGF-I levels. In contrast to non-syndromal obesity, the majority of patients with PWS have reduced serum IGF-I levels.[2, 28, 68, 74, 78] The GH deficiency in PWS has often been suggested to be of hypothalamic origin.[2, 68, 74, 76]

1.3.3 Growth hormone treatment

In 2000, an international group of pediatric endocrinologists agreed that medical evidence indicated that dysregulation of the GH/IGF-I axis is nearly universal in children with Prader-Willi syndrome (PWS), and thus, these patients should be offered GH treatment.[79] Many studies have shown that short-term GH treatment, administered for one or two years, improves gain in height.[28, 73, 74, 80-88] However, only few patients have been followed until adult height.[66, 89] It has been suggested that a normal adult height may be attained, particularly when GH treatment is started before onset of puberty.

Short-term GH treatment improves, but does not normalize, body composition by decreasing body fat percentage and increasing lean body mass.[74, 82-84] Furthermore, GH treatment

improves psychomotor development in the very young and has psychological and behavioral benefits.[74, 85, 88, 90] Although GH has been proven beneficial for children with PWS during one or two years of treatment, reports on long-term efficacy and safety were very scarce.[66, 89, 91] One study showed a dose-dependent effect on height, body composition and resting energy expenditure.[91] In that study, short-term metabolic effects of GH treatment were optimal with a dose of at least 1 mg/m^2·day. However, the decreased fat percentage was not sustained with 1.0 mg/m^2·day. Lean body mass persisted to increase after 2 years of GH treatment, but SD-scores for both fat percentage and lean body mass were not calculated. As lean body mass is highly correlated with height, it is unknown whether the increase in lean body mass was merely the result of the increase in height or reflected an increase in lean body mass SD-score.

Glucose homeostasis and serum lipids were neither adversely affected by short-term GH treatment, nor by long-term GH treatment in a small group of patients.[66] Effects of GH treatment on blood pressure in children with PWS were never reported. Thus, although GH treatment seems beneficial for children with PWS, results on long-term GH treatment in a large group of children, followed for 4 consecutive years and continuously treated with one standardized dose of 1 mg/m^2·day before onset of puberty, have not been reported. Notably, GH treatment does not resolve all symptoms of PWS, but is part of the multidisciplinary care involving many medical and allied health disciplines, with the ultimate goal of promoting health and increasing quality of life.

1.4 Sudden death in children with Prader-Willi syndrome

The annual death rate in PWS patients is very high (3% under 30 years).[3] Among the causes of death in older children and adults are cor pulmonale,[26, 40] fatal apneas,[92] unexpected bathtub drownings,[93] and gastric necrosis.[94] In 2002, the first reports were published on sudden death in young children with PWS.[95, 96] Importantly, these patients died of respiratory and cardiorespiratory insufficiency. An interesting international series of cases and a review reported that sudden death in children with PWS occurred particularly during mild to moderate respiratory tract infection and primarily in the early morning during sleep.[92, 97]

Patients with PWS suffer from sleep-related breathing disorders, consisting of obstructive and central sleep apneas. The sleep apneas are the result of a reduction in upper airway diameter, hypoventilation, decreased pulmonary function, and/or a decreased ventilatory and arousal response during hypercapnia.[21-23, 65, 92, 98] Therefore, several authors hypothesized that fatal apneas could be a cause of sudden death in children with PWS (Figure 6). It was postulated that GH treatment could compromise sleep-related breathing by causing tonsillar hypertrophy resulting in increased pharyngeal narrowness. In 2003, a warning label was

applied stating that GH treatment is contraindicated in patients with severe obesity and/or respiratory impairment. However, several studies, including ours, have shown that GH treatment has no effect on sleep-related breathing in children with PWS.[21-23] Interestingly, two of these studies showed a marked increase in the number of obstructive and central apneas during upper respiratory tract infections (URTI).[22, 23] Thus, illness rather than GH treatment seemed to cause an increase in preexisting sleep apneas resulting in sudden death (Figure 6).

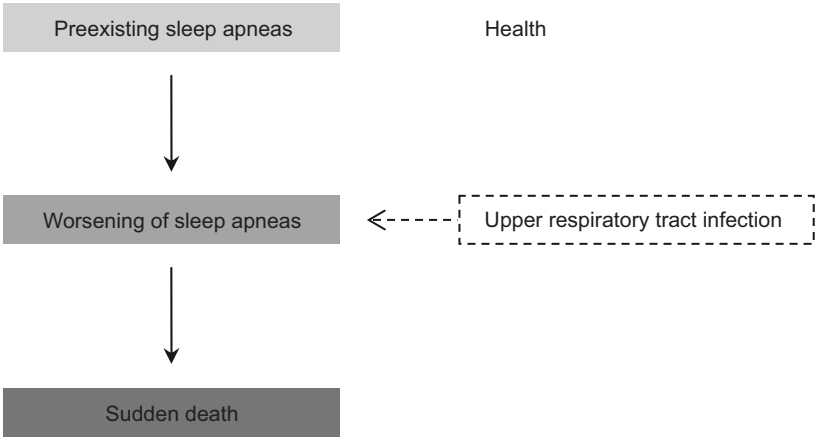

Figure 6. Schematic overview of the hypothesis of sudden death in children with PWS.

There are however some limitations to this hypothesis. Not all children who died unexpectedly had enlarged tonsils or an upper respiratory tract infection.[97, 99] Furthermore, sudden deaths also occurred in non-obese children without preexisting sleep apneas.[22] In fact, the sudden deaths in children with PWS often share a similar pattern: a sudden deterioration during a mild or moderate infection, which is often – but not always – an upper respiratory tract infection. One study reported small adrenal glands during autopsies in children who died unexpectedly.[99] These findings, coinciding with hypothalamic dysregulation in patients with PWS, might be indicative of central adrenal insufficiency. However, studies investigating the integrity of the hypothalamus-pituitary-adrenal axis had not been conducted. Furthermore, there were no published studies investigating the relationship between sleep-related breathing and central adrenal insufficiency.

1.5 Central adrenal insufficiency

The adrenal gland is the end organ of the hypothalamus-pituitary-adrenal axis (HPA-axis) and produces and secretes cortisol. Cortisol is essential for survival, particularly during stress, and regulates cardiovascular, metabolic, immunologic, and homeostatic functions. Inability to produce and secrete sufficient amounts of cortisol in reaction to stress, termed adrenal insufficiency, may lead to an Addisonian crisis, ultimately resulting in death.

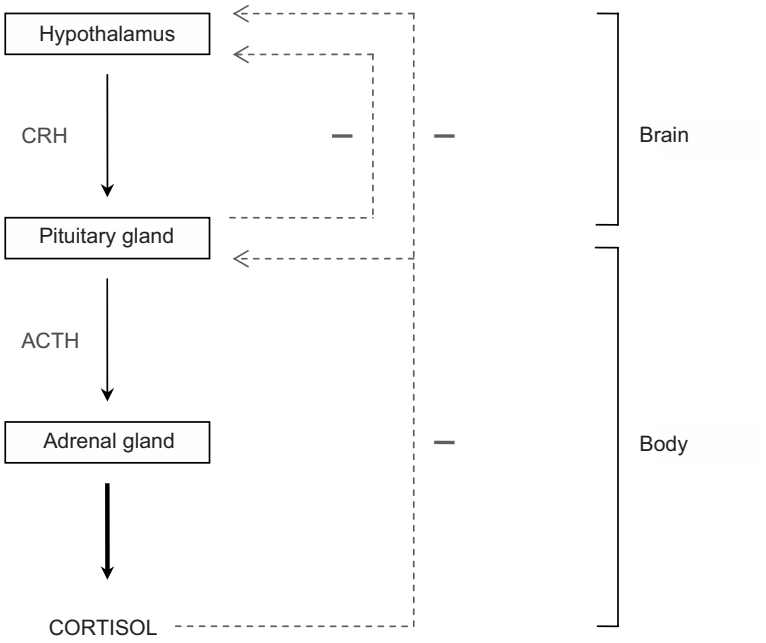

Figure 7. Simplified schematic overview of the HPA-axis.

Corticotropin releasing hormone (CRH) is secreted by the paraventricular nucleus of the hypothalamus in response to stress (Figure 7). The hypothalamo-pituitary portal system carries the CRH to the anterior lobe of the pituitary gland, where it stimulates corticotropes to secrete adrenocorticotropic hormone (ACTH). ACTH is secreted in the blood stream and transported to the adrenal glands where it induces production and secretion of cortisol. The HPA-axis has a feedback mechanism. The increase or decrease in CRH, ACTH, and cortisol levels automatically trigger a stimulation or inhibition of the HPA-axis thereby restoring the important equilibriums between these hormones.

Adrenal insufficiency may originate from the hypothalamus or pituitary gland (central) or from the adrenal gland itself (peripheral or primary).[100] Adrenal insufficiency may be diagnosed by measuring the diurnal cortisol rhythm or by testing the integrity of the HPA-axis with a variety of dynamic tests.[101] The metyrapone test has the highest sensitivity and specificity for measuring central adrenal insufficiency (Appendix A).[101-107]

1.6 Scoliosis

Spinal deformity is a major concern for patients with PWS. Scoliosis is a spinal curve with a Cobb's angle of more than 10° on a standing posteroanterior radiograph (Figure 8). The Cobb's angle is the angle between the two steepest vertebrae, i.e. the upper border of the upper vertebra in the curve and the lower border of the lower vertebra.[108] The prevalence of adolescent idiopathic scoliosis in the general Dutch population is 2.7%,[109] but the prevalence of scoliosis in PWS was not precisely known.

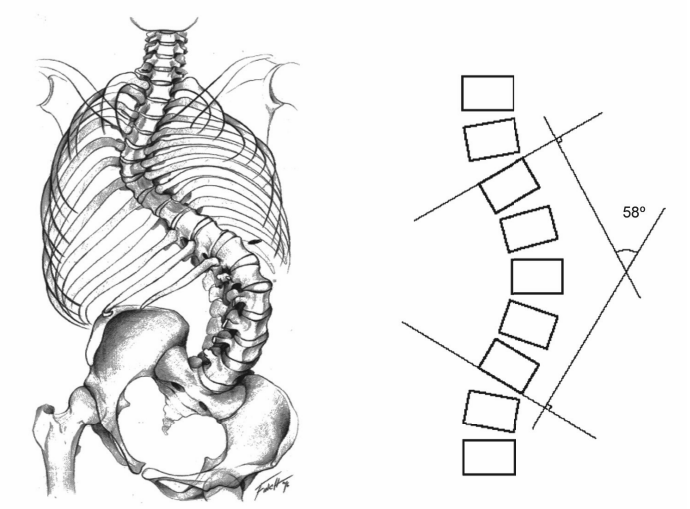

Figure 8. A severe scoliosis (left) and measurement of Cobb's angle (right).

Information on scoliosis in PWS was scarce and varied greatly.[24, 25, 40, 110, 111] The prevalence was estimated to be between 15% and 86%.[24, 25, 40, 110, 111] All studies were retrospective and included both adults and children, without and during GH treatment. Some calculated prevalences from questionnaires, whereas others studied material assembled over a great

time span. Thus, no accurate data concerning prevalence of scoliosis were available for children with PWS. Accelerated growth, either spontaneous or during GH treatment, has been associated with the onset of scoliosis and scoliotic curve progression.[112-117] Scoliosis was therefore often considered a contraindication for GH treatment. However, a randomized controlled trial investigating the effect of GH treatment on onset and progression of scoliosis had never been performed.

1.7 Bone mineral density

Bone mineral density (BMD) is the result of the equilibrium between bone formation and bone resorption. Adequate mineral acquisition during childhood is important for reaching peak BMD in late adolescence. A decreased peak BMD is a major determinant for osteoporosis and fracture risk later in life. Adequate gonadal hormone levels in pubertal development are vital for attainment of peak BMD.[118, 119] In cross-sectional studies of adults with PWS, BMD was decreased in 60% to 90% of subjects.[120-122] Furthermore, osteoporosis is considered a supportive finding in the diagnostic criteria of adult PWS.[14] The decreased BMD may account for the higher risk of fractures during life and complications during spinal surgery in patients with PWS.[20, 24, 120, 122] Although childhood is the critical period for bone accumulation, reports on BMD in children with PWS were very scarce.[85, 123, 124] Due to short stature in children with PWS, true BMD is underestimated by the standard areal measurement and should be corrected for bone size by calculating lumbar spine bone mineral apparent density ($BMAD_{LS}$).[125-129] As evaluation of BMD was not included in the aims of previous studies in children with PWS, a correction for short stature was never applied and $BMAD_{LS}$ was never reported.[85, 123, 124]

In patients with GH deficiency, BMD is decreased but normalizes during GH treatment.[127, 128] However, GH and IGF-I secretion are usually more affected in GH-deficient children than in those with PWS.[77] The effects of GH treatment on BMD in children with PWS were unknown.

1.8 Cardiovascular and metabolic risk factors

The prevalence of cardiovascular disease and diabetes mellitus type II is increased in adult patients with PWS, resulting in increased mortality.[5, 26, 40, 97, 121, 130] Microcirculatory dysfunction may be present at an early age.[131] To reduce morbidity and prolong life expectancy, it is important to have a clear view on the prevalence of cardiovascular risk factors in children with Prader-Willi syndrome.

1.8.1 Body composition, blood pressure, and serum lipids

Children with PWS have an unfavorable body composition, with a high fat percentage (fat%), even in underweight infants.[2, 74, 87, 91, 132, 133] The increased fat% in patients with PWS is most likely in part due to GH insufficiency.[2, 74, 87, 91, 132, 133] However, although fat%SDS decreased in response to GH treatment, it did not completely normalize. This suggests that GH insufficiency is not the only cause of the high fat%.

There was a paucity of knowledge regarding the metabolic and cardiovascular risk profile in young children with PWS. The high fat% is considered a cardiovascular and metabolic risk factor, as well as a high blood pressure, but this was never investigated in children with PWS. Dyslipidemia may be present, also before puberty.[75, 134] In contrast, insulin resistance was less than expected based on the degree of obesity, with higher adiponectin levels compared to obese controls but still lower than lean controls.[135-137] In addition, the presence of the metabolic syndrome was never investigated in children with PWS. Furthermore, reports describing effects of GH treatment on cardiovascular and metabolic risk factors were very scarce.

1.8.2 Acylation stimulating protein

Adipose tissue is a metabolically active organ producing numerous proteins, enzymes, and hormones. Acylation stimulating protein (ASP, Figure 9), also known as C3adesArg, is a hormone generated through activation of the alternative complement pathway proteins C3, B, and adipsin, and is produced by adipocytes.[138] ASP stimulates free fatty acid incorporation into adipose tissue by increasing triglyceride synthesis and storage and inhibiting hormone-sensitive lipase mediated triglyceride lypolysis.[139-144] Furthermore, ASP increases glucose uptake through enhanced translocation of glucose transporters to the plasma membrane surface. Effects of ASP are both additive and independent of those of insulin.[141-143] By controlling the storage of triglycerides, ASP is an important factor in keeping lipid levels within normal range.

In healthy subjects, an increase in ASP levels results in lower triglyceride levels and accumulation of fat tissue. However, in obesity, insulin resistance, dyslipidemia, and cardiovascular disease, both ASP and triglyceride levels are increased, suggestive of ASP resistance.[145-149] In vitro, this is demonstrated by reduced specific binding and response to ASP of cells from subjects with high ASP levels.[150, 151] Thus, ASP is an important marker for abnormal lipid metabolism and accumulation of fat tissue, whereas high ASP levels are associated with an increased risk of cardiovascular disease.[138, 147, 149]

As earlier discussed, the cause of the high fat% in patients with PWS is multifactorial, suggested by the fact that GH treatment does not completely normalize fat%.[74, 87, 91, 132, 133] Given its critical role in triglyceride storage in adipose tissue, ASP may contribute to the high fat% and may play a role in the lack of complete normalization of body composition

during GH. As ASP increases glucose uptake, it might also be involved in keeping glucose and insulin levels within normal range, despite the high fat% in children with PWS. However, there were no reports on ASP levels in children with PWS.

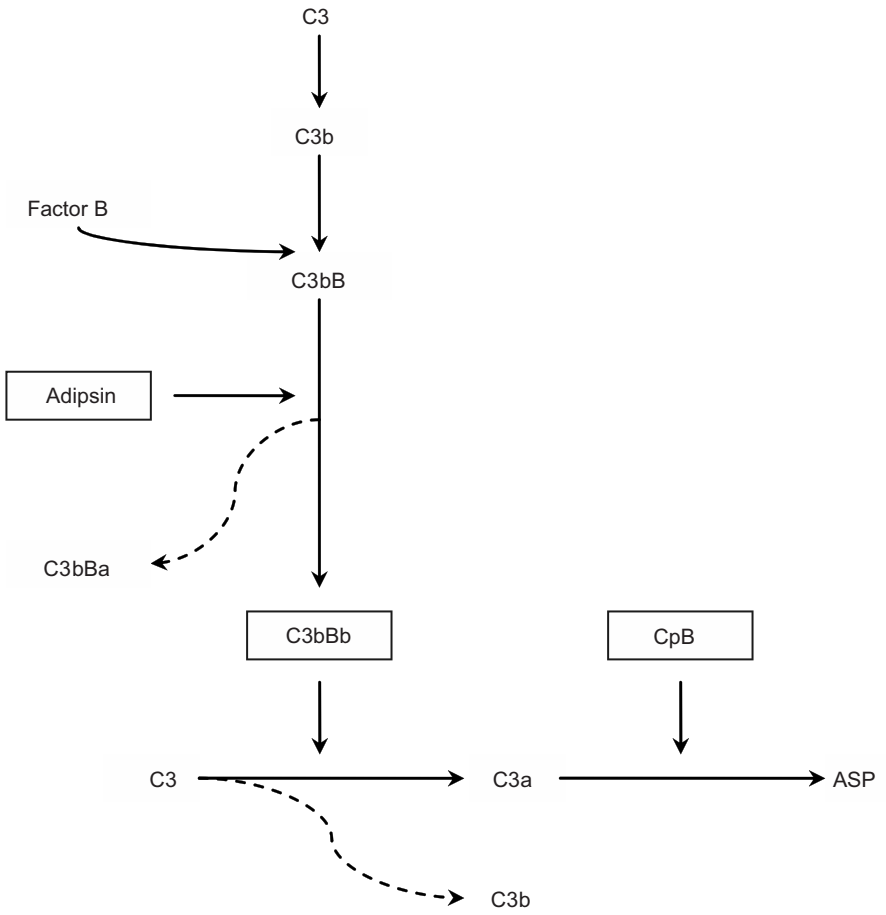

Figure 9. Synthesis of ASP. C3bBb is the active convertase that cleaves C3 to C3a, of which the N-terminal arginine is cleaved of to produce ASP (C3adesArg). C3bBb is generated by spontaneous conversion of C3 to activated C3b. C3b combines with factor B (C3bB), which is cleaved by the enzyme adipsin to form C3bB. Enzymes are depicted in boxes.

1.9 Aims of the studies and outline of the thesis

This thesis gives a detailed account of the various studies performed to improve the knowledge of PWS and care for patients with PWS. Studies are not necessarily described in the sequence in which these were carried out. The aims of the studies described in this thesis were to evaluate general aspects of children with Prader-Willi syndrome and to investigate the efficacy and safety of GH treatment. The study populations consisted of either a the total group of children initially recruited for the Dutch national randomized controlled GH trial or a subset thereof. The design of the Dutch national GH trial and the 4-year multicenter follow-up study (the Dutch Cohort Study) are described in Appendix A. In Appendix B, the metyrapone test is described.

Chapter 2 describes the study assessing the integrity of the HPA-axis (cross-sectional study).
Chapter 3 reports the results of the study investigating the relationship between central adrenal insufficiency and sleep-related breathing disorders (cross-sectional study).
Chapter 4 presents the outcomes of the study investigating the prevalence of scoliosis and effects of age, gender, body mass index, lean body mass and genotype (cross-sectional study).
Chapter 5 reports the effects of GH treatment on onset and progression of scoliosis (multicenter randomized controlled trial).
Chapter 6 describes the effects of one or two years GH treatment on anthropometry and body composition (randomized controlled trial).
Chapter 7 presents the outcomes of the study assessing bone mineral density and the effects of GH treatment on bone mineral density (cross-sectional study and randomized controlled trial).
Chapter 8 describes the cardiovascular and metabolic risk factors and ASP levels and reports the effects of GH treatment on these outcomes (cross-sectional study and randomized controlled trial).
Chapter 9 describes the long-term effects of 4 years GH treatment on body composition, growth, bone maturation, and safety parameters (Dutch Cohort Study).

In **Chapter 10**, results and conclusions are discussed in light of the present literature. **Chapter 11 and 12** summarize the studies and the results described in this thesis in English and Dutch, respectively.

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Appendix A: Metyrapone test

Rationale of the metyrapone test

The metyrapone test is used to evaluate whether the ACTH secretion by the pituitary gland will increase sufficiently in response to reduced cortisol secretion from the adrenal glands, thereby mimicking a situation of stress, which causes a sudden demand for stimulation of the hypothalamus-pituitary-adrenal (HPA-) axis. Thus, the metyrapone test assesses the quality of the hypothalamic CRH and pituitary ACTH response in case of stress. When the response to metyrapone is inadequate, demonstrated by an insufficient increase in ACTH levels, central adrenal insufficiency is diagnosed.

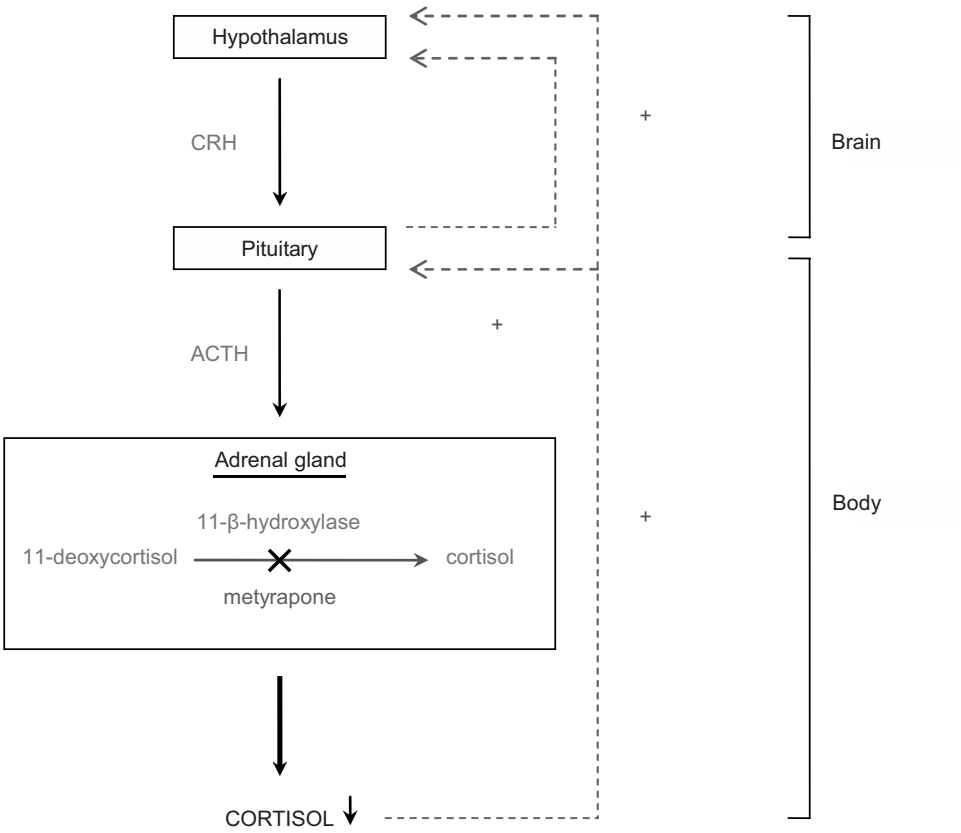

Figure 1. Simplified schematic overview of the HPA-axis activation in response to metyrapone.

Figure 1 displays a simplified schematic overview of the HPA-axis. There are many steps involved in the conversion of cholesterol to cortisol, the last step being the conversion of 11-deoxycortisol to cortisol involving the enzyme 11-β-hydroxylase. Metyrapone blocks 11-β-hydroxylase, thereby causing a decrease of serum cortisol levels. Due to the feedback mechanism, the hypothalamus and pituitary gland should subsequently secrete CRH and ACTH, respectively. In patients with central adrenal insufficiency, the increase in ACTH levels is insufficient. In those with primary adrenal insufficiency, the 11-deoxycortisol levels upstream of the enzyme blockade in the adrenal glands fail to accumulate, coinciding with sufficiently elevated ACTH levels.

Standard operating procedure of the metyrapone test

Metyrapone is administered orally at 2330 h in a dosage of 30 mg/kg with a maximum of 3 g. At 0400 h, 0600 h, and 0730 h blood is sampled for the measurement of ACTH, cortisol, and 11-deoxycortisol levels. For patient safety, serum glucose levels are measured and heart rate and oxygen saturation are monitored continuously, whereas blood pressure is measured every thirty minutes. Should a rise in heart rate (30% or more) or a decrease in blood pressure (10% or more) occur, blood pressure is measured every 15 minutes until normalization. After the last fasting blood samples are taken at 0730 h, a single-dose of hydrocortisone (25 mg) is administered. Central adrenal insufficiency is diagnosed when ACTH levels are below 33 pmol/l at 0730 h. Primary adrenal insufficiency is diagnosed when ACTH increases sufficiently, but 11-deoxycortisol levels fail to rise above 200 nmol/l (chromatography and radioimmunoassay) or 260 nmol/l (radioimmunoassay alone). Measurements of hormone levels performed throughout the night can be used to interpret whether the cortisol blockade was sufficient.

Appendix B: Study designs

Project coordination

Both the Dutch multicenter randomized controlled GH trial and the multicenter follow-up study (the Dutch Cohort Study) are coordinated by the Dutch Growth Research Foundation, Rotterdam, The Netherlands. The PWS research team consists of one or two physicians/ researchers, a research nurse, and a psychologist. Three-monthly, 18 hospitals throughout The Netherlands are visited by the researcher and research nurse, where children are examined, in collaboration with the local pediatrician or pediatric endocrinologist (Figure 1). Standardized measurements take place at the Erasmus University Medical Center / Sophia Children's Hospital, Rotterdam, The Netherlands at start, at 6 and 12 months, and subsequently each year.

Figure 1. Participating centers.

R.F.A. de Lind van Wijngaarden, E.P.C. Siemensma, D.A.M. Festen, P.M.C.C. van Eekelen, G.C.B. Bindels – de Heus, S.L.S. Drop, A.C.S. Hokken-Koelega, Erasmus University Medical Center Rotterdam / Sophia Children's Hospital, Rotterdam (in black); B.J. Otten, Radboud University Nijmegen Medical Center; E.G.A.H. van Mil, P.E. Jira, Hieronymus Bosch Medical Center, 's-Hertogenbosch; J. Rotteveel, VU University Medical Center, Amsterdam; R.J.H. Odink, St. Catharina Hospital, Eindhoven; M. van Leeuwen, St. Jansdal Hospital, Harderwijk; D.A.J.P. Haring, Diaconessenhuis, Leiden; G. Bocca, University Medical Center Groningen / Beatrix Children's Hospital, Groningen; E.C.A.M. Houdijk, Haga Hospitals / Juliana Children's Hospital, The Hague; J.J.G. Hoorweg-Nijman, H. van Wieringen, St. Antonius Hospital, Utrecht;

R.C.F.M. Vreuls, Medical Center Twente, Enschede; A.S.P. van Trotsenburg, Amsterdam Medical Center, Amsterdam; E.J. Schroor, Isala Hospitals, Zwolle; J.W. Pilon, Ijsselmeer Hospitals, Lelystad; B.Bakker, T.C.J. Sas, D. Mul, J.M. Wit, Leiden University Medical Center, Leiden; C. Westerlaken, Canisius-Wilhelmina Hospital, Nijmegen; E. van Pinxteren-Nagler, Medical Center Leeuwarden, Leeuwarden; T.A. de Heer-Groen, Gelre Hospitals, Apeldoorn, The Netherlands.

Patients

About 120 patients have currently (1-4-2009) been included in the randomized controlled GH trial (Figure 2). All children continued GH treatment in a multicenter follow-up study (the Dutch Cohort Study). For both studies, the following criteria were met:

Inclusion criteria:

- Genetically confirmed diagnosis of PWS;
- Age between 6 months and 16 years;
- Maximal bone age of less than 14 years in girls, or 16 years in boys.

Exclusion criteria:

- Non-cooperative behavior;
- Extremely low dietary intake of less than minimal required intake according to guidelines set by the World Heatlh Organization;
- Medication to reduce weight (fat);
- In children above 3 years of age: height above 0 SDS, unless weight-for-height is above 2 SDS;
- Previous treatment with GH (not applicable for the Dutch Cohort Study).

Design

Infants

The infant group currently consists of 61 children aged between 6 months and 3 years at start of study. Stratified for age, they were randomized into either a GH-treated group or a control group for the duration of one year (Figure 2). The GH-treated group received somatropin 1 mg/m^2.day, whereas the control group was not treated with GH. From 12 months of study onwards, all children were treated with somatropin 1 mg/m^2·day and were prospectively followed in the Dutch Cohort Study in collaboration with pediatricians or pediatric endocrinologists throughout The Netherlands.

Prepubertal group

The prepubertal group consists of 50 children; girls are aged between 3 and 12 years with Tanner breast stage < 2 and boys between 3 and 14 years with Tanner genital stage < 2 and a testicular volume < 4 ml. Stratified for BMI, children were randomized into either a GH-treated group or a control group for the duration of two years (Figure 2). The GH-treated group received somatropin 1 mg/m^2·day, whereas the control group was not treated with GH. Dietary advice and exercise training were offered to both groups and started three months prior to study in order to minimize a priori between group differences. From 24 months of study onwards, all children were treated with somatropin 1 mg/m^2·day and were prospectively followed in the Dutch Cohort Study in collaboration with pediatricians pediatric endocrinologists throughout The Netherlands.

Pubertal group

The pubertal group consists of 9 children; girls > 12 years and boys > 14 years, both with spontaneous or induced puberty. All pubertal children were treated with GH, but were randomized to receive either 1 mg/m^2·day or 1.5 mg/m^2·day until adult height (Figure 2). Dietary advice and exercise training were offered to both groups and started three months prior to study in order to minimize a priori between group differences.

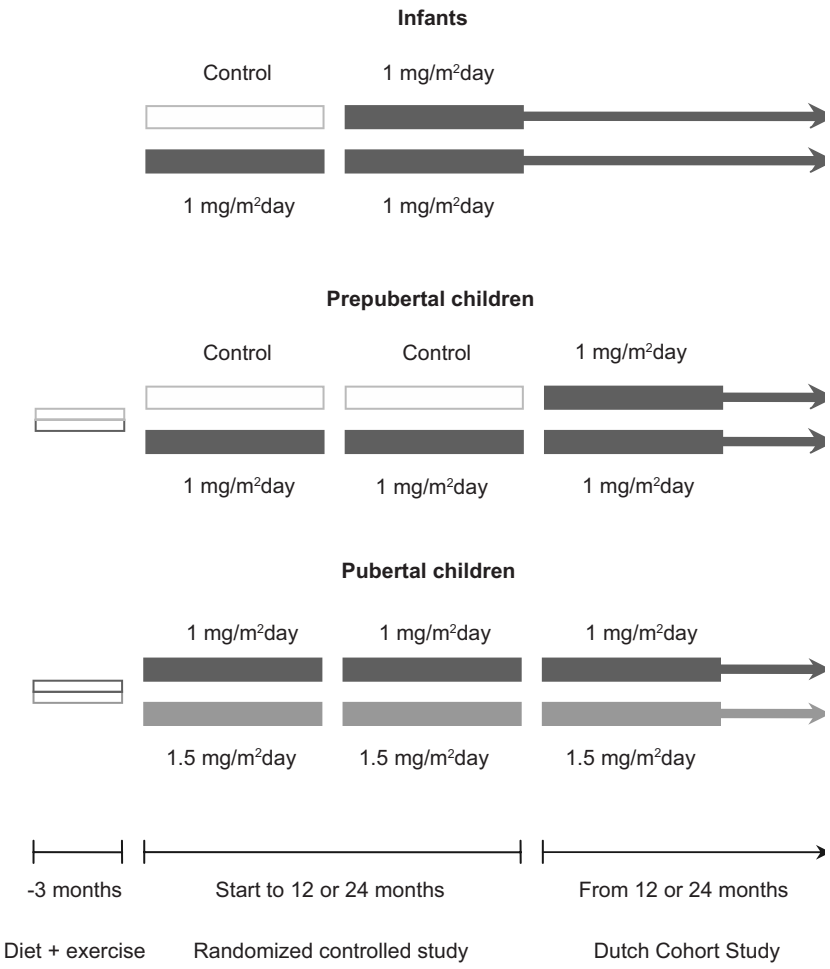

Figure 2. Design of the multicenter randomized controlled trial and the Dutch Cohort Study.

Chapter 2

High prevalence of central adrenal insufficiency in patients with Prader-Willi syndrome

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Abstract

Context: The annual death rate of PWS patients is very high (3%). Many of these deaths are sudden and unexplained.

Objective: Because most deaths occur during moderate infections and PWS patients suffer from various hypothalamic insufficiencies, we investigated whether PWS patients suffer from central adrenal insufficiency (CAI) during stressful conditions.

Design: Overnight single-dose metyrapone tests were performed. Metyrapone (30 mg/kg) was administered at 2330 h. At 0400, 0600, and 0730 h, ACTH, 11-deoxycortisol, cortisol and glucose levels were measured. Diurnal salivary cortisol profiles were assessed on a different day at wake-up, 30 minutes after wake-up, at 1400 h and at 2000 h.

Setting: The study was conducted in a pediatric intensive care unit.

Patients: Patients included 25 randomly selected PWS patients.

Main outcome measure: Patients were considered as having CAI when ACTH levels remained below 33 pmol/l at 0730 h.

Results: Median (interquartile range) age was 9.7 (6.8-13.6) years. Fifteen patients (60%) had an insufficient ACTH response (CAI, $p<0.001$). There was no significant difference in age, gender, genotype and BMISDS between patients with CAI and those without. Morning salivary cortisol levels and diurnal profiles were normal in all children, suggesting that CAI becomes apparent only during stressful conditions.

Conclusions: Strikingly, 60% of our PWS patients had central adrenal insufficiency. The high percentage of CAI in PWS patients might explain the high rate of sudden death in these patients, particularly during infection-related stress. Based on our data, one should consider treatment with hydrocortisone during acute illness in PWS patients, unless CAI has recently been ruled out with a metyrapone test.

Introduction

Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia, obesity, hypogonadism, psychomotor delay, and sleep-related breathing disorders.[1-4] PWS results from the lack of paternal expression of the q11-q13 region of chromosome 15 caused by deletion, uniparental disomy, imprinting center defect, or balanced translocation.[1, 5]
The annual death rate in PWS patients is very high (3%).[6] Among the causes of death in older children and adults are cor pulmonale,[7, 8] fatal apneas,[9] unexpected bathtub drownings,[10] and gastric necrosis.[11] In some patients, low adrenal weight was reported.[12, 13] Deaths in younger children are mostly related to only mild or moderate upper respiratory tract infections (URTIs).[9, 10] Many of the sudden deaths in PWS children are still unexplained.
PWS patients have hypothalamic dysregulations and show no or few signs of illness. Often, they do not get fever, cannot vomit, and have a higher pain threshold. Also during URTIs, PWS patients can appear less ill than they actually are.
As hypothalamic dysfunction is responsible for many other endocrine deficiencies in PWS patients,[14, 15] we hypothesized that PWS patients suffer from central adrenal insufficiency (CAI) during stressful conditions.
We used the overnight single-dose metyrapone test, as this is the best dynamic test for the diagnosis of CAI.[16-19] Metyrapone blocks the synthesis of cortisol by inhibiting 11-beta-hydroxylase type 1, which converts 11-deoxycortisol (compound-S) to cortisol. The decline in plasma cortisol stimulates ACTH production. The cut-off level for an appropriate ACTH response is 33 pmol/l at 0730 h.[19] Those failing to achieve such an ACTH response are considered as having CAI.[16, 17, 19-21]
To investigate the basal cortisol secretion and in the search for other parameters for the identification of patients at risk of CAI, diurnal salivary cortisol profiles were studied.

Methods

Patients

Twenty-five children with genetically confirmed PWS were randomly selected from our outpatient clinics. Patients underwent a metyrapone test during overnight stay at the Pediatric Intensive Care Unit of the Erasmus University Medical Center / Sophia Children's Hospital (Rotterdam, The Netherlands). Twelve patients had paternal deletion (63%), six had maternal disomy (32%), and one an imprinting center mutation (5%). All were treated with growth hormone, Genotropin 1.0 mg/m^2·day (Pfizer Inc., New York, NY), with a median (interquartile range) duration of 33 months (20-43). The protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center (International

Standard Randomized Controlled Trial Number 49726762). Informed consent was obtained from parents and children above 12 years of age.

Overnight single-dose metyrapone test

Metyrapone (30 mg/kg, Metopiron, Novartis Pharma BV, Arnhem, The Netherlands) was administered at 2330 h. The maximal cortisol suppression has been reported to occur at 0400 h.[18, 22] The decline in plasma cortisol stimulates ACTH production, which causes 11-deoxycortisol, before the enzyme blockade, to accumulate. Maximal levels of ACTH and 11-deoxycortisol are found at 0730 h.[16-19]

In 25 children, fasting blood samples were taken for the analysis of ACTH, 11-deoxycortisol, cortisol, and glucose at 0730 h,[16, 17, 19] and in 16 children also at 2330, 0400, and 0600 h.

During the metyrapone test, heart rate and oxygen saturation were measured continuously and blood pressure was measured every thirty minutes. If a rise in heart rate (30% or more) or a decrease in blood pressure (10% or more) occurred, blood pressure was measured every 15 minutes until normalization. After the last fasting blood samples were taken at 0730 h, a single-dose of hydrocortisone (25 mg) was administered.

Diurnal cortisol profile

Diurnal salivary cortisol profiles were assessed in 16 of the 25 PWS patients, on a different day and during health, using Salivettes (Sarstedt, Nümbrecht, Germany), at spontaneous wake-up (0600-0900 h), 30 min. after wake-up, at 1400 h, and at 2000 h. Maximal morning salivary cortisol levels were defined as the highest cortisol level in the morning (wake-up or 30 min. thereafter), and compared with those established in 237 healthy schoolchildren (same laboratory with same assay).

Assays

To rule out cross-reactivity of steroids,[16, 23, 24] cortisol was measured by radioimmunoassay (RIA) after extraction with dichloromethane and subsequent paper chromatography, according to the method described earlier for cortisol measurement in plasma and saliva.[24] The minimal detection level was 10 nmol/l for serum cortisol, 0.30 nmol/l for salivary cortisol, and 0.17 nmol/l for serum 11-deoxycortisol. Plasma ACTH levels were measured with an immunoradiometric assay (BioInternational, Gif sur Yvette, France) with a minimal detection level of 1.1 pmol/l. Glucose levels were measured with the Hitachi 917 (Hitachi Device Development Center, Tokyo, Japan), detecting glucose levels between 0 and 42 mmol/l.

Data analysis

Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 15.0, Chicago, Illinois, USA). Data are expressed as medians and interquartile ranges

(iqr). Standard deviation scores (SDS) for height, weight, and BMI were calculated based on Dutch reference data by Growth Analyser 3.0 (available at www.growthanalyser.org).[25] Blood pressure SDS was calculated according to reference values reported by Rosner et al.[26] Spearman's rho was calculated for the analysis of correlations.

Results

Median age of the PWS patients was 9.7 years (range: 3.7-18.6 years). Median (iqr) BMI SDS was 0.8 (0.2-1.3); weight-for-height SDS 1.1 (0.3-1.6); and height SDS -0.9 (-1.8 to 0.5).

At start of the metyrapone test (2330 h), hormone levels were not significantly different between patients later diagnosed with central adrenal insufficiency (CAI) and those who were not. In all children, metyrapone maximally suppressed cortisol concentrations at 0400 h (p=0.005 compared to baseline, Table 1). After 0400 h, cortisol levels increased as the effect of metyrapone declined. ACTH and 11-deoxycortisol levels increased during the entire test, with a maximum increase between 0400 and 0600 h (Table 1, Figure 1).

Adrenal insufficiency

Fifteen patients (60%, p<0.001) showed an insufficient ACTH response at the end of the metyrapone test (0730 h, Table 1 and Figure 1). As metyrapone blocks cortisol synthesis, it causes a sudden increased demand for ACTH production, a situation mimicking stress. Patients with an insufficient ACTH response during the metyrapone test are, therefore, considered as having CAI during stressful conditions. Directly from start of the enzyme blockade, patients with CAI had a significantly lower increase in ACTH levels than those without CAI (Figure 1).

ACTH levels correlated significantly with 11-deoxycortisol levels (r=0.5, p=0.03). Children with CAI had lower 11-deoxycortisol levels than those without, but this did not reach statistical significance (p=0.08 at 0730 h, Table 1). All children without CAI had 11-deoxycortisol levels above the classical cut-off level of 200 nmol/l at 0730 h. Seven of 11 children with CAI had 11-deoxycortisol levels below 200 nmol/l, suggesting adrenal atrophy.

At 0730 h, cortisol levels were still low in all children. Children with CAI had higher levels of cortisol at 0730 h than children without CAI (p=0.08), as was previously reported.[19] Levels did not differ at 2330, 0400, and 0600 h (p=0.2, p=0.4, and p=0.2, respectively).

Table 1. Results of the metyrapone test in PWS children.

	2330 h			0400 h			0730 h		
	ACTH (pmol/l)	11-DOC (nmol/l)	Cortisol (nmol/l)	ACTH$^{\alpha}$ (pmol/l)	11-DOC$^{\gamma}$ (nmol/l)	Cortisol$^{\varepsilon}$ (nmol/l)	ACTH$^{\beta}$ (pmol/l)	11-DOC$^{\delta}$ (nmol/l)	Cortisol (nmol/l)
CAI	3 (3-4)	0.4 (0-4.0)	75 (28-193)	9 (7-10)*	167 (160-281)	30 (10-61)	19 (14-26)†	172 (139-320)	215 (130-301)
Non-CAI	4 (4-5)	0.5 (0.2-1.2)	150 (100-333)	32 (12-180)*	206 (162-320)	40 (26-95)	63 (49-76)†	269 (230-313)	130 (70-190)

ACTH, 11-deoxycortisol and cortisol levels at different time points in PWS children with central adrenal insufficiency (CAI) and in those without (non-CAI). At 2330 h and 0400 h: N=16; at 0730 h: N=25.
αβ ACTH levels increased significantly over time compared with baseline (total group: α: p=0.003, β: p=0.001). γδ 11-deoxycortisol levels increased significantly over time compared with baseline values (total group: γ: p=0.012, δ: p=0.008). ε Maximal cortisol suppression at 0400 h occurred in both groups (total group: ε: p=0.005). *† Differences in ACTH levels between CAI and non-CAI were significant at 0400 h and 0730 h (*: p=0.04, †: p<0.001).
To convert ACTH in pmol/l to pg/ml, divide by 0.22; to convert 11-deoxycortisol in nmol/l to µg/dl, divide by 28.99; to convert cortisol in nmol/l to µg/dl, divide by 27.59.

Cortisol suppression did, therefore, not differ between children with CAI and those without. There was no significant difference in age, gender, BMI SDS, weight-for-height SDS, height SDS, and genotype between patients with CAI and those without (data not shown).

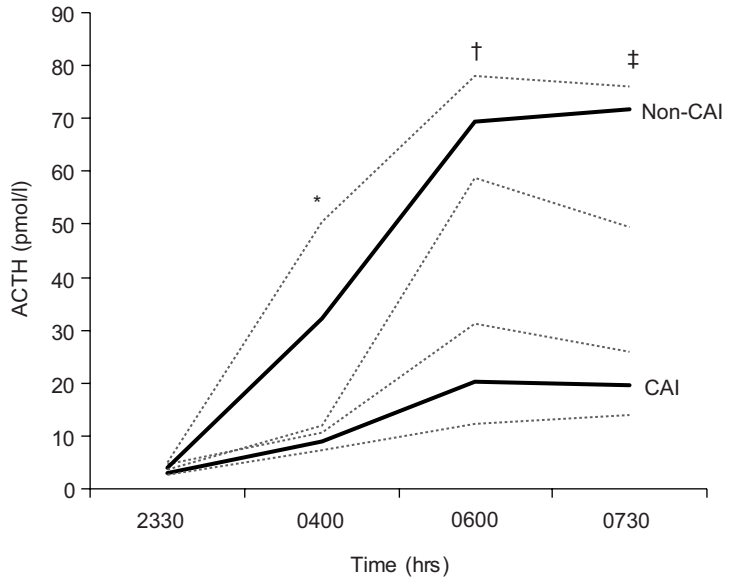

Figure 1. ACTH-response in PWS patients during a metyrapone test. Median (iqr) ACTH levels during the metyrapone test in children with central adrenal insufficiency (CAI) and those without (non-CAI). The cut-off level for a normal ACTH level is 33 pmol/l at 0730 h. In 16 children, ACTH levels were measured at 2330 h, 0400 h and 0600 h. *: p=0.048, †: p=0.01, ‡: p<0.001, all CAI vs. normal. To convert ACTH in pmol/l to pg/ml, divide by 0.22.

Diurnal cortisol profile

Figure 2 shows that all PWS patients had normal morning cortisol levels and a normal diurnal rhythm, with high levels in the morning decreasing during the day. This indicates that CAI only occurs during stressful conditions. Patients with an insufficient ACTH response during the metyrapone test had lower salivary cortisol levels at wake-up (2.7 (1.8-5.1) vs. 6.8 (3.5-12.3) nmol/l, p=0.05). Cortisol levels at wake-up correlated with ACTH levels at 0730 h during the metyrapone test, although this did not reach statistical significance (rho=0.5 with p=0.1). Salivary cortisol levels were not useful for identifying PWS patients

at risk of CAI, due to low sensitivity. The highest accuracy was obtained at a cut-off of 2.8 nmol/l, with 99.2% specificity, but only 55.6% sensitivity. Levels at wake-up of 12 nmol/l or above seemed indicative for absence of CAI (100% sensitivity), but such high levels were only found in one patient.

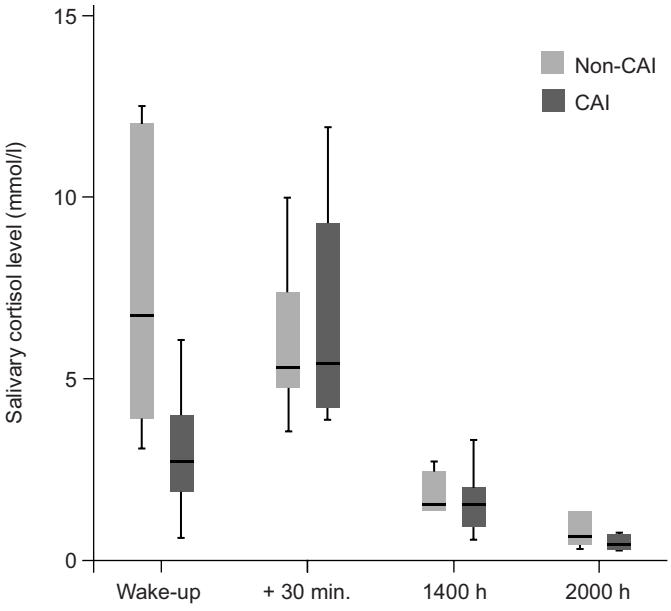

Figure 2. Diurnal salivary cortisol levels in PWS patients. Box-whisker plot of salivary cortisol levels at wake-up, 30 minutes after wake-up (+30 min), 1400 h and 2000 h in PWS children with central adrenal insufficiency (CAI) and without (Non-CAI), expressed as median (iqr). Differences at wake-up did not reach statistical significance (p=0.053). Median (iqr) reference values for maximal morning cortisol levels (either at wake-up or 30 minutes thereafter): 5.7 (1.9-16.2) nmol/l. To convert cortisol in nmol/l to µg/dl, divide by 27.59.

Side effects

None of the patients reported side effects during the metyrapone test, such as nausea and headache. Diastolic and systolic blood pressure SDS decreased significantly between 0030 and 0730 h (Figure 3, p-values: 0.002-0.03 and 0.004-0.05, respectively). The overall median systolic blood pressure SDS was lower in patients with CAI than in those without (-0.5 (-1.6 to 0.5) vs. 0.2 (-0.5 to 1.3), p=0.07). The overall diastolic blood pressure SDS correlated with cortisol (diastolic: rho=0.5, p=0.004; systolic: rho=0.3, p=0.07). Blood

pressure SDS did not significantly correlate with ACTH or 11-deoxycortisol.
All other vital parameters remained within the normal range: pulse ≥ 49/min, breathing ≥ 13/min, saturation ≥ 94%. Naturally, while fasting, glucose levels decreased significantly during the night, but were never below 3.9 mmol/l in any patient.

Figure 3. Blood pressure SDS during the metyrapone test. Median diastolic and systolic blood pressure SDS decreased during the metyrapone test. Blood pressures at 2330 h, 0400 h, and 0600 h were excluded, because patients were awake.

Discussion

Strikingly, 60% of PWS patients had central adrenal insufficiency, with ACTH levels failing to increase sufficiently during a metyrapone test. As metyrapone blocks cortisol synthesis, it causes a sudden increased demand for ACTH production, a situation mimicking stress. Patients with an insufficient ACTH response during the metyrapone test are, therefore, considered as having CAI during stressful conditions. The insufficient ACTH response was apparent directly from start of the metyrapone test. The diagnosis of CAI is in line with the presence of other hypothalamic insufficiencies and oral reports about hypoglycemias during surgery in PWS patients.

We are the first to report an inappropriate ACTH response during a metyrapone test in a large percentage of PWS children. In view of the importance of an adequate function of the hypothalamus-pituitary-adrenal (HPA) axis for survival, the high prevalence of CAI may be an explanation for the high death rate in PWS patients (3%).[6] In addition to CAI, the condition of acutely ill PWS patients is further compromised by an increase in the number of sleep apneas during upper respiratory tract infections (URTIs)[2] and a vague clinical presentation, because PWS patients often have an increased pain threshold and do not vomit or develop fever.

During a metyrapone test, only the ACTH response is measured. During illness, other factors than ACTH will influence the HPA-axis, such as cytokines and vasopressin.[23, 27] Nevertheless, an adequate increase of ACTH is a prerequisite for an appropriate response to stress.

Naturally, the metyrapone test was only performed in healthy PWS children and no signs of shock were present during the test. Despite the absence of acute illness, blood pressure was low in the early morning hours in all patients, as previously reported,[20] and correlated significantly with serum cortisol levels. The early morning hours might be the critical period during which PWS patients with CAI are at risk of dying. This hypothesis is in line with the fact that most PWS patients with mild infections (mostly URTIs) decease during the early morning hours.[9]

Interestingly, Stevenson et al.[12] reported autopsies in 4 children in whom the condition rapidly worsened after mild or moderate infections. Adrenal weight was low in one and severely low in the other three patients. Other reports also show low adrenal weight in deceased patients.[13] Adrenal size is related to the cause and duration of the various disease states leading to adrenal insufficiency.[28]

In 11 of 15 children with CAI (ACTH < 33 pmol/l), enough blood was available for measurement of 11-deoxycortisol levels. Of these 11 children with CAI, 7 had 11-deoxycortisol levels below the classical cut-off level of 200 nmol/l,[16, 17, 19, 21] suggesting that these children have CAI with adrenal atrophy. Four children had CAI with 11-deoxycortisol levels above 200 nmol/l, suggesting that these patients have partial or no adrenal atrophy. This is in line with reports showing that some but not all patients who died after mild or moderate infections had low adrenal weight during autopsy.[12, 13]

All patients received growth hormone (GH) treatment. We do not believe that this influenced the outcome of our study. Data on relationships between the GH-IGF-I system and the HPA-axis are contradictory.[29, 30] The prevalence of CAI is not increased in GH-treated children with isolated GH deficiency, Turner syndrome, or other disorders.[29] l'Allemand et al.[31] reported a decrease in random morning cortisol levels at 12 and 42 months of GH treatment. However, as stated by the authors, their study was not designed to examine the effects of GH treatment on the HPA-axis and the statistical power for such an analysis was

too low. Importantly, all cortisol levels remained within the normal range, as was also shown by our normal diurnal salivary cortisol profiles during health. l'Allemand et al.[31] found no correlation between IGF-I and cortisol levels. Interestingly, there was a tendency towards a negative correlation of cortisol levels with fat mass (r=0.48, p=0.082). Since GH treatment is known to reduce fat mass in PWS patients,[15, 32] GH could indirectly increase cortisol levels. Possibly, in patients with PWS, any direct negative effect of GH and/or IGF-I on cortisol levels may be outweighed by the positive effect of the reduction of fat mass on cortisol levels. This may explain why a correlation between GH/IGF-I and cortisol was not found.
Moreover, the similar death rate in PWS patient with and without GH treatment,[9] suggests an intrinsic rather than an extrinsic cause of CAI.
Morning salivary cortisol levels were normal in all PWS children, as has been previously reported,[31] indicating that CAI occurs only during stressful conditions.[17] Therefore, diurnal salivary cortisol profiles are not useful in identifying children at risk. In our study, salivary cortisol levels at wake-up (≥ 12 nmol/l) provided a sensitivity of 100%, but only one patient met this criterion.
Based on our data, 60% of PWS patients worldwide may be at risk of CAI. There are two possibilities for the therapeutical approach to this problem. The first option is that all PWS patients undergo a metyrapone test and should, in case of an impaired ACTH response, receive hydrocortisone treatment during stressful conditions. However, PWS patients suffer from (hypothalamic) hormonal insufficiencies of varying severity, not only between patients,[33] but also within patients.[34] In non-PWS patients, CAI may become more severe over time.[19] Due to these inconsistencies, even an appropriate ACTH response during a single metyrapone test will not rule out future development of CAI. More research needs to be performed on this matter.
The second option is to treat all PWS patients with hydrocortisone during stress. In our study, 40% of PWS patients would have been treated unnecessarily. However, the prevalence of CAI, a life threatening illness, is very high in PWS patients (60%). During moderate stress, patients should take hydrocortisone capsules, in total 30-50 mg/m^2·d in two to four times. During severe stress, parents should always contact a pediatrician or pediatric-endocrinologist and a higher dose of hydrocortisone should be administered (in total 75 mg/m^2·d in two to four times). For young children, suppositories are available. If the route of administration is compromised and/or during severe stress, the physician may decide to administer hydrocortisone intramuscularly or intravenously (50-100 mg/m^2). PWS patients show few signs of illness. Often, they cannot vomit and do not develop fever. The severity of stress can, therefore, not be read from these classical symptoms. In our experience, parents of children with PWS often know how the to classify the severity of stress of their child (mild, moderate, or severe). In order to guard the safety of PWS patients, a good collaboration between parents and physicians is crucial.

To our opinion, PWS patients should be considered to have CAI during stress, until proven otherwise with a metyrapone test.

Conclusion

Strikingly, 60% of our PWS patients have central adrenal insufficiency. We expect that the combination of an insufficient ACTH response during stress with an increased number of sleep apneas during illness, in the presence of reduced clinical symptoms, might lead to an increased risk of sudden death in PWS patients. More research is required, but at this moment it is important to consider hydrocortisone treatment for PWS patients during stressful conditions, including mild upper respiratory tract infections.

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Chapter 3

The relationship between central adrenal insufficiency and sleep-related breathing disorders in children with Prader-Willi syndrome

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Abstract

Background: The annual death rate of patients with Prader-Willi syndrome (PWS) is high (3%). Many deaths of children are sudden and unexplained. Sleep apneas have been suggested to play a role in sudden deaths. Recently, we discovered that 60% of patients with PWS suffer from central adrenal insufficiency (CAI) during stress.

Objective: To study the relationship between CAI and sleep-related breathing disorders (SRBDs).

Design: In 20 children with PWS who underwent a metyrapone test (30 mg/kg at 2330 h), sleep-related breathing was evaluated by polysomnography prior to the metyrapone test. In addition, we recorded sleep-related breathing in 10 children with PWS during their metyrapone test. CAI was diagnosed when ACTH levels during the metyrapone test were below 33 pmol/l at 0730 h. All tests were performed during healthy condition.

Setting*:* Pediatric Intensive Care Unit and specialized sleep center.

Results: Median (inter-quartile range, iqr) age was 8.4 years (6.5-10.2). After metyrapone administration, median (iqr) central apnea index (number/hour) increased significantly, from 2.2 (0.4-4.7) to 5.2 (1.5-7.9) (p=0.007). The increase tended to be higher in children with CAI [2.8 (2.0-3.9) vs. 1.0 (-0.2 to 2.6), p=0.09]. During polysomnography prior to the metyrapone test, sleep-related breathing was worse in children with CAI, who had a significantly higher central apnea index and tended to have a lower minimum oxygen saturation compared to those without CAI (p=0.03 and p=0.07).

Conclusions: In children with PWS, the central apnea index increased significantly after metyrapone administration, particularly in those with CAI during stress. In addition, children with CAI had a higher central apnea index compared to those without, several months prior to the metyrapone test.

Introduction

Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia, hypogonadism, psychomotor delay, and temper tantrums.[1-5] PWS results from the lack of expression of the paternally derived chromosome 15q11-q13 caused by deletion, maternal uniparental disomy, imprinting center defect, or balanced translocation.[3,6] The annual death rate in patients with PWS is high [3%[7]]. Deaths in children are often related to only mild or moderate upper respiratory tract infections and many deaths remain unexplained.[8-11]

Patients with PWS often have sleep-related breathing disorders (SRBDs), consisting of obstructive and central sleep apneas. Sleep apneas are the result of a reduction in upper airway diameter, hypoventilation, decreased pulmonary function, and a decreased ventilatory and arousal response during hypercapnia.[11-17] The most common cause of sudden death in children with PWS is respiratory or cardiorespiratory failure, often in association with a pulmonary or systemic infection and exacerbated by apneas.[3,10-13] During upper respiratory tract infections, children with PWS show a marked increase in the number of obstructive and central apneas.[12,13] The increase in central apneas may indicate a change in central control of sleep-related breathing during illness.

We recently discovered a high prevalence (60%) of central adrenal insufficiency (CAI) during a metyrapone test in children with PWS, shown by an inappropriate adrenocorticotrophic hormone (ACTH) response.[18] Our findings are put in clinical perspective by the low adrenal weight observed during autopsies in patients with PWS.[9,19] The diurnal salivary cortisol rhythms, measured on a different day during healthy condition, were normal in all children in our study.[18] Therefore, children with PWS are considered to be at risk of CAI only during stressful conditions, whereas CAI is not present during healthy conditions. The combination of an inappropriate ACTH response and mild or moderate infection may lead to sudden unexpected death.

In this study, we investigated the relationship between CAI and SRBD. A metyrapone test mimics a period of stress by causing a sudden increased demand for stimulation of the hypothalamus-pituitary-adrenal axis. We recorded sleep-related breathing during a metyrapone test. Our aims were to study the change in sleep-related breathing during the metyrapone test and to compare the change between children with and without CAI. Additionally, we evaluated whether sleep-related breathing during healthy condition some time before the metyrapone test was different in children with and without CAI and whether the severity of SRBD could be indicative for CAI.

Methods

Patients

In 20 children with PWS, sleep-related breathing was evaluated by complete overnight polysomnography prior to a metyrapone test. In 10 of these 20 children, sleep-related breathing was also recorded during a metyrapone test. All children were in healthy condition during the polysomnography and the metyrapone test. Prader-Willi syndrome was genetically confirmed in all children: 13 had a deletion, 6 a uniparental disomy and 1 an imprinting center defect. All children were treated with growth hormone, Genotropin 1 mg/m^2·day (Pfizer Inc., New York). All were naïve to glucocorticoid therapy.

Design

Of 20 children with PWS who underwent a metyrapone test,[18] sleep-related breathing was evaluated by complete overnight polysomnography in a specialized sleep center at 20.7 months (12.8-28.4) before the metyrapone test. The metyrapone test was performed in the Pediatric Intensive Care Unit (PICU) of the Erasmus University Medical Center Rotterdam / Sophia Children's Hospital, Rotterdam, The Netherlands. During the metyrapone test in the PICU, sleep-related breathing was recorded simultaneously in 10 of 20 children, i.e. in those for whom the sleep-recording device was available in the PICU. All tests were performed during healthy condition.

The protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center Rotterdam (ISRCTN 49726762). Written informed consent was obtained from parents and from children above 12 years of age. Assent was obtained from children under 12 years of age.

Metyrapone test

Metyrapone (30 mg/kg, Metopiron, Novartis Pharma BV, Arnhem, The Netherlands) was administered orally at 2330 h. Metyrapone blocks cortisol synthesis by inhibiting 11-beta-hydroxylase type 1, which converts 11-deoxycortisol (compound-S) to cortisol. The decline in plasma cortisol stimulates ACTH production, which causes an accumulation of 11-deoxycortisol prior to the enzyme blockade. The maximal cortisol suppression occurs at 0400 h.[18,20,21] Maximal levels of ACTH and 11-deoxycortisol are found at 0730 h.[20,22-27] The cut-off level for an appropriate ACTH response is 33 pmol/l at 0730 h.[23,24,26] Subjects failing to achieve such an ACTH response are considered as having CAI.[18,22-27] Fasting blood samples were taken for the analysis of ACTH, 11-deoxycortisol, and cortisol at 2330 h, 0400 h, 0600 h, and 0730 h. During the metyrapone test, heart rate and oxygen saturation were measured continuously and blood pressure was measured every thirty minutes. If a rise in heart rate (30% or more) or a decline in blood pressure (10% or more) occurred, blood

pressure was measured every 15 minutes until normalization.[18] After the last fasting blood samples were taken at 0730 h, a single-dose of hydrocortisone (25 mg) was administered. At time of the metyrapone test, children had been treated with growth hormone for a median (interquartile range, iqr) duration of 32.8 months (19.7-43.2).

Assays

To rule out cross-reactivity of steroids[22,28,29], cortisol and 11-deoxycortisol were measured by radioimmunoassay (RIA) after extraction with dichloromethane and subsequent paper chromatography, as previously described.[18, 9] The minimal detection level was 10 nmol/l for serum cortisol and 0.17 nmol/l for serum 11-deoxycortisol. Plasma ACTH levels were measured with an immunoradiometric assay (BioInternational, Gif sur Yvette, France) with a minimal detection level of 1.1 pmol/l.

Serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, California). The intra-assay CV was 4% and the inter-assay CV was 6%. Because of age and sex dependency, IGF-I levels were transformed into SDS.[30]

Complete overnight polysomnography in a specialized sleep center

Complete overnight polysomnographic evaluation of sleep-related breathing was performed in 20 children in one specialized sleep center (A.W.d.W., sleep specialist). Recordings included an electroencephalogram, an electrooculogram, a one channel derivation of electrocardiogram, and a surface electromyography of the submental muscle and both anterior tibial muscles. Nasal-oral airflow was monitored by nasal pressure prongs fixed in the nose, respiratory effort by thoracoabdominal strain gauges, and oxygen saturation (SaO_2) by pulse oximetry. The polygraphic records were scored according to criteria set by Rechtschaffen and Kales and revised by the American Academy of Sleep Medicine.[31,32] A period of apnea or hypopnea was defined as a reduction in airflow of at least 90% (apnea) or 50% (hypopnea) for the duration of three breaths or longer. For hypopneas, the additional criterion was a reduction of SaO_2 of 4% or more. An apnea was considered central when the airflow was decreased in concordance with a temporary cessation in respiratory effort. Apneas were considered obstructive when absence of airflow occurred without a decrease in respiratory effort. The central apnea index was defined as the total number of central apneas divided by the total time span of sleep in hours (no/hr). The obstructive apnea and hypopnea indices were calculated in a similar manner. If the value of the combined apnea indices (apnea-hypopnea-index) was greater than one, sleep-related breathing was considered pathological.[33,34] Abnormal SaO_2 was defined as a decrease in SaO_2 below 92% or a reduction of 4% or more below nightly baseline values. We evaluated the following polysomnographic results: the central apnea, obstructive apnea, and hypopnea indices (no/

hr); the duration of the longest apnea (sec); the lowest SaO_2 measured (%); the percentage rapid-eye-movement (REM) sleep and slow wave sleep; and the number of awakenings per hour.

At time of the polysomnography, children had been treated with growth hormone for a median (iqr) duration of 7.1 months (6.1-10.5).

Metyrapone-related sleep registration at the PICU

Sleep-related breathing was recorded overnight during the metyrapone test in 10 of 20 children in the PICU with the Embla (Embla, Denver, CO, USA). Only one child had onset of sleep at 2100 h, whereas all others had onset of sleep before 2000 h. Recordings included a three channel derivation of an electrocardiogram, nasal airflow measured by a nasal canula, respiratory effort measured by thoracoabdominal strain gauges, and SaO_2 measured by pulse oximetry. All recordings were evaluated with the software Somnologica 3 (Embla, Denver, CO, USA) by one person (S.v.d.B.). The polygraphic records were scored according to criteria set by Rechtschaffen and Kales and revised by the American Academy of Sleep Medicine[31,32], as described above. A central apnea index greater than one was considered pathological.[33,34] The oxygen-desaturation index (ODI) was calculated by dividing the total number of oxygen desaturations by the time span in hours. We evaluated the following sleep-related breathing parameters: the central apnea index (no/hr), the duration of the longest apnea (sec), the oxygen-desaturation-index (ODI: no/hr), and the lowest SaO_2 measured (%).

Data analysis

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS 15.0, Chicago, Illinois, USA). As data were not normally distributed according to Levene's test, they are expressed as median with an inter-quartile range (iqr). Standard deviation scores (SDS) for height and BMI were calculated with Growth Analyser 3.0 (available at www.growthanalyser.org), based on Dutch reference data reported by Fredriks et al.[35] Three sets of analyses were performed. First we compared data of sleep-related breathing during the metyrapone test between patients with CAI and those without. P-values for differences were calculated with the Mann-Whitney U test (non-normal distribution). Secondly, we studied the change in sleep-related breathing after metyrapone administration and whether this was different between children with CAI and those without. P-values for differences between the two time periods were calculated with the Wilcoxon Signed Ranks Test (non-normal distribution). P-values for differences in change were calculated with the Mann-Whitney U test. Thirdly, we evaluated the differences in previous polysomnographic records between children with CAI and those without. P-values for these differences were calculated with the Mann-Whitney U test. Correlation analyses were performed with Spearman's tests. P-values ≤0.05 were considered statistically significant.

Results

Eleven boys and nine girls with PWS were included. The median (iqr) age during polysomnography and during the metyrapone test was of 6.0 years (4.2-9.2) and 8.4 years (6.5-10.2), respectively (Table 1). As children were longer treated with GH at time of the metyrapone test, heightSDS was significantly higher than at time of the polysomnography. BMISDS and IGF-I SDS at time of the metyrapone test did not significantly differ from levels at time of the polysomnography.

Table 1. Clinical characteristics during the polysomnography and metyrapone test.

	Polysomnography	Metyrapone test	P-value
Age (years)	6.0 (4.2 to 9.2)	8.4 (6.5 to 10.2)	<0.0001
HeightSDS	-2.1 (-2.7 to -0.9)	-0.8 (-2.0 to 0.2)	0.004
BMISDS	0.7 (0.3 to 1.3)	1.0 (0.3 to 1.5)	0.44
IGF-I SDS	2.2 (1.0 to 2.9)	2.4 (1.8 to 3.5)	0.72

Data are expressed as median (iqr). SDS, SD-score; BMI, body mass index; IGF-I, insulin-like growth factor I.

Metyrapone test

CAI, defined as an ACTH level <33 pmol/l at 0730 h,[23,24,26] was present in 13 of 20 children with PWS (65%). Before the administration of metyrapone at 2330 h, there were no significant differences in ACTH and cortisol levels between children later diagnosed with CAI and those not (both p=0.20). The enzyme blockade maximally suppressed cortisol levels at 0400 h, causing a decrease of median (iqr) cortisol levels from 100.0 nmol/l (40.0-241.5) at 2330 h to 34.0 nmol/l (23.3-55.8) at 0400 h (p=0.005). The decline in serum cortisol levels was similar in children later diagnosed with CAI compared to those not, and resulted in increased ACTH secretion in children with and without CAI, but the ACTH response was significantly lower in children with CAI. The difference in ACTH levels between children with CAI and those without was already significant at 0400 h [9.0 pmol/l (7.3-10.5) vs. 32.2 pmol/l (12.0-55.9), respectively; p=0.04] and became greater over time [0730 h: 18.9 pmol/l (12.8-26.8) vs. 74.2 pmol/l (56.4-81.5), respectively; p<0.0001]. In both groups, the increase in ACTH caused an accumulation of 11-deoxycortisol upstream of the enzyme blockade, increasing from 0.41 nmol/l (0.09-2.49) at 2330 h to 229.5 nmol/l (166.8-319.3) at 0730 h (p=0.008). Glucose levels and heart rate did not significantly change during the metyrapone test. However, systolic and diastolic blood pressures were significantly lower during early morning hours than at start. No metyrapone test had to be discontinued. Results of the metyrapone test in this group of children with PWS have previously been described in detail.[18]

Metyrapone-related sleep registration

During the night of the metyrapone test conducted in the PICU, sleep-related breathing was recorded in 10 children from onset of sleep (before metyrapone administration) until wake-up. Six children had CAI and four had not. During total sleep time in the PICU, children with CAI had a central apnea index of 5.7 (3.1-6.8), compared to 1.1 (0.9-5.1) in those without CAI (p=0.09). Before metyrapone administration, the maximum duration of apneas was significantly longer in children with CAI than in those without (p=0.03). The central apnea index, oxygen-desaturation-index, and lowest SaO_2 were not significantly different (all p=0.52). The obstructive apnea index was less than 1 in all children, similar to previously published results,[12] and did not change after metyrapone administration.

Table 2 shows the effects of metyrapone administration on sleep-related breathing. It compares the results of the recordings of sleep-related breathing between two time intervals: the time interval from onset of sleep until the administration of metyrapone at 2330 h, and the time interval after metyrapone administration until wake-up when cortisol levels were suppressed. In the total group, sleep-related breathing worsened after metyrapone administration, as the median (iqr) central apnea index in the total group significantly increased from 2.2 (0.4-4.7) before 2330 h to 5.2 (1.5-7.9) after 2330 h (p=0.007). In children with CAI, the median (iqr) central apnea index significantly increased from 3.6 (0.7-4.7) before metyrapone administration to 6.0 (4.4-7.9) after administration (p=0.03), compared to an increase from 0.7 (0.4-4.2) before 2330 h to 1.4 (0.7-6.7) after 2330 h in children without CAI (p=0.14). The difference in increase between children with CAI and those without did not reach statistical significance in our group of 10 children (Figure 1, p=0.09).

In the total group, the maximum duration of apneas was 15.2 sec. (11.2-21.0) before 2330 h and 16.8 sec. (15.2-27.6) after 2330 h (p=0.12). The lowest SaO_2 was 87.5% (82.8-91.0) before 2330 h and 85.5% (76.0-87.5) after 2330 h (p=0.14). The change in duration of the longest apnea and lowest SaO_2 did not significantly differ between children with and without CAI (p=0.62 and p=0.29, respectively). In children with CAI, the median oxygen-desaturation-index increased with 2.1% after metyrapone administration, whereas the median ODI decreased with 2.3% in the non-CAI group (p=0.09 for the difference in change of ODI).

Table 2. Results of sleep recording before and after metyrapone administration.

		Before metyrapone	After metyrapone	P-value[a] Before vs. After	P-value[b] Δ CAI vs. non-CAI
Total	Central apnea index	2.2 (0.4-4.7)	5.2 (1.5-7.9)	0.007	
	Longest apnea	15.2 (11.2-21.0)	16.8 (15.2-27.6)	0.12	
	ODI	3.8 (1.7-6.7)	4.0 (2.4-6.4)	0.58	
	Lowest SaO_2	87.5 (82.8-91.0)	85.5 (76.0-87.5)	0.14	
CAI	Central apnea index	3.6 (0.7-4.7)	6.0 (4.4-7.9)	0.03	0.09
	Longest apnea	19.0 (15.0-24.6)[†]	17.7 (14.1-40.1)	0.47	0.62
	ODI	2.9 (1.7-6.6)	5.0 (2.4-13.5)	0.17	0.09
	Lowest SaO_2	86.5 (82.0-90.3)	85.5 (74.0-87.5)	0.50	0.29
Non-CAI	Central apnea index	0.7 (0.4-4.2)	1.4 (0.7-6.7)	0.14	0.09
	Longest apnea	11.2 (9.9-14.5)[†]	16.1 (15.1-20.9)	0.07	0.62
	ODI	5.9 (1.4-7.2)	3.6 (1.2-4.7)	0.14	0.09
	Lowest SaO_2	88.5 (83.2-94.5)	85.5 (75.8-92.3)	0.07	0.29

Results of the recordings of sleep-related breathing before and after metyrapone administration at 2330 h. Data are expressed as median (iqr). [a] P-values for differences in variables before and after metyrapone administration at 2330 h (Wilcoxon Signed Ranks Test); [b]P-values for differences in change after metyrapone administration between children with CAI and those without (Mann-Whitney U test). [†] Before metyrapone administration, the maximum duration of apneas was significantly longer in children with CAI than in those without (p=0.03). ODI, oxygen-desaturation-index: the number of oxygen desaturations per hour; SaO_2, oxygen saturation.

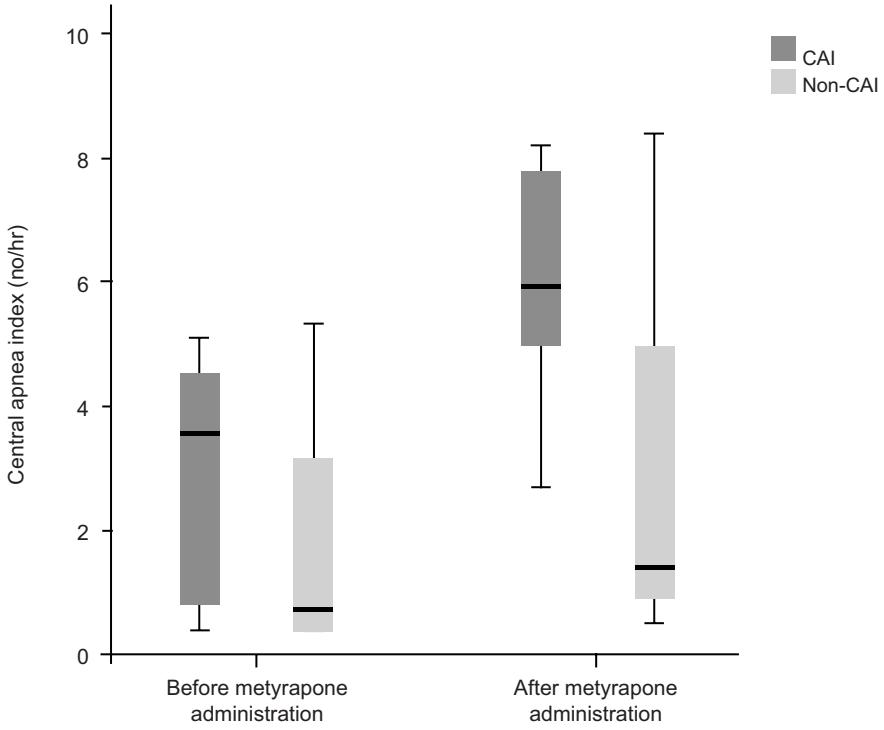

Figure 1. Box-Whiskers plot depicting the central apnea index (no/hr) in children with CAI and in those without, before and after the administration of metyrapone at 2330 h (30 mg/kg). The increase in median (iqr) central apnea index was 2.8 (2.0-3.9) in children with CAI compared to 1.0 (-0.2 to 2.6) in those without (p=0.09).

Complete overnight polysomnography prior to the metyrapone test

Twenty children who underwent a metyrapone test also had a complete overnight polysomnography in a specialized sleep center at 20.7 months (12.8-28.4) before the test (Table 3). During polysomnography, sleep-related breathing was considered pathological in all children, defined as an apnea-hypopnea-index above 1.

Children who were later diagnosed with CAI had a significantly higher central apnea index than those not (p=0.03). The median (iqr) hypopnea index was 1.7 (0.6-3.9) in children with CAI and 0.7 (0.0-1.7) in those without (p=0.14). The median (iqr) lowest SaO_2 was 91.0% (85.0-95.0) in children with CAI and 97.0% (90.0-100.0) in those without (p=0.07). Thus, sleep-related breathing seemed less favorable in those later diagnosed with CAI compared to those not. A polysomnography was also performed before start of GH treatment in 8 children with CAI and in 5 without. During this polysomnography performed 33.4 months

(25.4-37.3) prior to the metyrapone test, the median (iqr) central apnea index was 4.6 (1.9-6.0) in children with CAI versus 1.8 (1.5-3.1) in children without CAI (p=0.24).
Although some time had elapsed between the polysomnography performed during GH treatment and the sleep recording during overnight stay at the PICU, we found a significant correlation between the central apnea index during polysomnography and the central apnea index during the metyrapone test, particularly after metyrapone administration (r=0.79, p=0.006, Figure 2), but also before administration (r=0.73, p=0.02).

Table 3. Results of complete overnight polysomnography.

	CAI	No CAI	P-value
N (m/f)	13 (7/6)	7 (4/3)	
Age	6.0 (4.1-8.8)	6.1 (4.2-10.1)	
Central apnea index (no/hr)	3.0 (2.2-4.9)	1.4 (1.0-1.9)	0.03
Obstructive apnea index (no/hr)	0.0 (0.0-0.1)	0.1 (0.0-0.3)	0.07
Hypopnea index (no/hr)	1.7 (0.6-3.9)	0.7 (0.0-1.7)	0.14
Longest apnea (sec)	18.0 (15.0-27.0)	19.0 (17.0-22.0)	0.81
Lowest SaO_2 (%)	91.0 (85.0-95.0)	97.0 (90.0-100.0)	0.07
% REM sleep	24.0 (19.5-30.0)	22.0 (19.0-24.0)	0.28
% slow wave sleep	24.0 (20.8-29.0)	22.0 (18.0-31.0)	0.50
No. awakenings per hour	1.4 (1.1-1.8)	1.3 (1.0-1.9)	0.55

Polysomnographic records compared between patients with and without CAI. Data are expressed as median (iqr). SaO_2, oxygen saturation.

We also investigated if polysomnographic results were predictive for the later diagnosis of CAI and the severity thereof, reflected by the ACTH response during a metyrapone test. Notably, in our group of 20 children with PWS, the ACTH response during the metyrapone test tended to correlate negatively with the central apnea index previously assessed by polysomnography (r=-0.41, p=0.07). Two children without CAI had a central apnea index below 1 during polysomnography. However, this was also seen in one girl later diagnosed with CAI, who had the lowest central apnea index during polysomnography of all children (0.42 per hour). It is not known whether this girl already suffered from CAI during the polysomnography or that she acquired CAI afterwards. Unfortunately, a cut-off level for a central apnea index indicating a normal stress response during a later performed metyrapone test could not be provided. On the other hand, the four highest central apnea indices were found in children later diagnosed with CAI, ranging from 4.15 to 9.31 per hour. The highest central apnea index found in a child without CAI was 4.0 per hour. Thus, although a cut-off level for a central apnea index ruling out CAI could not be provided, a high central apnea index seemed indicative for a high risk of having CAI.

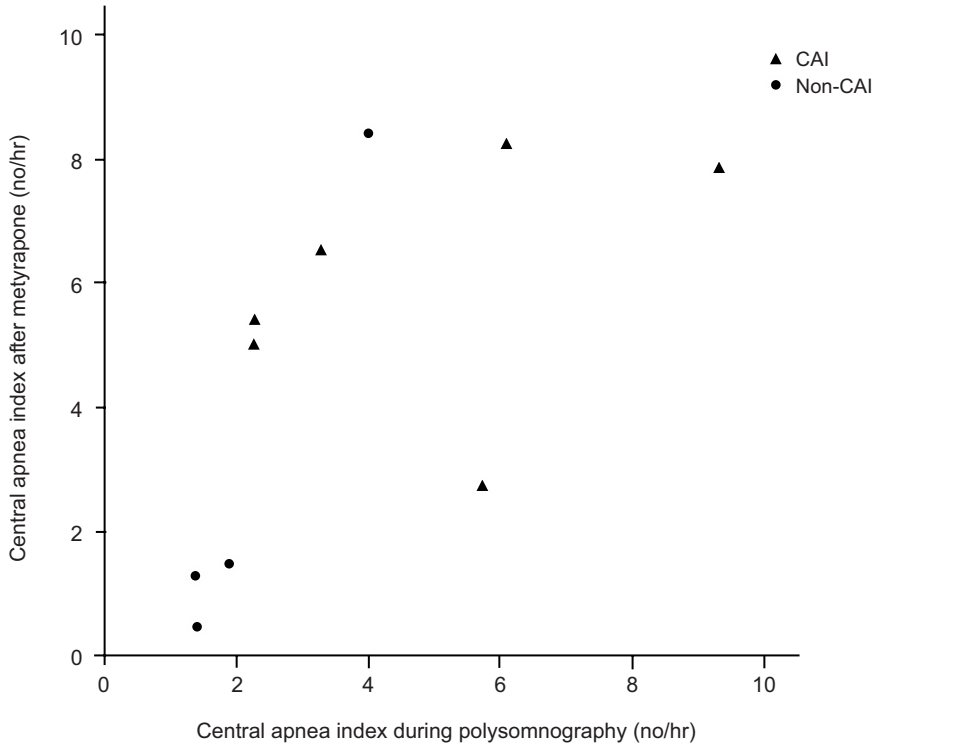

Figure 2. Scatterplot depicting the correlation between the central apnea index during polysomnography prior to the metyrapone test and the central apnea index recorded in the PICU after metyrapone administration. Spearman's correlation coefficient r was 0.79, with a corresponding p-value of 0.006.

Discussion

We performed metyrapone tests in twenty children with PWS, who had undergone polysomnographic evaluation of sleep-related breathing in a specialized sleep center prior to the metyrapone test. In ten of them, we also recorded sleep-related breathing during their metyrapone test in the Pediatric Intensive Care Unit. All tests were performed during healthy condition. Sleep apneas have been suggested to play a role in the sudden death of patients with PWS.[3,10-13] We recently reported a prevalence of CAI during stress in children with PWS of 60%.[18] Further research should be performed to confirm these results. CAI during mild or moderate infection may contribute or lead to unexpected death. In children with PWS, both CAI as well as the pathologic sleep-related breathing are likely to originate

from the hypothalamus.[18] In the present study, we hypothesized that PWS children with CAI have more central sleep apneas than those without CAI, both before and during stress. The combination of CAI and increased central apneas during stress might increase the risk for a fatal cascade leading to unexpected death.

A metyrapone test mimics a period of stress by causing a sudden increased demand for stimulation of the hypothalamus-pituitary-adrenal axis, but without a concomitant increase in cortisol response. Our results show a significant increase of the central apnea index after metyrapone administration in all ten children, irrespective of adrenal status. This increase could either reflect a difference in sleep architecture between the period before and after metyrapone administration, or an alteration in the central ventilatory regulation due to cortisol suppression which occurred in all children, both with and without CAI. In children with an increased central apnea index due to a central hypoventilation disorder, central apneas are most common during NREM sleep stages which are most abundant during the first part of the night.[36] In contrast, our results showed more central apneas during the second part of the night. Thus, the increased central apnea index during the second part of the night after metyrapone administration suggests an alteration in central ventilatory regulation during stress in children with and without CAI. More research investigating the change in sleep-related breathing during stress is warranted.

Our data showed that the increase in central apnea index as a response to metyrapone administration tended to be higher in children later diagnosed with CAI than in those not. As cortisol levels were maximally suppressed in all children, cortisol levels are not likely to be related to these differences. The decreased cortisol levels caused a subsequent sudden demand for hypothalamic corticotropin-releasing hormone (CRH) and pituitary ACTH secretion. As patients with PWS suffer from hypothalamic dysregulations,[3,18,37,38] CAI is likely to result from an inappropriate CRH secretion by the hypothalamus. Low CRH levels have been demonstrated to decrease EEG frequency, causing deeper sleep and less wakefulness.[39] A deeper stage of sleep is associated with a higher arousal threshold.[40] Possibly, children with CAI may be more prone to have central sleep apneas due to lower CRH levels resulting in deeper sleep and an increased arousal threshold. This may also account for the tendency toward a higher increase in oxygen-desaturation-index in response to metyrapone in children with CAI.

Before metyrapone administration, the duration of the longest apnea was the only difference between children with CAI and those without, whereas no significant difference was found after metyrapone administration. This may be due to the limited number of patients. However, the number of patients that underwent a polysomnography prior to the metyrapone test was larger. Our results show that during healthy condition prior to the metyrapone test, sleep-related breathing was more severely disturbed in children with a later diagnosis of CAI, demonstrated by the significantly higher central apnea index. Children with CAI during

stress may possibly have lower CRH levels during healthy condition as well. As explained above, lower CRH levels during health in CAI may result in children being more prone to disturbed sleep-related breathing. On the other hand, CAI during stress and a high central apnea index during health may be considered two independent symptoms, both resulting from more severe dysfunction of the hypothalamus. Possibly, a more severe hypothalamic dysregulation could result in a more severely disturbed adrenal function causing CAI during stress, and concomitantly in more severely disturbed sleep-related breathing during health.

Adequacy of cortisol suppression was assured by low cortisol levels at 0400 h and 0600 h. All children had maximally suppressed cortisol levels at 0400 h and there was no significant difference between children with CAI and those without, neither in the decline of cortisol levels from 2330 h to 0400 h, nor in the rise of cortisol levels thereafter. Thus, cortisol synthesis was equally inhibited by metyrapone in both groups. Some authors suggested a cut-off level of 220 nmol/l at 0730 h as a criterion for adequate cortisol suppression, although it is used without any regard for cortisol levels at 0400 h and 0600 h. Application of this criterion resulted in exclusion of only 1 and 2 children from analysis of the data of the metyrapone-related sleep registration and polysomnography, respectively. These analyses yielded similar results as in the total group.

In our study, we also investigated whether polysomnographic results could be indicative for the outcome of CAI in a metyrapone test. Interestingly, the ACTH response during a metyrapone test tended to correlate with the central apnea index measured by polysomnography prior to the metyrapone test. A central apnea index of 4.15 per hour and higher seemed indicative for a higher risk of having CAI. However, this finding is based on polysomnographic records of only 20 children with PWS. Thus, more research in a larger cohort is warranted. Our results could not provide a cut-off level for a central apnea index ruling out CAI. We found a low central apnea index of 0.4 per hour in a girl later diagnosed with CAI, indicating that either CAI and a concomitant increase in central apnea index developed after the initial polysomnography, or that CAI can also be present without an increased central apnea index. In the same line, we previously reported on the sudden death of a three-year-old boy after a moderate upper respiratory tract infection.[12] Because serum cortisol one hour after death was undetectable, we suspected CAI as the cause of death. During a polysomnography seven months prior to his demise, he had a central apnea index of only 0.9 per hour. Thus, a low central apnea index is not a guarantee for a normal ACTH stress response. Also, tests performed during healthy condition, such as a metyrapone test, do not reflect all changes in biological equilibriums during illness. Other factors such as cytokines, interleukins and vasopressin play an important role during illness.[28,41] Changes in nocturnal respiratory parameters during illness may be much greater than those observed during a metyrapone test. Future research should focus on the effect of illness on ACTH response in combination with recording of sleep-related breathing.

In conclusion, children with PWS and CAI during stress have a significantly higher central apnea index during healthy condition and a higher increase in central apnea index during stress. The combination of CAI and compromised sleep-related breathing could aggravate the deterioration of the condition of an acutely ill patient with PWS. This combination might be the prelude of a fatal cascade during illness.

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Chapter 4

Scoliosis in patients with Prader-Willi syndrome: Effects of age, gender, body mass index, lean body mass, and genotype

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Abstract

Objective: The reported prevalence of scoliosis in Prader-Willi Syndrome (PWS) children varies from 15% to 86%. We studied the prevalence of scoliosis and the effects of age, gender, body mass index (BMI), total lean body mass (LBM), LBM of the trunk (trunkLBM) and genotype.

Design: Children visited our hospital where X-rays were taken, length and weight were measured (BMI standard deviation scores (BMISDS) and body surface area (BSA)) and dual energy x-ray absorptiometry (DXA) was performed, measuring LBM and trunkLBM.

Patients: Ninety-six children, median (interquartile range, iqr) age 4.8 years (2.1 to 7.5), were included in a multi-centre study. None of them received growth hormone treatment.

Main outcome measures: Two types of scoliosis were identified: 1) “long C-curve type” scoliosis (LCS) and 2) “idiopathic” scoliosis (IS). Children were divided into age categories (infants: 0-3 years, juveniles: 3-10 years, adolescents 10-16 years).

Results: The prevalence of scoliosis was 37.5% and increased with age (infants and juveniles: ~30%, adolescents: 80%), 50% of children with scoliosis had a Cobb angle above 20°. Children with scoliosis were significantly older than those without. Children with LCS were younger and more hypotonic than those with IS: median (iqr) age 4.4 years (1.7-5.9) vs. 11.1 years (6.5-12.1) (p=0.002) and trunkLBM:BSA 7080 (6745-7571) vs. 7830 (6932-8157) (p=0.043).

Conclusions: The prevalence of scoliosis in PWS children is high (37.5%) and many children with scoliosis (13%) had undergone brace treatment or surgery. The type of scoliosis is affected by age and trunkLBM:BSA ratio.

Introduction

Prader-Willi syndrome (PWS) is characterised by hypotonia, hypogonadism, short stature, hyperphagia with obesity and psychological and behavioural problems.[1-4]
PWS results from the lack of paternal expression of the q11-q13 region of chromosome 15, caused by deletion, uniparental disomy (UPD), imprinting centre defect causing maternal imprinting (ICD), or balanced translocation.[5-12] Hypothalamic dysfunction may be responsible for many features of PWS.[13-15] The birth incidence of PWS is 1:22.000 to 1:29.000. The overall annual death rate under the age of 30 years is 3%.[16-18]
Children with PWS may develop scoliosis.[17,19-22] Scoliosis is a spinal curve with a Cobb angle of more than 10° on a standing posteroanterior radiograph. The Cobb angle is the angle between the two steepest vertebrae, i.e. the upper border of the upper vertebra in the curve and the lower border of the lower vertebra.[23]
Information on scoliosis in PWS is limited and varies greatly. The prevalence of scoliosis in PWS children was estimated to be between 15% and 86%.[17,19-22] All studies were retrospective and included both adults and children, without and during GH treatment.[17,19-21] Some calculated prevalences from questionnaires, whereas others studied material assembled over a great time span.[17,19-22]
We conducted a multi-centre study to investigate the prevalence and severity of scoliosis in a large group of PWS children without growth hormone treatment and studied the effects of age, gender, body mass index (BMI), lean body mass (LBM) and genotype on scoliosis.

Methods

Patients

Patients were included from April 2002 until November 2006. Ninety-six children with PWS (Table 1) were recruited through their paediatricians and paediatric endocrinologists for a national study and fulfilled the following inclusion criteria: 1) genetically confirmed diagnosis of PWS by positive methylation test; 2) age between 6 months and 16 years; and 3) bone age less than 14 years (girls) or 16 years (boys). None of the children were treated with growth hormone. All children visited the Erasmus Medical Centre / Sophia Children's Hospital in Rotterdam, The Netherlands.
The study protocol was approved by the Medical Ethics Committee of ErasmusMC, Rotterdam, The Netherlands. Informed consent was obtained from parents and children above 12 years.

Radiographics

Standing posteroanterior X-rays were taken of children who were able to stand. In young and/or hypotonic children who were not able to stand, posteroanterior X-rays in supine position were taken. Cobb angles were measured by two observers, a clinical research fellow (RdL) and an experienced paediatric orthopaedic surgeon (LdK). A triage system was used: one observer (RdL) measured all spinal X-rays, the other observer (LdK) examined all scoliotic curves. Intra-observer variability (Intra-OV, RdL: mean (SD) difference –0.05° (1.78°), ICC=0.998, rho=0.994, p=0.01, 95%C.I.=-0.91 to 0.80) and inter-OV (mean (SD) difference -0.02° (3.1°), r=0.94, ICC=0.97, p=0.01, 95%C.I.=-0.89 to 0.85) were minimal. Consistency between conventional and digital measurements was very high (mean (SD) difference 0.03° (1.54°), r=0.969, ICC=0.98, p=0.01, 95%C.I.=-0.52 to 0.59).

A thoracic scoliosis was defined as a scoliosis with its apex between the second and eleventh thoracic vertebra. A thoracolumbar scoliosis has its apex at either the twelfth thoracic or first lumbar vertebra. The apex is the most displaced vertebra in the scoliotic curve.

Two types of scoliosis were identified: 1) “long C-curve type” scoliosis, often seen in children with neuromuscular disorders (LCS); and 2) scoliosis behaving much like idiopathic scoliosis and which could therefore be classified according to the Lenke classification system (IS, Figures 1 and 2).[24]

Anthropometrics

Supine length was recorded below the age of 2.5 years. Thereafter, standing height was measured with a Harpenden stadiometer. Weight was assessed on an accurate scale, and BMI (kilograms per square meter) was calculated. Height and BMI were converted into SDS according to Dutch references for age.[25,26] Body surface area (BSA) was calculated. Calculations were performed with Growth Analyser version 3.0 (www.growthanalyser.org).

DXA

Lean body mass was measured with Dual Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare). Since no reference values for LBM in very young children were available, a “lean body mass:body surface area” ratio (LBM:BSA) was used for total LBM calculations and a trunkLBM:BSA ratio for calculations using only the LBM of the trunk. TrunkLBM, a measure of central lean body mass, is the total amount of LBM in the chest, abdomen and pelvis.

Data analysis

Data were analyzed for all children and for different age categories: infantile (0-3 years), juvenile (3-10 years) and adolescent scoliosis (10-18 years).[27,28] Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 11.0, Chicago, IL). Most of

the data obtained in our patients were not Gaussian distributed. We therefore expressed our data as median and interquartile range (iqr). Nonparametric tests (Mann-Whitney U test) and Chi-square tests were used to compare results between different age categories and types of scoliosis. Correlations were calculated using Spearman's correlation coefficient (r).

Results

Ninety-six children were included in this study (Table 1). Median (iqr) age was 4.8 years (2.1 years to 7.5 years) and BMISDS was 1.1 (-0.2 to 1.9). Height SDS was significantly different between the infant and juvenile group (p=0.02) and between the juvenile and adolescent group (p=0.002). Weight SDS was significantly different between all groups (infant and juvenile p<0.0001, infant and adolescent p<0.0001, juvenile and adolescent p=0.002). Of 82 children, the detailed genotype was known. Thirty-eight children had a deletion (46.3%), 35 UPD (42.7%), 8 ICD (9.8%) and one a balanced chromosomal translocation (1.2%).

Scoliosis in the total group of PWS children

Of 96 children, 36 had scoliosis (21 boys, 15 girls) at a median (iqr) age of 6.9 years (3.0-11.5). The total prevalence of scoliosis in our group of PWS children was 37.5% (Table 1). The prevalence was much higher than in the Dutch non-PWS population (scoliosis 2.7%, 10°-19° 2% and 20-39° 0.5%).[29] Thirteen scoliosis (36%) were classified as "long C-curve type" scoliosis (LCS). Twenty-three (64%) were classified as scoliosis resembling idiopathic scoliosis (IS, Figures 1 and 2).

The median (iqr) Cobb angle of all scoliosis was 18.3° (12.9°-35.0°). Eighteen children (50% of all with scoliosis, Table 1) had Cobb angles of 20° and more, the severity of scoliosis for which children are generally referred to an orthopaedic surgeon. Twelve children (13%) had undergone conventional (Boston brace) or surgical treatment.

There was no difference in the number of children with thoracic and thoracolumbar scoliosis between those with IS and those with LCS.

Lenke classification of 15 children with IS indicated that most of them (7 children, 47%) had type 1B scoliosis (Figure 2), in which the main curve is thoracic and the centre sacral vertical line (CSVL) is out of alignment, just touching the apical bodies.[24]

Table 1. Overview of the study population and the prevalence and severity of scoliosis.

	Total	Infants	Juveniles	Adolescents
Age range	0.5-16	0-3	3-10	10-16
N (m/f)	96 (52/44)	30 (24/6)	51 (20/31)	15 (8/7)
Age	4.8 (2.1 to 7.5)	1.6 (1.2 to 2.0)	5.8 (3.9 to 7.0)	11.9 (11.5 to 14.8)
Height SDS	-2.3 (-3.1 to -1.4)	-1.5 (-2.6 to -0.7)	-2.5 (-3.2 to -1.8)	-2.2 (-3.8 to -1.8)
Weight SDS	1.2 (-0.4 to 2.0)	-0.9 (-1.7 to 1.2)	1.3 (0.3 to 1.9)	2.2 (1.9 to 3.3)
TrunkLBM:BSA ($\cdot 10^3$)	7.3 (6.8 to 7.8)	6.9 (6.6 to 7.6)	7.3 (6.9 to 7.7)	8.1 (7.8 to 8.4)
Scoliosis (%)	36 (38)	9 (30)	15 (29)	12 (80)
LCS (%)	13 (36)	6 (67)	7 (47)	0
IS (%)	23 (64)	3 (33)	8 (53)	12 (100)
10°-19°	18 (19)	7 (23)	9 (18)	2 (13)
20°-39°	6 (6)	2 (7)	1 (2)	3 (20)
Brace	4 (4)	0	2 (4)	2 (13)
Surgery	8 (8)	0	3 (6)	5 (33)

Overview of the total number of children (N), the median (iqr) age, height SDS, weight SDS and trunkLBM:BSA ratio of children included in the study. Also, the number (%) of children with scoliosis divided by type of scoliosis, the Cobb angles and the number (%) of children treated for scoliosis are stated.

There was no difference in prevalence, severity, location of the apex or Lenke classification between boys and girls and between different genotypes. Girls showed more IS than LCS when compared to boys. This, however, did not reach statistical significance (p=0.089), possibly due to small numbers.
We did not find congenital spinal anomalies, which is in line with Kroonen et al.[20]

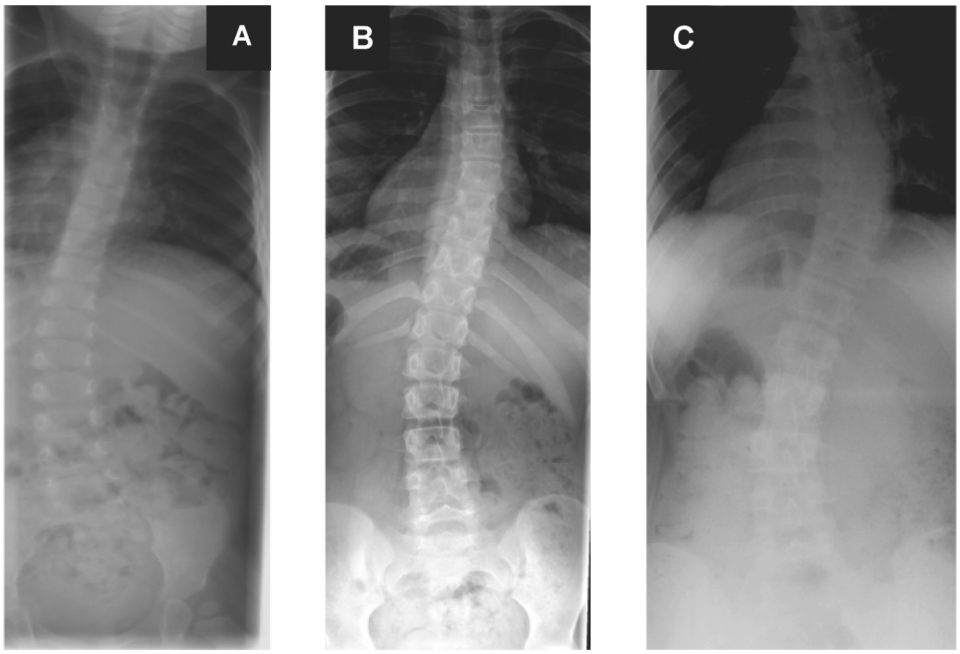

Figure 1. Examples of "long C-curve type" scoliosis (LCS, Figure 1A) and scoliosis resembling idiopathic scoliosis (IS, Figures 1B and 1C).

Infantile scoliosis

Nine of 30 PWS infants (30%) had scoliosis (Table 1). Six children had LCS (67%) and 3 had IS (33%, Figure 1). Two children had thoracic scoliosis and seven had thoracolumbar scoliosis. The median (iqr) Cobb angle of all infantile scoliosis was 15.5° (13.3°-21.5°). Two children had Cobb angles greater than 20° and were, therefore, monitored by an orthopaedic surgeon. At the time of measurement, none of them needed treatment yet (Table 1).

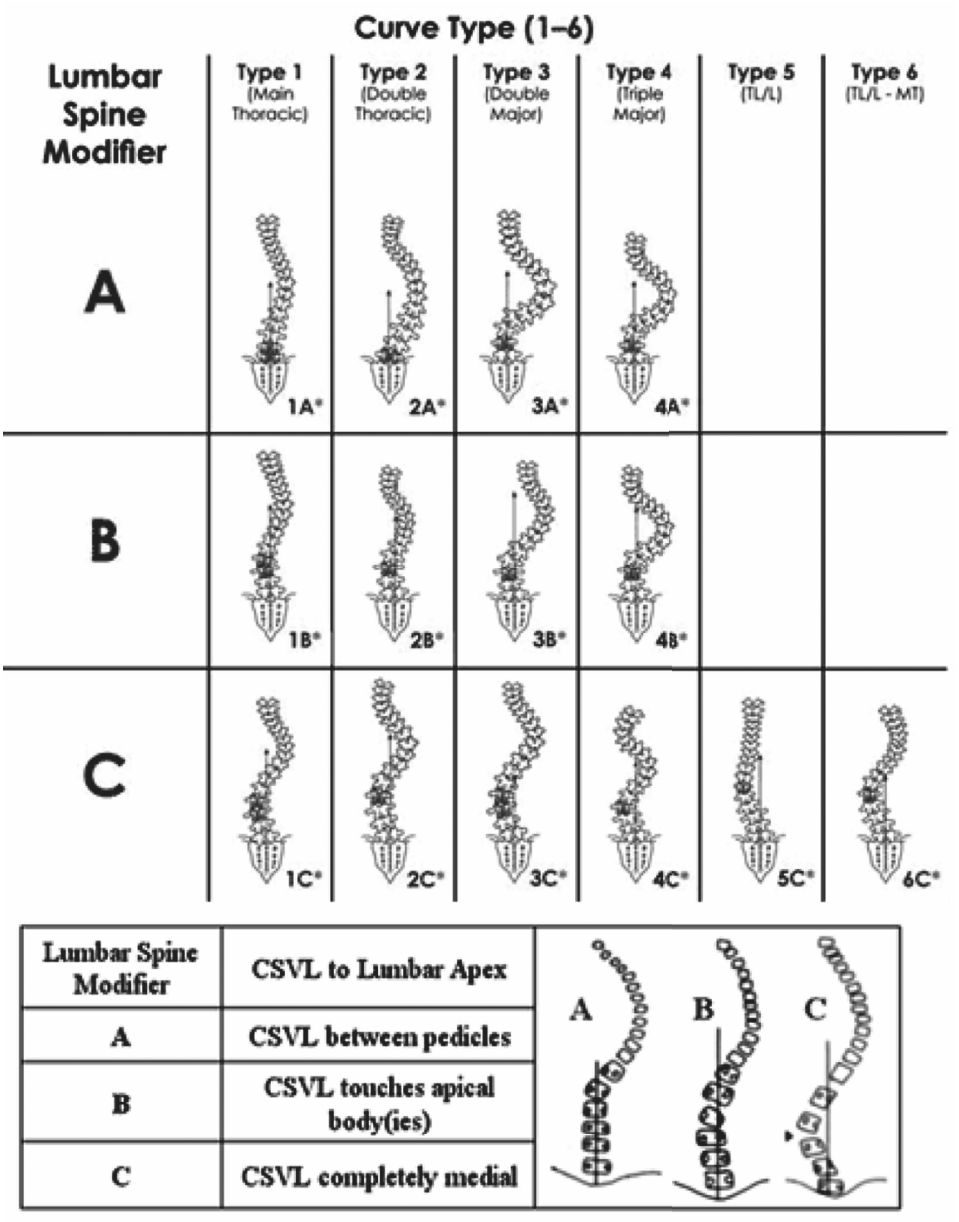

Figure 2. Lenke Classification system for idiopathic scoliosis: from http://www.spinal-deformity-surgeon.com/classification.html by L.G. Lenke, MD. CSVL: center sacral vertical line. Most children suffered from type 1B scoliosis.

Juvenile scoliosis

Of 51 children aged 3 to 10 years, 15 had juvenile scoliosis (29%). Seven showed LCS (47%) and 8 had IS (53%, Figure 1). Four children had thoracic scoliosis and eight had thoracolumbar scoliosis. The median (iqr) Cobb angle of all juvenile scoliosis was 14.5° (12.5°-35.0°). Six children had Cobb angles greater than 20°. Five of them (10% of children with juvenile scoliosis) were treated, while one was still being monitored (Table 1).

Adolescent scoliosis

The prevalence of scoliosis in children of 10 years and older was 80%. Adolescent scoliosis were all classified as IS (Table 1, Figure 1). The median (iqr) Cobb angle of all adolescent scoliosis was 35.0° (19.0°-41.5°). Ten children had Cobb angles greater than 20°. Seven children (47% of children with adolescent scoliosis) were treated, while two were still being monitored (Table 1).

Effects of age

Children with scoliosis were significantly older than those without scoliosis (p=0.007, Table 2). Adolescent children showed significantly more scoliosis than infant and juvenile PWS children (p=0.002 and p=0.0001, respectively). There was no significant difference in the number of children with scoliosis between the infant and juvenile group (p=0.96).

Children with LCS were significantly younger than children with IS (p=0.002, Table 2). Adolescent children showed significantly more IS than infant and juvenile PWS children (p=0.001 and p=0.006, respectively). There was no significant difference in type of scoliosis (LCS or IS) between the infant and juvenile group (p=0.34).

A higher percentage of children was treated in the adolescent group than in the infantile and juvenile group (p=0.0001 and p=0.001, respectively, Table 1). This difference did not reach significance between the infantile and juvenile group, probably due to small numbers (p=0.077). Our data indicate that scoliosis in PWS children is progressive with age.

The difference in location of the apex between infants (mainly thoracolumbar scoliosis) and adolescents (mainly thoracic scoliosis) was significant (p=0.036).

BMI and LBM

The ratio of lean body mass of the trunk and body surface area (trunkLBM:BSA) was significantly different between infants and adolescents ($p<0.0001$) and between the juvenile and adolescent group ($p<0.0001$, Table 1). BMISDS and trunkLBM:BSA were significantly correlated with age ($r=0.40$ with $p=0.01$ and $r=0.54$ with $p=0.01$, respectively).

Children with scoliosis had a significantly higher BMISDS than children without scoliosis (p=0.05, Table 2). Children with LCS had significantly lower BMISDS and trunkLBM:BSA than those with IS (p=0.026 and p=0.043, respectively).

Table 2. Differences between PWS children with and without scoliosis.

	No scoliosis	Scoliosis	LCS	IS
Age (years)*	3.7 (1.9 to 6.5)	6.9 (3.0 to 11.5)	4.4 (1.7 to 5.9)	11.1 (6.5 to 12.1)
BMI (SDS)**	0.6 (-0.2 to 1.7)	1.3 (-0.4 to 2.4)	1.1 (-0.8 to 1.5)	1.9 (1.0 to 2.6)
LBM:BSA ($\cdot 10^4$)	1.5 (1.5 to 1.6)	1.6 (1.5 to 1.7)	1.6 (1.5 to 1.6)	1.6 (1.5 to 1.7)
TrunkLBM:BSA ($\cdot 10^3$)†	7.3 (6.8 to 7.8)	7.5 (6.9 to 7.9)	7.1 (6.7 to 7.6)	7.8 (6.9 to 8.2)

Differences in age, BMISDS, LBM:BSA and trunkLBM:BSA for children with and without scoliosis, also divided by type of scoliosis. Values are expressed as median (iqr).
* Children with scoliosis were older than those without (p=0.007) and children with LCS were younger than those with IS (p=0.002).
** Children with scoliosis had a higher BMISDS than those without (p=0.05). Children with IS had a higher BMISDS than children with IS (p=0.026).
† Children with LCS had a significantly lower LBM:BSA than children with IS (p=0.043).

Discussion

Our study shows a prevalence of scoliosis in PWS patients of 37.5%, increasing in adolescence (30% in infants, 29% in juveniles and 80% in adolescents). Fifty percent of children with scoliosis had Cobb angles greater than 20° and 13% of them was treated. Our data show that scoliosis is a progressive deformity and that, therefore, the percentage of treated scoliosis increased with age. Children with scoliosis were older with higher BMISDS than those without.

We identified two types of scoliosis: “long C-curve type” scoliosis often seen in neuromuscular disorders (LCS) and scoliosis resembling idiopathic scoliosis (IS). Girls had more IS than LCS when compared to boys, but this did not reach statistical significance. More importantly, infants mainly showed LCS, whereas adolescents only showed IS; juvenile children showed both types of scoliosis. This could indicate a shift in a single patient from a LCS type to an IS type scoliosis, or a transition as a total group evolving from regression of LCS and new development of IS. The LCS type scoliosis showed a strong association with a low trunkLBM:BSA, used as a proxy for hypotonia of the trunk. The decreasing number of LCS type scoliosis with progressing age in PWS children might therefore be associated with an increase in lean body mass of the trunk. This is an interesting matter for future research.

The prevalence of scoliosis of 37.5% was much higher than in the non-PWS Dutch population (2.7%).[29] Importantly, our study also shows a very high prevalence of both infantile and juvenile scoliosis (~30%). Interestingly, Nagai et al.[21] reported a prevalence of 25% in patients aged 6-11 years. This study also reported that females were at greater risk of developing scoliosis,[21] while Kroonen et al.[20] treated more male patients. As could be expected from these results, gender did not affect the prevalence of scoliosis in our

study. This is in contrast to the Dutch non-PWS population, in which the male:female ratio is 1:1.2.[29]

Due to the high prevalence of scoliosis, it is advisable to perform frequent physical examination of the spine (Adam's Forward Bend Test) of PWS children during routine visits. However, physical examination may not be reliable to detect scoliosis in PWS children with obesity and/or hypotonia. In such children and in those with suspected scoliosis, yearly radiographic examination (preferably standing posteroanterior radiographs) is advisable. We recommend that all PWS patients with scoliosis of 20° or more be referred to an orthopaedic surgeon. In our clinic, non-PWS children with progressive scoliosis of 20-25° or more are treated with a Boston brace. In children with PWS, however, efficacy of the bracing is very limited due to hypotonia and obesity. And if effective, psychological problems (e.g. temper tantrums) may complicate the patients compliance. Children with a progressive scoliosis despite bracing or with a scoliosis of 45° or more are a candidate for spinal surgery. Surgery may be high-risk and contra-indicated in some PWS patients. However, severe progressive scoliosis may become a life-threatening deformity in itself. In short, the choice for treatment is complicated and the potential result should be weighed against possible complications.

Increased growth velocity during puberty and catch-up growth initiated by GH treatment have caused development or progression of scoliosis in non-PWS patients, prone to developing scoliosis.[30-32] Because many PWS patients have a reduced growth velocity, they are often treated with growth hormone. It would be interesting to perform a longitudinal study on the effects of growth and growth hormone treatment on the development or progression of scoliosis.

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Chapter 5

Randomized controlled trial to investigate the effects growth hormone treatment on scoliosis in children with Prader-Willi syndrome

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Abstract

Context: The prevalence of scoliosis in children with Prader-Willi syndrome (PWS) is 30-80%, depending on age. Although reports about effects of growth hormone (GH) treatment on scoliosis in children with PWS are limited, scoliosis is generally considered a contraindication for GH treatment.

Objective: To study the effects of GH treatment on the onset of scoliosis and curve progression in children with PWS.

Design: A multi-center, randomized controlled GH study in infants, prepubertal and pubertal children. Infants and prepubertal children were randomized into a GH-treated group (1.0 mg/m^2·day) and a control group for one and two years, respectively. Pubertal children were randomized to receive somatropin 1.0 or 1.5 mg/m^2·day. Yearly, X-rays of the spine were taken and height, weight, truncal lean body mass (trunkLBM with DXA), and IGF-I were measured.

Patients: 91 children with PWS, median (iqr) age 4.7 (2.1-7.4) years.

Main outcome measures: Onset of scoliosis (Cobb>10°) and scoliotic curve progression.

Results: GH-treated children had similar onset of scoliosis and curve progression as randomized controls (p=0.27-0.79 and p=0.18-0.98, respectively). GH treatment, IGF-I SDS, and catch-up growth had no adverse effect on the onset of scoliosis or curve progression, also not after adjustment for confounders. HeightSDS, trunkLBM and IGF-I SDS were significantly higher in GH-treated children than in randomized controls. At baseline, a higher IGF-I SDS was associated with a lower severity of scoliosis.

Conclusions: Scoliosis should no longer be considered a contraindication for GH treatment in children with PWS.

Introduction

Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia with obesity, hypogonadism, and psychological and behavioral problems.[1-7] PWS results from the lack of expression of the paternally derived chromosome 15q11-q13 caused by deletion, uniparental disomy (UPD), imprinting center defect, or balanced translocation.[1,8] Hypothalamic dysfunction may be responsible for many features of PWS.[9-11]

Spinal deformity is a major concern for patients with PWS. Scoliosis is defined as a spinal curve with a Cobb angle of more than 10° on a standing posteroanterior radiograph. The Cobb angle is the angle between the two steepest vertebrae, i.e. the upper border of the upper vertebra in the curve and the lower border of the lower vertebra.[12] The prevalence of scoliosis in PWS is high (30% <10 years, 80% >10 years[13-15] vs. 2.7% in the general Dutch adolescent population[16]). Children with PWS show two types of scoliosis (Figure 1): "Long C-curve type" scoliosis (LCS) often seen in children with neuromuscular disorders causing hypotonia, and scoliosis resembling idiopathic scoliosis (IS). Young children mainly show LCS, associated with a low ratio of truncal lean body mass versus body surface area (trunkLBM:BSA), which is a proxy for hypotonia. Older children mainly show IS.[13]

Growth hormone (GH) treatment is beneficial for children with PWS, as it improves body composition (increase in lean body mass, decrease in fat percentage) and psychomotor development.[17-23] In a previous report by our group, the effects of GH treatment on height and body composition of children with PWS have been described in detail.[23] Accelerated growth, either spontaneous or during GH treatment, has been associated with the onset of scoliosis and scoliotic curve progression.[24-29] As scoliosis is often considered a contraindication for GH treatment in children with PWS, the need for controlled data about the effect of GH treatment on scoliosis was emphasized.[30-32] We therefore performed a large randomized controlled trial. We hypothesized that GH treatment would not affect scoliosis, because it also increases truncal lean body mass which may counteract the adverse effects of accelerated growth on scoliosis. The primary aim of our study was to investigate the effects of GH treatment on the onset of scoliosis. The secondary aim was to study the effects of GH treatment on scoliotic curve progression. As age and gender are known to affect onset of scoliosis, while age, gender, and severity of scoliosis affect curve progression, we adjusted for these factors in our analyses.

Subjects and Methods

Subjects

Between April 2002 and January 2008, 104 children were enrolled in a large randomized controlled trial investigating the effects of GH treatment in children with PWS (Table 1), after fulfilling the following inclusion criteria: genetically confirmed diagnosis of PWS by positive methylation test and age between 6 months and 16 years. The study group was divided in infants, prepubertal and pubertal children. The infant group consisted of children aged 6 months to 3.5 years. The prepubertal group consisted of girls aged 3.5 to 12 years with Tanner breast stage < 2, and boys aged 3.5 to 14 years with Tanner genital stage < 2 and a testicular volume < 4 ml.[33] The pubertal group consisted of girls aged 12 to 16 years and boys aged 14 to 16 years with spontaneous or induced puberty. Caloric intake and activity level of all participants were standardized. All children were naïve to GH treatment at start of study. Children visited the Erasmus University Medical Center / Sophia Children's Hospital in Rotterdam, The Netherlands, and the study protocol was approved by the Medical Ethics Committee. Written informed consent was obtained from parents and children over 12 years of age. Assent was obtained for children between 4 and 12 years of age.

Design

The primary objective of our study was to investigate the effects of GH treatment on the onset of scoliosis. The secondary objective was to study the effects of GH treatment on progression of scoliosis. Infants and prepubertal children were randomized into a GH-treated group (1.0 mg/m^2·day) and a control group for one and two years, respectively. Pubertal children were randomly assigned to receive somatropin 1.0 or 1.5 mg/m^2·day (Genotropin, Pfizer, New York) for a follow-up period of two years. The first four weeks of treatment, children received 0.5 mg/m^2·day to prevent fluid retention. In January 2008, 38 infants (<3.5 years) had completed the one-year follow-up, and 44 prepubertal and 9 pubertal children had completed the two-year follow-up. Thus, 91 children were eligible for analysis (Table 1).

Radiographics

At start and subsequently each year, standardized posteroanterior X-rays were taken. In young and/or hypotonic children who were not able to sit or stand, posteroanterior X-rays were taken in supine position. All X-rays were taken in one center (EMCR). Cobb angles were measured independently by two observers (R.F.A.d.L.v.W. and L.W.L.d.K.), as previously reported, with minimal intra- and inter-observer variance (ICC=0.998 and 0.97, respectively).[13] The orthopedic surgeon was fully blinded to the assigned treatment. If the

independent measurements of Cobb angles differed between the two observers, the mean of the Cobb angles was used for analysis. Onset of scoliosis was defined as the presence of a Cobb angle of 10° or higher at 12 or 24 months of study in those without scoliosis at baseline (outcome: yes/no). Progression of scoliosis was evaluated as the change in Cobb angle over time in those with scoliosis at baseline and in those that developed scoliosis during study. Because treatment of scoliosis (bracing and surgery) prevents further curve progression, the effects of GH treatment on curve progression was only investigated in children with untreated scoliosis. For baseline characteristics of the total study population (Table 1), the Cobb angle of the scoliotic curve of children treated with a brace was set at 35° and the Cobb angle of those surgically treated at 55°. None of the children needed to start treatment of scoliosis during the study.

Anthropometrics

Standing height was measured with a Harpenden Stadiometer and supine length with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was assessed on an accurate scale. Height and BMI SDS were calculated, adjusted for sex and age according to Dutch references.[34,35] Height SDS, BMI SDS and body surface area (BSA) was calculated with Growth Analyser 3.0 (available at www.growthanalyser.org). Growth was calculated as the increase in heightSDS per year (ΔheightSDS) or the increase in centimeters per year (Δheight).

Severe scoliosis interferes with height and therefore also with Δheight(SDS). Lean body mass is known to be highly correlated with height.[23,36,37] In our study, these two parameters also showed a strong correlation (rho=0.82, p<0.0001 and rho=0.67, p<0.0001 at 12 and 24 months, respectively). To analyze the effect of Δheight on the onset of scoliosis and curve progression, we therefore also used the change in truncal lean body mass (ΔtrunkLBM) as a proxy for Δheight.

DXA

Dual Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare) was performed to measure the lean body mass of the trunk (trunkLBM), defined as the total amount of LBM in the chest, abdomen and pelvis. Reference values of trunkLBM in very young children were not available. To analyze the effects of GH treatment on relative muscle mass, we used a ratio of trunkLBM versus body surface area (trunkLBM:BSA ratio), as previously described.[13]

Assay

Serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano,

California). The intra-assay CV was 4% and the inter-assay CV was 6%. Because of age and sex dependency, IGF-I levels were transformed into SDS.[38]

Data analysis

Data were analyzed for all children together as well as for different age categories. Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 15.0, Chicago, IL). Data are presented as median and inter-quartile range (iqr). A change in Cobb angle of 5° or more was considered clinically relevant. Power calculation estimated a total number of 40 patients (comprising infants and prepubertal children) to yield a power of 0.80, in line with the international convention: Assuming a clinically relevant difference between GH-treated children and controls of 0.80 in terms of Cohen's d, an alpha level of 0.05 (one-tailed) and a total number of required patients of 40, the power of the study was 0.80.[39]
In our primary analyses, effects of GH treatment on onset and progression of scoliosis were analyzed after adjustment for confounders, using binary logistic regression models for onset of scoliosis (Table 3, odds ratios) and linear regression models for curve progression (Table 4, in β). In order to allow comparison with other reports regarding scoliosis in PWS in which these adjustments were not performed, we additionally analyzed differences in onset of scoliosis and curve progression between GH-treated children and randomized controls with Chi-square tests and Mann-Whitney U tests.
To investigate risk of onset of scoliosis during study, we included all children without scoliosis at start of study. Goodness of fit of binary logistic regression models was assured by performing the Hosmer and Lemeshow test (correct fitting when $p>0.05$). R^2 was calculated as a measure of explained variance. To investigate curve progression, we included all children with untreated scoliosis at start of study and those who had their onset of scoliosis during the study. Tolerance of all variables within the linear regression models was assured by calculating the variable inflation factor as a measure of multicollinearity. Nagelkerkes R^2 was calculated for all binary logistic and R^2 for all linear regression models as a measure of explained variance.
Mann-Whitney U tests and Chi-square tests were performed to compare outcomes between two groups. Data obtained in the smaller pubertal group were analyzed separately. A p-value below 0.05 was considered statistically significant.

Results

Table 1 shows the baseline clinical characteristics of the 91 children with PWS in our randomized controlled trial who completed the one- or two-year follow-up. The median (interquartile range, iqr) age was 4.7 years (2.1-7.4). The genotype was specified in 77 children: 35 had a deletion (46%), 33 had an UPD (43%), 8 an imprinting center defect (10%), and one a balanced translocation (1%). Positive methylation test was demonstrated in the remaining 14 patients, but the underlying genetic defect was not identified.

Baseline data

At start of study (Table 1), 36% of children had scoliosis with a median (iqr) Cobb angle of 19.0° (13.3°-36.0°). The prevalence of scoliosis increased with age (infants vs. pubertal: p=0.03). With increasing age, there was a shift from a predominance of "long C-curve type" scoliosis (LCS-type, Figure 1) in infants towards a predominance of scoliosis resembling idiopathic scoliosis (IS-type, Figure 1) in older children (infants vs. pubertal: p<0.03). The differences in prevalence of scoliosis and predominance of IS-type between prepubertal and pubertal children did not reach statistical significance, most likely due to the limited number of pubertal children (p=0.09 and p=0.07, respectively). The number of children treated for scoliosis was higher in older children.

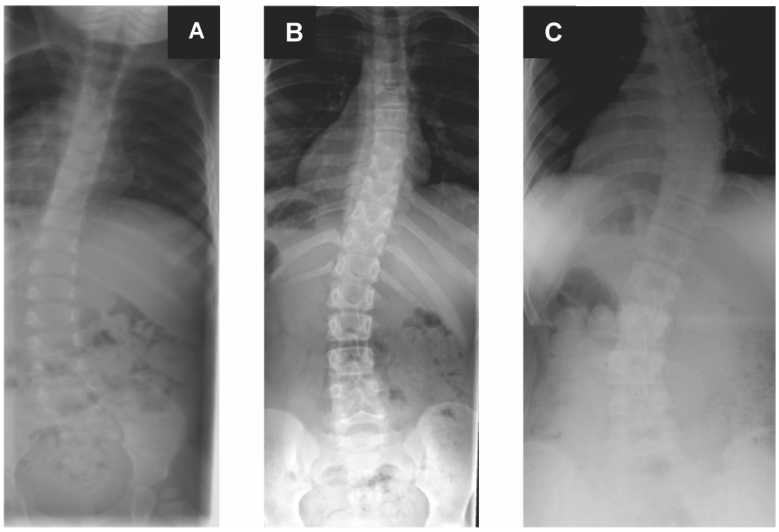

Figure 1. Examples of "Long C-curve type" scoliosis (LCS, A) and scoliosis resembling idiopathic scoliosis (IS, B). Younger children mainly showed LCS, while pubertal children only showed IS.

Table 1. Baseline data.

	Total	Infants		Prepubertal		Pubertal	
		GH-treated	Controls	GH-treated	Controls	GH-treated 1.0 mg/m^2·day	GH-treated 1.5 mg/m^2·day
N (m/f)	91 (49/42)	19 (11/8)	19 (14/5)	23 (13/10)	21 (8/13)	6 (3/3)	3 (0/3)
Age	4.7 (2.1 to 7.4)	2.0 (1.6 to 3.1)	1.5 (1.1 to 3.0)	6.8 (5.6 to 9.7)	6.0 (4.6 to 7.5)	13.4 (11.8 to 15.9)	13.9 (12.0 to 14.8)
Height SDS	-2.2 (-3.1 to -1.3)	-2.5 (-2.8 to -0.6)	-2.0 (-3.2 to -0.9)	-1.9 (-2.8 to -0.9)	-2.6 (-3.3 to -1.9)	-3.7 (-4.0 to -2.9)	-2.4 (-4.8 to -1.8)
BMI SDS	1.1 (-0.6 to 1.9)	-0.7 (-1.1 to 1.5)	-0.8 (-1.7 to 1.7)	1.2 (0.2 to 2.6)	1.2 (1.1 to 1.7)	1.2 (-0.1 to 2.1)	1.6 (-0.6 to 1.7)
TrunkLBM:BSA	7.6 (7.2 to 8.1)	7.4 (6.9 to 8.0)	7.3 (7.0 to 7.7)	8.0 (7.5 to 8.4)	7.6 (7.1 to 8.1)	8.7 (8.1 to 8.9)	8.1 (7.5 to 8.4)
IGF-I SDS	-2.1 (-3.1 to -1.4)	-2.5 (-3.9 to -1.4)	-2.6 (-5.7 to -0.7)	-1.7 (-2.3 to -1.2)	-1.9 (-2.6 to -1.2)	-3.0 (-4.9 to -2.5)	-2.1 (-3.3 to -2.0)
Scoliosis (%)	33 (36)	7 (37)	4 (21)	7 (30)	9 (43)	4 (67)	2 (67)
LCS-type (%)	13 (37)	3 (43)	3 (75)	2 (25)	5 (50)	0 (0)	0 (0)
IS-type (%)	22 (63)	4 (57)	1 (25)	6 (75)	5 (50)	4 (100)	2 (100)
Treated scoliosis (%)	12 (13)	1 (5)	1 (5)	3 (13)	2 (10)	4 (100)	1 (33)
Cobb angle (°)	19.0 (13.3 to 36.0)	15.5 (14.0 to 25.5)	18.5 (13.8 to 46.1)	14.5 (12.0-55.0)	16.0 (12.8 to 20.5)	36.0 (35.0-50.5)	21.3 (13.0-29.5)

Overview of study population depicted as total numbers, median (iqr) or percentage. N (m/f): total number of children (male/female). TrunkLBM:BSA: ratio of truncal lean body mass divided by body surface area (kg/m^2). Scoliosis (LCS/IS): total number of children with scoliosis and the number of children divided by type of scoliosis. Treated for scoliosis (%): total number of children treated for scoliosis and the percentage of children with treated scoliosis within the total group of children. At baseline, there were no significant differences between GH-treated children and randomized controls.

Prepubertal children had a significantly higher BMI SDS than infants (p<0.0001). Pubertal children also had a higher BMI SDS than infants, but this did not reach statistical significance (p=0.07). Prepubertal children had a significantly higher IGF-I SDS compared to pubertal children (p=0.004), and compared to infants, but this did not reach statistical significance (p=0.06). The trunkLBM:BSA ratio increased with age and was significantly different between all age categories (p<0.0001 to p=0.02).

Children who were treated for scoliosis at start of study had lower IGF-I SDS than children without scoliosis (-3.3 (-4.5 to -2.1) vs. -2.0 (-2.8 to -1.3), p=0.02), suggesting a protective effect of higher IGF-I levels. Linear regression modeling for 91 children at start of study showed a tendency for a less severe scoliosis in case of higher IGF-I levels (age: β=3.83, p=0.059; IGF-I SDS: β=–0.26, p=0.08).

Effects of GH treatment on growth and IGF-I levels

At baseline, there were no significant differences in age, heightSDS, BMI SDS, trunkLBM:BSA ratio, IGF-I SDS, and prevalence and severity of scoliosis between the GH treatment group and the randomized controls. In all children, GH treatment significantly increased heightSDS (ΔheightSDS) and IGF-I SDS compared to randomized controls (Table 2). Growth in prepubertal GH-treated children was greatest during the first year: median (iqr) ΔheightSDS 0.9 (0.7-1.3) during the first year versus 0.6 (0.3-0.7) during the second year (p<0.0001). Thus, catch-up growth was the most prominent during the first year of GH treatment. Compared to controls, BMI SDS tended to be lower in GH-treated children at 12 months of study (p=0.05), but was not significantly different at 24 months of study (p=0.19). GH treatment significantly decreased the hypotonia of the truncal muscles, shown by an increase in ΔtrunkLBM and ΔtrunkLBM:BSA. There was a significant correlation between IGF-I SDS and trunkLBM:BSA ratio (r=0.51 with p<0.0001 and r=0.41 with p<0.0001, at 12 and 24 months of study, respectively). During our study, there were no adverse effects of GH treatment.

Table 2. Effects of GH treatment versus randomized controls.

	Infants			Prepubertal					
	At 12 months			At 12 months			At 24 months		
	GH-treated	Controls	*P*[a]	GH-treated	Controls	*P*[a]	GH-treated	Controls	*P*[a]
N	19	19		23	21		23	21	
Height SDS	-0.9 (-1.6 to 0.1)	-1.8 (-3.5 to -1.4)	**0.003**	-1.0 (-1.5 to -0.3)	-2.5 (-3.4 to -2.3)	**<0.0001**	-0.5 (-0.8 to 0.0)	-2.6 (-3.4 to -2.3)	**<0.0001**
ΔheightSDS	1.2 (1.0 to 1.6)	-0.2 (-0.6 to 0.3)	**<0.0001**	0.9 (0.7 to 1.3)	-0.1 (-0.2 to 0.1)	**<0.0001**	1.4 (1.3 to 1.8)	-0.1 (-0.4 to 0.1)	**<0.0001**
BMI SDS	0.3 (-0.1 to 1.6)	0.3 (-0.6 to 1.6)	0.72	0.8 (-0.1 to 2.1)	1.4 (1.0 to 1.6)	0.05	1.1 (-0.2 to 1.7)	1.4 (1.1 to 1.6)	0.19
ΔtrunkLBM	1.7 (1.3 to 2.1)	0.7 (0.4 to 0.9)	**<0.0001**	1.8 (1.4 to 2.3)	0.7 (0.1 to 0.8)	**<0.0001**	2.8 (2.6 to 3.5)	0.8 (0.4 to 1.0)	**<0.0001**
ΔtrunkLBM:BSA	1.2 (0.7 to 1.8)	0.3 (-0.3 to 0.6)	**0.002**	1.3 (0.7 to 1.7)	0.0 (-0.4 to 0.3)	**<0.0001**	1.4 (0.5 to 1.7)	-0.2 (-0.5 to -0.1)	**<0.0001**
IGF-I SDS	2.5 (1.4 to 2.9)	-2.6 (-4.1 to -0.7)	**<0.0001**	2.3 (1.5 to 2.8)	-2.5 (-3.1 to -1.5)	**<0.0001**	2.4 (2.1 to 2.8)	-1.6 (-2.5 to -1.0)	**<0.0001**
Onset scoliosis (%)	4 (21)	2 (11)	0.71	5 (22)	6 (29)	0.52	5 (22)	7 (33)	0.14
Progression	-6.0 (-12.5 to 12.8)	-7.5 (-7.5 to -5.0)	0.48	-3.5 (-7.3 to 1.8)	0.0 (-1.0 to 1.0)	0.60	3.3 (-4.3 to 11.9)	-5.0 (-9.0 to -2.0)	0.27

Effects of GH-treatment on different parameters expressed as median (iqr) or numbers (%). ΔheightSDS, ΔtrunkLBM and ΔtrunkLBM:BSA are the gain in heightSDS, trunkLBM (kg) and trunkLBM:BSA ratio (kg/m^2) after 12 or 24 months of study compared to baseline. Progression is the change in Cobb angle during 12 or 24 months of study.

[a] p-values of Mann-Whitney U tests, GH-treated versus controls.

Table 3. Odds ratios for the risk of onset of scoliosis.

	Infants						Prepubertal children											
	N=27						N=28											
	12 months						12 months						24 months					
	Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
	OR	*P*	OR	*P*	OR	*P*	OR	*P*	OR	*P*	OR	*P*	OR	*P*	OR	*P*	OR	*P*
Age	0.68	0.54	0.49	0.32	0.50	0.37	0.62	0.06	0.63	0.06	0.63	0.06	1.00	1.00	0.99	0.95	0.97	0.86
Gender	4.16	0.18	12.53	0.06	12.80	0.08	0.38	0.31	0.28	0.21	0.28	0.20	0.56	0.49	0.34	0.26	0.28	0.22
GH	3.33	0.26			0.89	0.96	0.42	0.36			1.46	0.80	0.28	0.13			2.81	0.59
ΔtrunkLBM			2.06	0.45	2.25	0.69			0.46	0.17	0.38	0.29			0.47	0.06	0.30	0.19
R^2		0.19		0.29		0.29		0.29		0.33		0.34		0.12		0.19		0.20

Binary logistic regression models depicting the effects of parameters on the risk of onset of scoliosis, expressed in odds ratios. Gender: 0=male, 1=female. GH: growth hormone treatment; 0= no GH treatment, 1=GH treatment. ΔtrunkLBM: the increase in truncal muscle mass in kilograms. R^2: explained variance by the model.

Table 4. Multiple linear regression models (β) for influences on curve progression.

	Infants						Prepubertal children											
	N=15						N=26											
	0-12 months						0-12 months						12-24 months					
	Model 1		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**		**Model 1**		**Model 2**		**Model 3**	
	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*	β	*P*
Age	3.05	0.34	4.75	0.40	4.73	0.44	-1.32	0.07	-0.87	0.24	-0.50	0.44	1.19	0.14	0.87	0.29	1.00	0.25
Gender	11.85	0.06	12.81	0.09	8.27	0.46	-2.94	0.40	-4.71	0.20	-3.29	0.30	3.32	0.35	4.98	0.19	5.18	0.18
IGF-I SDS	1.20	0.24	0.38	0.81	0.41	0.81	-0.24	0.69	0.59	0.47	0.45	0.52	1.36	0.09	0.03	0.98	-0.26	0.85
ΔtrunkLBM			7.19	0.51	7.84	0.52			-3.95	0.16	-4.13	0.09			2.91	0.22	3.30	0.18
Cobb at start					-0.47	0.59					-0.71	**0.03**					-0.24	0.52
R^2		0.47		0.52		0.56		0.28		0.37		0.57		0.25		0.31		0.33

Multiple linear regression models depicting the effects of parameters on curve progression, defined as the Cobb angle of the main scoliotic curve, expressed in β. Gender: 0=male, 1=female. ΔtrunkLBM: the increase in truncal muscle mass in kilograms. Cobb at start: The Cobb angle of the scoliotic curve at start of study. R^2: explained variance by the model.

Effects of GH treatment on scoliosis

Infants (0-3.5 years)

During 12 months of study, there was no significant difference between GH-treated infants and randomized controls with regard to onset of scoliosis, curve progression (p=0.71 and p=0.48, Table 2), and start of treatment for scoliosis (p=1.00).

Table 3 shows the odds ratios for the risk of onset of scoliosis. Corrected for age and gender, GH treatment had no significant effect on the risk of onset of scoliosis, with an OR (95% confidence interval) of 3.33 (0.41-27.2) (p=0.26, Model 1). Also, ΔtrunkLBM as a proxy for Δheight did not affect the risk of onset of scoliosis, with an OR (95% CI) of 2.1 (0.3-13.7) (p=0.45, Model 2). In our final model (Model 3), both GH treatment and ΔtrunkLBM did not increase the risk of onset of scoliosis in infants with PWS.

Table 4 shows the effect (β) of different variables on the progression of scoliosis. Corrected for age and gender, IGF-I SDS had no significant effect on the progression of scoliosis during 12 months of study, with a β (95% CI) of 1.20 (-1.0 to 3.4) (p=0.24, Model 1). Also, ΔtrunkLBM as a proxy for Δheight did not affect the progression of scoliosis during 12 months of study (β (95% CI): 7.19 (-19.1 to 33.5), p=0.51; Model 2). In our final model (Model 3), IGF-I SDS, ΔtrunkLBM, and the severity of scoliosis at start of study had no significant effect on the progression of scoliosis in infants with PWS.

Results were similar when ΔheightSDS was included in our models instead of ΔtrunkLBM.

Prepubertal children (3.5-12/14 years)

During 12 and 24 months of study, there was no significant difference between GH-treated prepubertal children and randomized controls with regard to onset of scoliosis, curve progression (12 months: p=0.52 and p=0.60; 24 months: p=0.14 and p=0.27; Table 2), and start of treatment for scoliosis (both p=1.00).

Table 3 shows the odds ratios for the risk of onset of scoliosis. Corrected for age and gender, GH treatment had no significant effect on the risk of onset of scoliosis after 12 and 24 months of study with an OR (95% CI) of 0.42 (0.07-2.7) at 12 months and 0.3 (0.05-1.5) at 24 months of study (p=0.36 and p=0.16, respectively; Model 1). Also, ΔtrunkLBM as a proxy for Δheight did not affect the risk of onset of scoliosis after 12 and 24 months of study, with an OR (95% CI) of 0.46 (0.1-1.4) at 12 months and 0.47 (0.2-1.0) at 24 months of study (p=0.17 and p=0.06, respectively; Model 2). In our final model (Model 3), both GH treatment and ΔtrunkLBM did not increase the risk of onset of scoliosis in prepubertal children with PWS after 12 and 24 months of study.

Table 4 shows the effect (β) of different variables on the progression of scoliosis. Corrected for age and gender, IGF-I SDS had no significant effect on the progression of scoliosis during the first and second year of the study, with a β (95% CI) of -0.24 (-1.5 to 1.0) during the first year and 1.3 (-0.3 to 2.9) during the second year of study (p=0.69 and p=0.10,

respectively; Model 1). Also, ΔtrunkLBM as a proxy for Δheight did not significantly affect the progression of scoliosis, with a β (95% CI) of -3.95 (-9.6 to 1.8) during the first year and 3.7 (-3.9 to 11.3) during the second year of study (p=0.16 and p=0.32, respectively; Model 2). In our final model with the highest explained variance (Model 3, R^2=0.57), a more severe scoliosis at start of study and a higher ΔtrunkLBM during the first year were associated with a tendency for regression of scoliosis (β (95% CI) of ΔtrunkLBM: -4.1 (-9.1 to 0.8) with p=0.09; β (95% CI) of severity at start: -0.71 (-1.30 to -0.11) with p=0.03). During the second year of GH treatment, IGF-I SDS, ΔtrunkLBM and Cobb angle at start of study had no significant effect on curve progression in prepubertal children with PWS.
Results were similar when ΔheightSDS was included in our models instead of ΔtrunkLBM.

Pubertal children (12/14-16 years)
A GH dose of 1.5 mg/m^2·day in pubertal children (N=3) resulted in a higher height velocity and IGF-I SDS compared with those treated with 1.0 mg/m^2·day (N=6; p=0.046 and p=0.08, respectively; data not shown). Three of nine pubertal children had no scoliosis at start of study and had no onset of scoliosis during study. Six pubertal children had scoliosis at start of study, but there was no difference in the number of children treated for scoliosis or in curve progression between those treated with a dose of 1.0 and 1.5 mg/m^2·day (data not shown).

Discussion

Our randomized controlled trial shows that there was no significant difference between GH-treated children and randomized controls with regard to onset of scoliosis, curve progression, and start of treatment of scoliosis. In both the infant and prepubertal group, GH treatment, ΔheightSDS, ΔtrunkLBM (used as a proxy for Δheight), and IGF-I SDS were not associated with an increased risk of onset of scoliosis or curve progression, both before and after correction for confounders. Thus, GH treatment not only improves heightSDS and trunkLBM of children with PWS,[17-23] but has also no adverse effects on the onset of scoliosis and curve progression.
Some authors have described an association between increased GH levels and a higher rate of curve progression in children without PWS.[27-29] In contrast to these reports, our data show that a higher baseline IGF-I SDS was associated with a lower severity of scoliosis, suggesting a protective effect of higher IGF-I SDS in children with PWS. As IGF-I SDS was also positively associated with the trunkLBM:BSA ratio, the protective effect may be due to a higher truncal lean body mass. In our randomized controlled trial, GH-treated children had a significantly higher IGF-I SDS and ΔtrunkLBM, but IGF-I SDS was not associated

with progression of scoliosis. The ΔtrunkLBM, however, was associated with a tendency for regression of scoliosis, but only during the first year of study.

The prepubertal group provides the most accurate information about the effects of GH treatment on scoliosis, because all X-rays were taken in standing position and children were followed in a two-year randomized controlled trial. GH treatment and catch-up growth had no adverse effect on the onset of scoliosis or curve progression. Notably, our results show that those with a more severe scoliosis at start of study and a higher catch-up growth had a tendency for regression of scoliosis during the first year of study. This effect was not seen during the second year. Our study is the first randomized controlled trial investigating the effects of GH treatment on scoliosis in a large group of children with PWS. Our data indicate that even severe scoliosis should not be considered a contraindication for GH treatment in children with PWS. The findings are in line with a retrospective study demonstrating that GH treatment did not affect scoliosis in these children.[14]

Although our main aim was to investigate the onset and progression of scoliosis in a randomized controlled GH trial in infants and prepubertal children, we did not want to withhold GH treatment from a small group of pubertal children PWS. In this group of pubertal children we found that a higher dose of GH (1.5 mg/m^2·day) increased height velocity and IGF-I SDS and was not associated with an accelerated onset of scoliosis or curve progression.

Infants had a controlled period of one year and prepubertal children of two years. This period is not very long for follow-up. However, in orthopedic practice visits are scheduled every four to six months to monitor progression. Therefore, changes occurring during GH treatment can easily be noted during one or two years of follow-up. Moreover, if GH treatment would have adverse effects on scoliosis by stimulating growth, one would especially notice this during the period with the highest gain in heightSDS, i.e. catch-up growth during the first year of GH treatment. In our opinion, one or two years of follow-up is sufficient to identify the effects of GH-induced catch-up growth on the onset or progression of scoliosis. Our final models in infants and prepubertal children explained 20-57% of variances (R^2). In future, when more data on the pathogenesis of scoliosis become available, perhaps our models might be improved.

In conclusion, our randomized controlled GH trial in a large group of children with PWS shows that GH treatment had no adverse effects on the onset of scoliosis and curve progression. A higher baseline IGF-I SDS was associated with a lower severity of scoliosis. Thus, scoliosis should not be considered a contraindication for GH treatment in children with Prader-Willi syndrome. Because of the high prevalence of scoliosis and the potential associated morbidities in patients with PWS, it is recommended to perform frequent physical examination and yearly radiographic examination, independently from GH treatment.

Acknowledgements

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Chapter 6

Randomized controlled GH trial: Effects on anthropometry, body composition, and body proportions in a large group of children with Prader-Willi syndrome

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Abstract

Background: Prader-Willi syndrome (PWS) children have impaired growth, and abnormal body composition. Previous 1-year controlled studies showed improvement of height and body composition during GH treatment.

Objective: To evaluate growth, body composition and body proportions during GH treatment in a large group of PWS children.

Design and patients: We performed a randomized controlled GH trial in 91 prepubertal PWS children (42 infants, 49 children, aged 3-14 years). After stratification for age, infants were randomized to GH treatment (GH-group; 1 mg/m^2·day; N=20), or no treatment (control group; N=22) for 1 year. In the second year all infants were treated with GH. After stratification for BMI, children >3 years of age were randomized to GH treatment (GH-group; 1 mg/m^2·day; N=27) or no treatment (control group; N=22) for 2 years. Anthropometric parameters were assessed once in every 3 months. Body composition was measured by Dual Energy X-ray Absorptiometry.

Results: Median (interquartile range, iqr) height SDS increased during 2 years of GH in infants from -2.3 (-2.8 to -0.7) to -0.4 (-1.1 to 0.0) and in prepubertal children from -2.0 (-3.1 to -1.7) to -0.6 (-1.1 to -0.1). In non-GH-treated children height SDS did not increase. Head circumference improved during 1 and 2 years of GH in infants and children, respectively. Body fat percentage and body proportions improved in GH-treated children, but did not completely normalize. Lean body mass SDS improved compared to the control group. Serum IGF-I increased to levels above the normal range in most GH-treated children.

Conclusions: Our randomized study shows that GH treatment in PWS children significantly improves height, BMI, head circumference, body composition and body proportions. PWS children are highly sensitive to GH, suggesting that monitoring of serum IGF-I is indicated.

Introduction

Prader-Willi Syndrome (PWS) is a genetic disorder, caused by either a microdeletion on the paternally derived chromosome 15q11-13 or a uniparental maternal disomy affecting the same region.[1] In rare cases, PWS is due to an imprinting centre mutation[2] which results in silencing of genes that are normally active in the paternally inherited chromosome 15q11-13. PWS is characterized by muscular hypotonia, psychomotor delay, short stature and feeding difficulties in infancy. After the age of 2-4 years, excessive appetite may result in rapid weight gain and obesity. Most of the characteristic features of PWS are thought to result from hypothalamic dysfunction.[3]

Several endocrine abnormalities have been reported, including GH deficiency and hypogonadotropic hypogonadism.[4] Twenty years ago, GH treatment was suggested to improve growth velocity in children with PWS.[5] Nowadays, studies are available, showing that GH treatment improves growth velocity in GH-deficient PWS children[6-8] or in PWS children treated irrespective of their GH status.[9] The largest uncontrolled GH-study in PWS children to date showed no change in BMI SDS, however, there was a wide variability in GH dosage and age at start of study between children.[10] In addition, body composition improved, that is, body fat percentage decreased and lean body mass (LBM) (expressed in kg) increased during 1 year of GH treatment.[7,11,12]

We hypothesized that GH treatment would normalize height, BMI and improve body composition, both after correction for sex and age, and after correction for sex and height, respectively. We therefore evaluated a large group of 91 not severely overweight PWS children, according to a 1- and 2-year controlled design in infants and children, respectively. We evaluated effects of GH on growth, LBM and body fat percentage, head circumference (primary outcome measures), and body proportions (secondary outcome measures).

Patients and methods

Patients

Ninety-one patients were included in our randomized controlled GH trial. Forty-two children below 3.5 years at start of study (infant group) were treated in a 1-year controlled study. Forty-nine prepubertal children over 3.5 years at start of study (pre-pubertal group) were treated in a 2-year controlled study. All participants fulfilled the following inclusion criteria: (i) genetically confirmed diagnosis of PWS; (ii) age between 6 months and 14 years at start of study; (iii) bone age <14 years (girls) or 16 years (boys); (iv) prepubertal at start of study, defined as Tanner breast stage ≤2 for girls and testicular volume <4 ml for boys[13] and with age below 12 or 14 years in girls or boys, respectively. Patients with noncooperative

behavior or patients receiving medication to reduce fat were excluded, but in this group, no patients were excluded for these reasons. Children were regularly seen by a dietician and physiotherapist. The caloric intake and activity level of all children were standardized at 3 months prior to start of study. Compliance to diet and exercise was evaluated by the research nurse (MvE), in close collaboration with the dietician and the physiotherapist. All children were naïve to GH treatment at the start of study. They were included irrespective of their GH secretory status. Prior to randomization, infants were stratified for age and children (>3.5 years) for BMI. All participants were randomized to GH treatment (GH-group) or no GH treatment (control group). A double blind placebo-controlled study was considered unethical. The GH-group received recombinant human GH (Genotropin, Pfizer, New York, NY) 1 mg/m^2 s.c. daily at bedtime.

The study protocol was approved by the Medical Ethics Committees of the 20 participating Dutch centers. Written informed consent was obtained from the parents and children over 12 years of age.

Anthropometry

At baseline, and at 3-monthly intervals, anthropometric measurements were performed. Height (or supine length), and sitting height (only in children >3.5 years) were obtained using a Harpenden stadiometer and a sitting height table. Weight was measured on an accurate scale. Left foot length, left tibia length and span width were measured according to Cameron[14] using a Harpenden anthropometer. The mean of three measurements was used for analysis. All measurements were obtained by three observers (DF, RdL, MvE). Height, body mass index (BMI), and head circumference were expressed as SDS, adjusting for age and sex.[15,16] BMI and SDS of BMI, height, sitting height, head circumference, foot length, span width and tibia length were calculated with Growth Analyser version 3.0 (www.growthanalyser.org)

Body composition

Dual energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK) was performed in all children and total fat mass, fat percentage and LBM were measured. Fat mass, fat percentage and LBM were transformed into SD-scores adjusting for sex and age (LBM_{age}).[17] As body composition, particularly LBM, is strongly related to height, LBM expressed as SDS for age and sex might result in an underestimation in short stature. For this reason, LBM was also adjusted for height and sex (LBM_{height}). LBM_{height} SDS was computed by comparing LBM of PWS children with LBM of healthy children with the same height and sex. Reference values were obtained using the same instrumentation and software.[17] Because reference data for DXA (Lunar Prodigy) were not available for children aged 0-4 years, only children >4 years were included in this analysis.

IGF-I and IGFBP-3

Blood samples were collected in the morning after a 12 h overnight fast, immediately centrifuged and stored at -20°C until assayed. Samples were pooled and assayed in one session, to minimize variation. Serum IGF-I and IGFBP-3 were measured using a specific RIA in one laboratory.[18] The intra-assay CV was 4% and the interassay CV was 6%. For IGF-I and IGFBP-3, sex- and age-matched Dutch references were available. Because of the age- and sex dependency, IGF-I and IGFBP-3 levels were transformed to SDS.

Statistical analysis

Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS Version 11.0, Chicago, IL). Most of our data were not Gaussian distributed, and therefore expressed as median (interquartile range, iqr). Differences compared to baseline within groups were calculated using Wilcoxon signed rank test. Differences in change compared to baseline between groups were calculated using Mann-Whitney U tests. We used Bonferroni's correction for multiple testing for both inter- and intra-individual testing. We used the binomial test to compare results of SD-scores in PWS children with healthy reference data (0 SDS). Correlations were calculated using Spearman's rank correlation coefficient (rho).

Results

Infants

Clinical charactheristics

Table 1 lists the characteristics of 42 PWS infants and toddlers. After 1 year, 31 of them were re-evaluated (11 did not complete 1 year of study). After 1 year, the control group also started with GH. After 2 years, 24 of them were re-evaluated (seven did not complete the second year of study) (Figure 1). Infants with repeated measurements had similar BMI SDS and height SDS compared to infants who did not yet finish 2 years of study. Infants with repeated measures were, however, older (p=0.025), which might reflect early diagnosis of PWS during recent years. One infant of the GH-treated group received T4 therapy for low free T4 levels prior to start of GH. Fifteen infants had a paternal deletion, 13 had uniparental maternal disomy, and in 14, PWS was confirmed by a positive methylation test, but the underlying genetic defect was yet unknown.

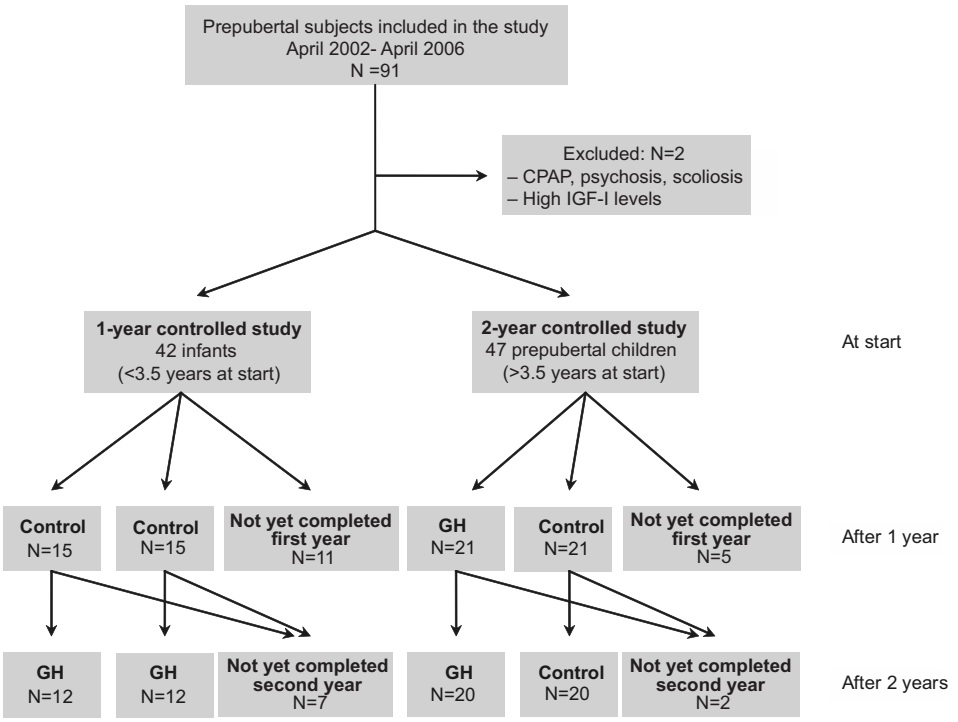

Figure 1. Flow chart of eligible patients.

Height, BMI, and head circumference

At start, BMI was not significantly different from 0 SDS, but all other median SD-scores were significantly below 0 (Table 1). Anthropometric parameters were similar in the two groups (Figure 2a-c). Median head circumference SDS was relatively high compared to height SDS: -0.8 versus -2.3 SDS in the GH-treated group and -1.1 versus -2.1 in the control group.

In the GH-treated group, median height SDS increased significantly after 1 and 2 years (Table 1, Figure 2a). After 2 years of GH, all infants had a height SDS above -2 SDS. In the control group, median height SDS remained low in the first year, but increased significantly, when GH was started in the second year (Figure 2a). Also median head circumference SDS increased accordingly (Figure 2c). BMI SDS increased progressively in both groups, but remained within the normal range in most patients (Figure 2b).

Table 1. Characteristics of PWS infants (6 months to 3 years) during 1-year controlled study.

	GH-treated			Controls		
	Baseline	1 year (GH)	2 year (GH)	Baseline	1 year (untreated)	2 year (GH)
N	20	16	12	22	15	12
Sex (m/f)	12/8			16/6		
Age (years)	2.0 (1.6 to 3.1)	3.1 (2.6 to 4.1)	4.5 (3.6 to 5.1)	1.3 (1.0 to 2.8)	2.6 (2.3 to 4.2)	4.2 (3.3 to 5.2)
Height (SDS)	-2.3 (-2.8 to -0.7)	-1.0 (-1.9 to 0.1)[4d]	-0.4 (-1.1 to 0.0)[3a]	-2.1 (-3.2 to -1.0)	-1.8 (-3.5 to -1.4)[d]	- 1.2 (-2.3 to 0.1)[2a]
BMI (kg/m^2)	16.4 (15.1 to 18.6)	16.3 (15.7 to 18.2)	16.7 (15.7 to 21.2)	16.1 (14.7 to 18.2)	16.4 (15.4 to 19.8)	17.1 (16.4 to 18.7)
BMI (SDS)	0.5 (-0.9 to 1.9)	0.3 (-0.1 to 1.6)	0.8 (0.1 to 2.8)[1]	-0.8 (-1.7 to 1.6)	0.1 (-0.7 to 2.6)[2]	0.9 (0.5 to 1.9)[1]
Head circ (SDS)	-0.8 (-1.6 to -0.3)	0.0 (-0.9 to 0.7)[3d]	0.4 (-0.5 to 1.1)[3]	-1.1 (-1.8 to -0.5)	-0.8 (-1.6 to -0.3)[1d]	0.0 (-0.6 to 0.6)[2]
IGF-I (ng/ml)	27.0 (22.0 to 35.0)	179.0 (119.5 to 241.0)	287.0 (227.0 to 419.0)	47.0 (17.0 to 52.0)	33.0 (22.5 to 47.8)	259.0 (164.0 to 292.0)
IGF-I (SDS)	-1.9 (-2.8 to -1.3)	2.5 (1.6 to 3.0)[3]	3.2 (1.9 to 4.3)	-1.6 (-2.6 to -0.4)	-2.1 (-3.1 to -0.5)	2.4 (2.2 to 3.0)
IGFBP-3 (SDSI)	0.8 (0.7 to 1.1)	2.2 (1.6 to 2.4)	2.7 (2.2 to 3.6)	1.1 (0.8 to 1.3)	0.9 (0.7 to 1.3)	2.5 (1.9 to 2.6)
IGFBP-3 (SDS)	-2.6 (-3.3 to -2.0)	0.5 (0.0 to 1.2)[2]	1.5 (0.4 to 2.3)[1]	-1.5 (-2.6 to -0.7)	-2.4 (-3.5 to -1.2)	0.7 (0.5 to 1.1)
IGF-I/BP3 (SDS)	-0.9 (-2.0 to -0.4)	2.3 (1.7 to 3.4)[2d]	2.4 (1.7 to 3.3)	-0.3 (-1.7 to 0.6)	-1.1 (-2.1 to 0.0)[d]	2.5 (2.0 to 3.5)

Data are expressed as median (iqr). IGF-I and IGFBP-3 levels were available in 11, 12 and 8 patients of the GH-treated group and in 11, 12 and 7 children of the control group respectively. The GH-treated group received GH during the entire study. Infants in the control group were not treated during the first year, but were treated with GH in the second year. [1] p<0.05, [2] p<0.01, [3] p<0.005, [4] p<0.001 compared to baseline levels; [a] p<0.05, [d] p<0.001, change GH-treated group versus the control group. l

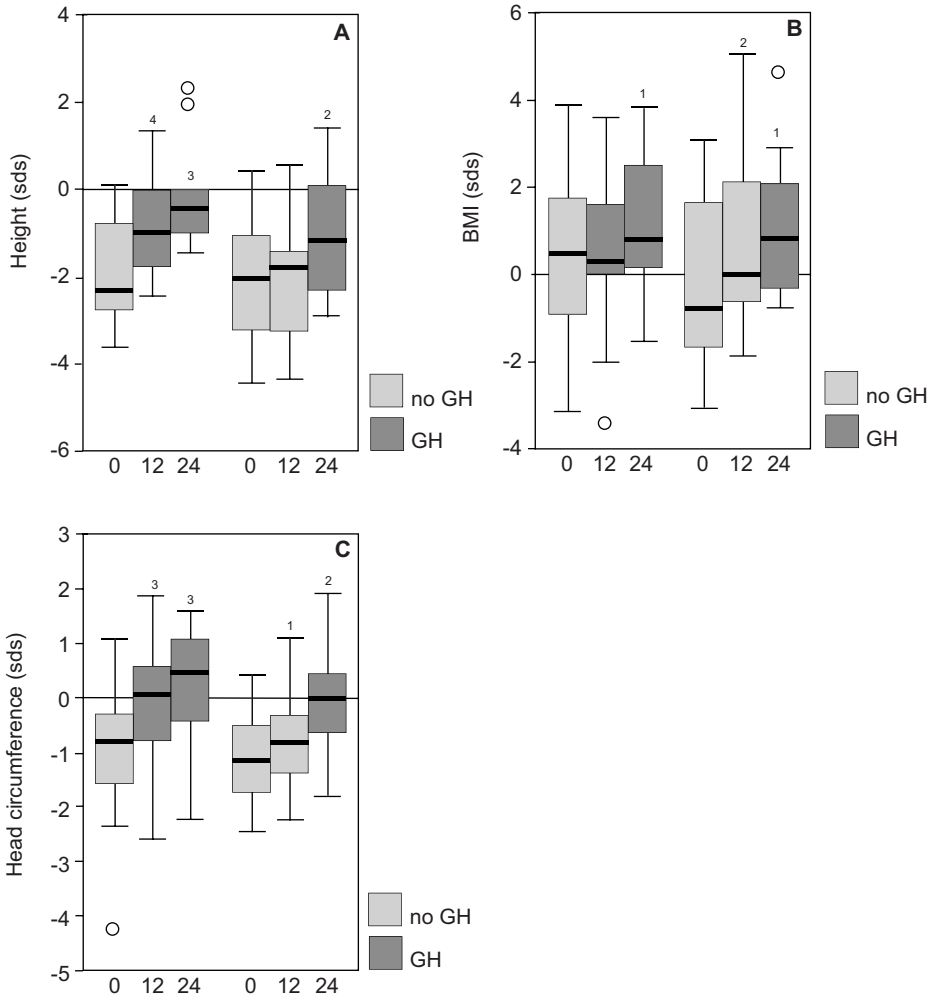

Figure 2 a-c. Anthropometric data in PWS infants, during 2 years of follow-up. In the first year only the GH group received GH treatment; during the second year both groups were treated. Data are expressed in SDS for age and sex and represented by boxplots. The lower boundary is the 25th percentile and the upper boundary is the 75th percentile. The line in the box represents the median. Lines are drawn from the smallest observed value to the largest observed value that is not an outlier. Open circles are outliers. Significantly different compared with baseline [1]p<0.05, [2]p<0.01, [3]p<0.005, [4]p<0.001. Significantly different change in GH group vs. control group: height SDS after 12 months (p<0.001) and after 24 months (p<0.05), head circumference after 12 months (p<0.001).

Table 2. Characteristics of prepubertal PWS children (3-14 years) during 2-year controlled study).

	GH-treated			Controls		
	Baseline	1 year (GH)	2 year (GH)	Baseline	1 year (untreated)	2 year (untreated)
N	25	21	20	22	21	20
Sex (m/f)	13/12			8/14		
Age (years)	6.8 (5.4 to 8.8)	7.8 (6.4 to 9.7)	8.7 (7.3 to 11.2)	5.9 (4.7 to 7.4)	7.0 (5.6 to 8.5)	8.0 (6.6 to 9.5)
Height (SDS)	-2.0 (-3.1 to -1.7)	-1.2 (-1.5 to -0.6)[4d]	-0.6 (-1.1 to -0.1)[4d]	-2.5 (-3.3 to -1.9)	-2.6 (-3.4 to -2.3)[d]	-2.6 (-3.4 to -2.3)[d]
BMI (kg/m^2)	17.7 (16.0 to 22.3)	17.5 (15.3 to 19.8)	17.5 (16.1 to 21.1)	18.1 (17.2 to 19.9)	18.6 (17.6 to 19.7)	19.1 (17.8 to 20.8)
BMI (SDS)	1.2 (0.1 to 2.2)	0.8 (-0.3 to 1.4)[4d]	0.6 (-0.4 to 1.6)[3c]	1.3 (1.1 to 1.6)	1.4 (1.0 to 1.6)[d]	1.3 (1.1 to 1.6)[c]
Head circ (SDS)	-0.8 (-1.5 to -0.2)	-0.2 (-1.2 to 0.2)[4]	-0.1 (-1.1 to 0.5)[3]	-0.6 (-1.2 to -0.1)	-0.6 (-0.9 to 0.3)[1]	-0.6 (-1.1 to 0.3)[a]
IGF-I (ng/ml)	60.0 (46.5 to 96.5)	337.0 (274.3 to 474.3)	424.0 (313.0 to 570.0)	56.0 (42.0 to 88.0)	55.0 (42.5 to 94.8)	92.0 (61.8 to 130.0)
IGF-I (SDS)	-1.7 (-2.3 to -1.2)	2.3 (1.5 to 2.7)[4d]	2.4 (2.1 to 2.8)[4d]	-1.9 (-2.6 to -1.2)	-2.5 (-2.8 to -1.4)[d]	-1.8 (-2.6 to -1.0)[d]
IGFBP-3 (SDSI)	1.3 (0.9 to 1.5)	2.5 (2.2 to 2.9)	2.8 (2.6 to 3.2)	1.2 (0.9 to 1.5)	1.3 (0.8 to 1.5)	1.5 (1.2 to 1.8)
IGFBP-3 (SDS)	-1.9 (-2.8 to -1.2)	0.4 (-0.1 to 0.8)[4d]	0.6 (0.3 to 1.1)[4d]	-2.2 (-3.1 to -1.4)	-2.4 (-3.5 to -1.8)[d]	-1.7 (-2.3 to -1.2)[d]
IGF-I/BP3 (SDS)	-0.5 (-1.0 to 0.5)	2.5 (2.0 to 3.0)[4d]	2.5 (1.8 to 2.9)[4d]	-0.6 (-1.6 to 0.3)	-0.8 (-1.4 to -0.2)[d]	-0.6 (-1.2 to -0.1)[d]

Data are expressed as median (iqr). IGF-I and IGFBP-3 levels were available in 21, 21 and 20 patients of the GH-treated group and in 18, 12 and 16 children of the control group respectively.

[1] p<0.05, [3] p<0.005, [4] p<0.001, compared to baseline levels, [a] p<0.05, [c] p<0.005, [d] p<0.001, change GH-treated group versus the control group.

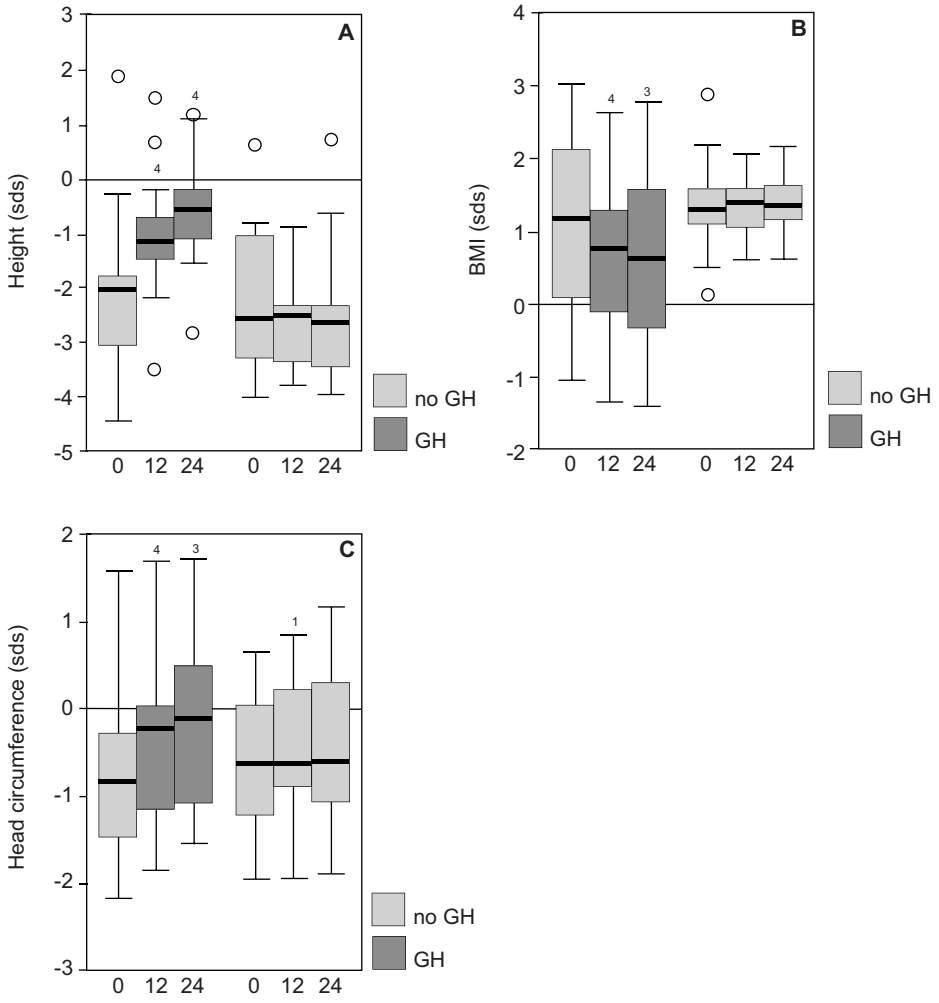

Figure 3 a-c. Anthropometric data in pre-pubertal PWS children, during the 2-year controlled study. Data are expressed in SDS for age and sex and represented by boxplots. Significantly different compared with baseline: [1]p<0.05, [2]p<0.01, [3]p<0.005, [4]p<0.001. Significantly different change in GH-treated group versus the control group: height SDS after 12 months (p<0.001) and after 24 months (p<0.001), BMI SDS after 12 months (p<0.001) and after 24 months (p<0.005), head circumference after 24 months (p<0.05).

Table 3. Body proportions and body composition of prepubertal PWS children (3-14 years) during 2-year controlled study.

	GH-treated			Controls		
	Baseline	1 year (GH)	2 year (GH)	Baseline	1 year (untreated)	2 year (untreated)
Tibia length (SDS)	-1.2 (-1.9 to -0.5)	-0.9 (-1.4 to -0.2)[1a]	-0.5 (-0.9 to -0.3)[1d]	-1.8 (-2.6 to -1.4)	-2.0 (-2.7 to -1.2)[a]	-2.4 (-3.0 to -1.7)[2d]
Foot length (SDS)	-1.8 (-3.3 to -1.6)	-1.3 (-2.4 to -0.9)[4d]	-1.4 (-2.1 to -0.5)[3d]	-2.2 (-2.7 to -1.6)	-2.5 (-3.4 to -1.7)[3d]	-3.2 (-3.7 to -2.2)[3d]
Sitt height (SDS)	-1.3 (-1.7 to -0.8)	-0.4 (-1.0 to 0.2)[4d]	-0.4 (-1.2 to 0.0)[4d]	-1.6 (-2.0 to -0.8)	-1.5 (-2.0 to -0.7)[d]	-2.1 (-2.5 to -0.8)[1d]
Arm span (SDS)	-1.6 (-2.3 to -0.8)	-1.0 (-1.4 to -0.5)[2d]	-0.9 (-1.6 to -0.5)[1d]	-1.8 (-2.8 to -1.2)	-2.0 (-3.3 to -1.6)[1d]	-2.4 (-3.6 to -1.7)[1d]
Fat% (SDS)	2.1 (1.7 to 2.7)	1.5 (0.7 to 2.1)[3d]	1.9 (0.7 to 2.3)[3d]	2.3 (1.9 to 2.6)	2.3 (2.0 to 2.6)[d]	2.4 (2.1 to 2.7)[d]
Fat (SDS)	1.2 (0.8 to 2.0)	0.9 (0.2 to 1.4)[3d]	1.1 (0.6 to 2.0)[1b]	1.2 (0.7-1.6)	1.3 (0.7 to 1.9)[d]	4.5 (0.9 to 2.0)[b]
LBM_{age} (SDS)	-1.7 (-3.0 to -1.0)	-0.5 (-1.3 to 0.7)[3d]	-0.1 (-1.3 to 0.6)[d]	-1.9 (-3.4 to -1.2)	-2.1 (-4.1 to -1.3)[1d]	-2.5 (-3.8 to -1.4)[3d]
LBM_{height} (SDS)	-1.7 (-3.8 to -0.6)	-1.5 (-2.3 to -0.7)[a]	-1.9 (-2.4 to -1.4)[a]	-1.4 (-2.9 to 0.9)	-1.9 (-2.9 to 0.0)[1a]	-2.3 (-2.7 to -1.3)[3a]
Trunk fat (%)	36.0 (24.8 to 46.2)	28.0 (16.9 to 36.7)[4d]	33.3 (17.3 to 40.9)[3d]	36.0 (29.2 to 41.2)	37.2 (32.0 to 42.5)[d]	37.9 (35.0 to 45.7)[1d]

Data are expressed as median (iqr). Body composition only in children >4 years at start of study. [1] $p<0.05$, [2] $p<0.01$, [3] $p<0.005$, [4] $p<0.001$, compared to baseline levels; [a] $p<0.05$, [b] $p<0.01$, [c] $p<0.005$, [d] $p<0.001$, change GH group versus the control group.

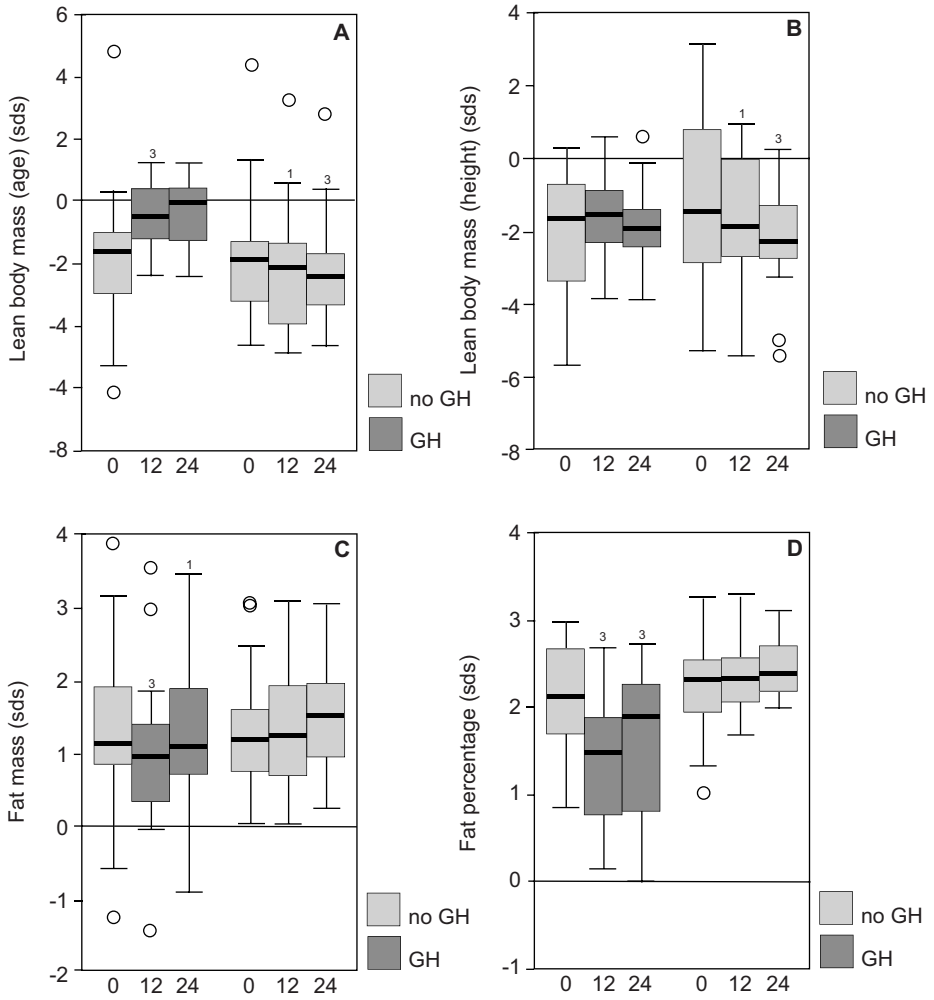

Figure 4 a-d. Body composition in pre-pubertal PWS children, during the 2 year controlled study. Data are expressed in SDS for age and sex or SDS for sex and height and represented by boxplots. Significantly different compared with baseline: [1]p<0.05, [2]p<0.01, [3]p<0.005, [4]p<0.001. Significantly different change in GH-treated group versus the control group: LBM_{age} SDS 12 months (p<0.001) and after 24 months (p<0.001); fat mass SDS 12 months (p<0.001) and after 24 months (p<0.01); body fat percentage 12 months (p<0.001) and after 24 months (p<0.001), and LBM_{height} SDS: 12 months (p<0.005) and after 24 months (p<0.05).

IGF-I and IGFBP-3 levels

IGF-I increased during GH treatment to a median above +2 SDS (Table 1). After 1 year of GH treatment, 8 of 12 infants (67%) had an IGF-I level >+2 SDS, and after 2 years, 5 of 7 (71%). In the control group, IGF-I only increased during GH in the second year, with 6 of 7 (86%) infants having an IGF-I level >+2 SDS. IGFBP-3 levels increased during GH treatment, but remained low during the first year in the control group. However, as IGFBP-3 levels remained within the normal range during GH, the IGF-I to IGFBP-3 ratio increased from -0.9 to 2.4 after 2 years (p=0.056) in the GH-treated group and from -0.3 to -1.1 after 1 year without GH to 2.5 after 1 year of GH (p=0.056) in the control group.

Prepubertal children

Clinical characteristics

Of the 49 prepubertal PWS children, two were excluded from the present analysis. In one patient, GH dose was reduced because IGF-I levels increased to +5 SDS. A second patient had spinal surgery for scoliosis, CPAP for sleep-related breathing disorders, and Risperdal after he had two psychotic episodes, all prior to start of GH. As a result, 47 children were eligible for baseline analysis. After 1 year, 42 were re-evaluated (21 in the GH-treated group and 21 in the control group), and after 2 years 40 of them were re-evaluated (20 in the GH-treated group and 20 in the control group) (Figure 1). Children with repeated measurements had similar SD-scores of BMI and height compared to those who did not yet finish 2 years of study. Three children (one in the GH-treated group and two in the control group) received T4 replacement therapy prior to start of GH to correct low free T4 levels.

Twelve children had a paternal deletion, 14 had a uniparental maternal disomy, and 5 had an imprinting centre mutation. In 16 patients, PWS was confirmed by a positive methylation test, but the underlying genetic defect was unknown.

Height, BMI, and body proportions

At start of study, BMI was significantly higher than 0 SDS. Ten out of 47 children (21%) were considered overweight, defined as BMI > +2 SDS. All other median SD-scores were significantly below zero (Table 2). Head circumference SDS, however, appeared to be relatively high compared to other anthropometric parameters. At start, height SDS and BMI SDS were highly correlated (r=0.44, p=0.002), but neither height SDS nor BMI SDS were related to IGF-I SDS. Baseline anthropometric parameters were not significantly different between the GH-treated and control group.

During GH treatment, median height SDS increased significantly compared to baseline after 1 and 2 years (Table 2). In the control group, who did not receive GH for 2 years, height SDS remained low (Figure 3a). BMI SDS decreased significantly during the first year of GH treatment and then stabilized, at a level that was not significantly higher than 0 SDS (p=0.08

and p=0.12 after 1 and 2 years, respectively), whereas in the control group BMI remained significantly higher than 0 SDS (Figure 3b). Head circumference increased significantly to completely normal values during GH treatment (Figure 3c), whereas tibia length, foot length, arm span and sitting height significantly improved, but remained significantly lower than 0 SDS (Table 3).

Body composition

At start of study, LBM corrected for age and sex (LBM_{age}), and for height and sex (LBM_{height}) was significantly below 0 SDS and body fat percentage was significantly higher than 0 SDS in the total group. During GH treatment, median LBM_{age} SDS increased significantly from -1.7 to -0.5 after 1 year, and to -0.1 (p=0.10 cmopared to baseline) after 2 years, resulting in a LBM_{age} not significantly below 0 SDS after 1 and 2 years of GH treatment (Table 3, Figure 4a). In the control group, LBM_{age} SDS significantly declined over time from -1.9 to -2.5 after 2 years and body fat percentage remained high. However, LBM corrected for height and sex, LBM_{height} SDS, did not significantly increase in the GH-treated group from -1.7 to -1.5 to -1.9 after 2 years (Figure 4b). Notably, in the control group there was a progressive and significant decrease in LBM_{height} SDS from -1.4 to -1.9 to -1.3, resulting in a significantly different change in LBM_{height} between the GH-treated group and the control group after 1 and 2 years. Median body fat percentage SDS decreased significantly from 2·1 to 1·5 to 1·9 but body fat percentage was still significantly higher than 0 SDS, after 1 and 2 years of GH (Figure 4d). Trunk fat (%) decreased significantly in the first year of GH, and increased in the second year to a level still significantly below baseline. In contrast, in the control group, trunk fat increased gradually, resulting in significantly higher levels after 2 years.

IGF-I and IGFBP-3

IGF-I SDS was significantly lower than 0 at start of study (Table 1). After 1 year of GH treatment, IGF-I SDS had significantly increased, and remained high. After 2 years, 17 of 19 children (89%) had IGF-I SDS above +2. IGF-I SDS remained low in the control group, with levels below 0 SDS during 2 years. During GH treatment, IGFBP-3 also increased, but not to the same SDS as IGF-I.

Safety aspects

GH was well tolerated. Compared to randomized controls, children with GH treatment did not show disadvantageous effects of GH on carbohydrate metabolism, sleep-related breathing disorders and thyroid hormone levels. The results of these safety aspects have been published separately.[19-21]

Discussion

Our study demonstrates that height and head circumference significantly increased in GH-treated infants and children, resulting in a complete normalization after 2 years of GH in prepubertal PWS children. Also length of foot and tibia, arm span, sitting height and body fat percentage improved in GH-treated children. LBM, corrected for sex and age, significantly improved. LBM corrected for height and sex (LBM_{height}) did not increase during GH treatment, but significantly declined in non-GH-treated controls. Thus, GH appeared to prevent reduction in LBM as seen in the controls. During GH-treatment, IGF-I levels increased to levels above normal ranges in many patients. Also, IGFBP-3 increased, but to a lesser extent, resulting in an increased IGF-I to IGFBP-3 ratio.

Median height SDS and IGF-I SDS, prior to start of GH treatment, were relatively low compared to previously reported levels.[7,11,22] This may be explained by the fact that our PWS children were not severely overweight in contrast to previously reported studies. In non-syndromal obese children, stimulated GH values are low as well as the 24-h GH secretion, but IGF-I levels are high or normal.[23,24] It might be that in more severely obese PWS children, the low IGF-I levels are counteracted by the obesity-related IGF-I increase.

Head circumference SDS was less depressed than median SD-scores of height, foot length, arm span and sitting height. This was in contrast to our expectations, because usually, in syndromal short stature, head circumference and other anthropometric parameters are equally or more impaired. Our results are in line with previous studies in young PWS children.[12,25]

Two years of GH-treatment resulted in a normal median height SDS, whereas height SDS in non-GH-treated children remained low. This is in line with previous studies[11,26,27] although so far, no 2-year controlled studies were available.

The most important effect of GH, in our opinion, is the prevention of loss of LBM as was found in the non-GH-treated children. Low LBM in PWS most likely reflects a reduced muscle mass, and may therefore contribute to clinical hypotonia, poor physical performance, and as a result, reduced energy expenditure.[4] LBM corrected for height and sex (LBM_{height}), did not increase during GH-treatment, LBM_{height} did significantly decrease in the control group, which suggests that GH prevents reduction in LBM. The decrease in LBM in non-GH-treated PWS children is in line with previous studies.[28] In previous GH studies, LBM was expressed in kilograms, not taking into account height-dependency of LBM. Only one study expressed LBM in SDS (for both height and age) in GH-treated PWS subjects compared to baseline.[29] Our study is the first one showing that, although LBM_{height} did not increase in the GH-treated group, loss of LBM_{height} was prevented by GH.

Trunk fat percentage decreased during GH-treatment and increased in the control group, indicating that GH decreases trunk fat percentage. However, trunkal fat percentage includes

both subcutaneous trunk fat and visceral fat. It remains to be established whether GH-treatment is beneficial for decreasing visceral fat in PWS children.

It has previous been suggested that a normalization of small hands and feet during GH-treatment, would suggest that those features are the result of GH deficiency rather than directly caused by the genetic defect.[26] Our results, however, show that the size of the feet, arm span and sitting height improve, but do not normalize during GH-treatment, indicating that GH deficiency cannot fully explain these features. We did not measure hand length, because in our experience, a reliable assessment of hand length is difficult in young PWS subjects.

During GH, median IGF-I levels rapidly increased to levels above the upper limit of normal, whereas IGFBP-3 increased more gradually to levels completely within the normal range. IGFBP-3 is the major carrier protein of IGF-I and binds 70-95% of IGF-I as a binary complex or as a ternary complex together with the acid labile subunit (ALS).[30] Only a minor fraction of IGF-I circulates in its free form, which is considered the biologically active form.[31] An increase of the IGF-I to IGFBP-3 ratio therefore suggests a large increase in free IGF-I. IGF-I levels in the supraphysiological range are in line with those reported by others in prepubertal PWS children and infants/ toddlers.[7,22,32] In one of our patients, the GH dose had to be decreased in order to keep IGF-I levels within reasonable limits. It is not known why PWS patients seem more responsive in terms of IGF-I than GH deficient children.[33] In our study, samples were collected and stored, as all assessments were performed afterwards. We therefore did not adjust GH dose in most children. Based on our data, we do now recommend to monitor IGF-I levels regularly, to prevent extremely high levels of IGF-I. One might consider treating PWS subjects with a lower GH dose. However, it has previously been shown that in order to optimally benefit the metabolic effects of GH, PWS children should be treated with at least 1 mg/m^2·day.[34] Future studies should evaluate the optimal GH dose in PWS children.

In conclusion, GH resulted in a significant improvement of height, BMI and head circumference, in PWS children. Foot length, arm span, tibia length, sitting height and body fat percentage also improved significantly, but did not completely normalize. GH appeared to prevent reduction in LBM, as was found in the controls. IGF-I levels increased to supraphysiological levels in most PWS children, indicating that PWS children are highly sensitive to GH and that monitoring of IGF-I is warranted to keep IGF-I levels within reasonable limits.

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Chapter 7

Randomized controlled trial investigating bone mineral density and effects of growth hormone treatment in prepubertal children with Prader-Willi syndrome

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Abstract

Background: Bone mineral density (BMD) is unknown in children with PWS, but is decreased in adults with PWS. In patients with growth hormone (GH) deficiency, BMD increases during GH treatment.

Objectives: To evaluate BMD in children with PWS and to study the effects of GH treatment.

Design: We conducted a randomized controlled GH trial. Forty-six prepubertal children were randomized into either a GH-treated group (1.0 mg/m^2·day) or a control group for two years. At start, 6, 12, and 24 months of study, total body and lumbar spine BMD (BMD_{TB} and BMD_{LS}) were measured by Dual-Energy X-ray Absorptiometry (DXA) and bone mineral apparent density ($BMAD_{LS}$) was calculated.

Results: Baseline BMD_{TB}SDS and BMD_{LS}SDS were normal [mean ± SD: -0.2 ± 1.1 and -0.4 ± 1.2 SDS, respectively]. $BMAD_{LS}$SDS, which corrects for short stature, was also normal [mean ± SD: 0.40 ± 1.1 SDS]. BMD_{TB}SDS decreased during the first six months of GH treatment ($p<0.0001$), but increased during the second year of GH treatment. After 24 months of study, BMD_{TB}SDS, BMD_{LS}SDS, and $BMAD_{LS}$SDS did not significantly differ between GH-treated children and randomized controls ($p=0.30$, $p=0.44$, and $p=0.47$, respectively). Results were similar when corrected for BMISDS. Repeated measurements analysis showed a significant positive association between IGF-I SDS and BMD_{TB}SDS and BMD_{LS}SDS, but not with $BMAD_{LS}$.

Conclusions: Our results show that prepubertal children with PWS have a normal BMD. GH treatment had no effect on BMD, except for a temporary decrease of BMD_{TB}SDS in the first 6 months.

Introduction

Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia, hypogonadism, scoliosis, psychomotor delay, and temper tantrums.[1-7] PWS results from the lack of expression of the paternally derived chromosome 15q11-q13.[3,6] Hypothalamic dysfunction may be responsible for many features of PWS.[8,9] Growth hormone (GH) treatment is beneficial for children with PWS, as it improves height, body composition (increase in lean body mass, decrease in fat percentage) and psychomotor development.[10-15]

Bone mineral density (BMD) is the result of the equilibrium between bone formation and bone resorption. Adequate mineral acquisition during childhood is important for reaching peak BMD in late adolescence. A decreased peak BMD is a major determinant for osteoporosis and fracture risk later in life. Adequate gonadal hormone levels during pubertal development are vital for attainment of peak BMD.[16,17] In cross-sectional studies of adults with PWS, BMD was decreased in 60% to 90% of subjects.[18-20] In addition, osteoporosis is considered a supportive finding in the diagnostic criteria of adult PWS.[2] The decreased BMD may account for the higher risk of fractures during life and complications during spinal surgery.[3,18,20-22] Although childhood is the critical period for bone accumulation, reports on BMD in children with PWS are very limited.[12,23,24] Due to short stature in children with PWS, true BMD is underestimated by the standard areal measurement and should be corrected for bone size by calculating lumbar spine bone mineral apparent density ($BMAD_{LS}$).[25-29] As evaluation of BMD was not included in the aims of previous studies in children with PWS,[12,23,24] a correction for short stature was never applied and $BMAD_{LS}$ was never reported.

Patients with PWS have many symptoms resembling GH deficiency, including short stature, small hands and feet, an increased fat percentage, a decreased lean body mass, and low IGF-I levels.[6,7,30,31] In patients with GH deficiency, BMD is decreased but normalizes during GH treatment.[27,28] However, GH and IGF-I secretion are usually more affected in GH-deficient children than in those with PWS.[7] The effects of GH treatment on BMD in children with PWS are unknown.

The aims of our study were to evaluate BMD and $BMAD_{LS}$ in prepubertal children with PWS and to investigate the effects of GH treatment. We hypothesized that the low BMD in adult patients with PWS is mainly the result of hypogonadotropic hypogonadism and only partly due to GH insufficiency. We therefore expected BMD and BMAD to be within the low-normal range in our group of prepubertal children and expected a small effect of GH treatment. To investigate our hypotheses, we performed the present study within our randomized controlled GH trial in prepubertal children with PWS.

Methods

Patients

The study population comprised 46 prepubertal children with a genetically confirmed diagnosis of PWS by positive methylation test. At time of inclusion, girls were 4 to 12 years old with Tanner breast stage <2 and boys were between 4 and 14 years with Tanner genital stage <2 and a testicular volume <4 ml.[32] None of the children had onset of puberty during 24 months of study. Caloric intake and activity level of all participants were standardized three months before inclusion. All children were naïve to GH treatment at start of study. The study protocol was approved by the Medical Ethics Committees of the Erasmus University Medical Center / Sophia Children's Hospital in Rotterdam, The Netherlands, and of collaborating centers. Written informed consent was obtained from parents and from children over 12 years of age. Assent was obtained from children under 12 years of age.

Design

The primary objective of our study was to evaluate total body and lumbar spine BMD standard deviation scores (BMD_{TB} SDS and BMD_{LS} SDS) and lumbar spine bone mineral apparent density SDS ($BMAD_{LS}$ SDS) in prepubertal children with PWS. The secondary objectives were to investigate the effects of GH treatment on BMD and to study the relationship between scoliosis and BMD. After stratification for age and BMI, all children were randomly assigned either to a GH-treated group (1.0 mg/m^2·day) or a control group for two years. Biosynthetic GH (Genotropin, Pfizer, New York) was administered subcutaneously once daily at bedtime. The first four weeks of GH treatment, children received 0.5 mg/m^2·day to prevent fluid retention. Three-monthly, children were seen by the PWS research team of the Dutch Growth Research Foundation in collaboration with pediatric endocrinologists and pediatricians. At each visit, the GH dose was corrected to the calculated body surface area.

All measurements described in this study were performed in the Erasmus University Medical Center Rotterdam / Sophia Children's Hospital, at start and at 6, 12, and 24 months of study.

Dual-Energy X-ray Absorptiometry

In all children, bone mineral content (BMC, grams), bone mineral density of the total body and the lumbar spine (BMD_{TB} and BMD_{LS}, grams/cm^2), fat mass, and lean body mass (LBM), were measured by Dual-Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare, Chalfont St. Giles, UK). Quality assurance was performed daily. The coefficient of variation (CV) was 0.64% for BMC and BMD_{TB} and 1.04% for BMD_{LS}. The CV for lean tissue and fat tissue was 1.57% to 4.49% and 0.41% to 0.88%, respectively. In children

with short stature, true BMD is underestimated by the areal presentation and should be corrected for bone size by calculating the bone mineral apparent density ($BMAD_{LS}$).[26] $BMAD_{LS}$ was calculated using the model: $BMAD_{LS} = BMD_{LS} \cdot [4 / (\pi \cdot width)]$, with the width as the mean width of the second to fourth lumbar vertebral body. This model has been extensively validated by in vivo volumetric data obtained from magnetic resonance imaging of the lumbar vertebrae.[26] BMD standard deviation scores were calculated according to age- and sex-matched reference values from the Dutch population.[33,34]
Fat mass was expressed as percentage of total body mass. Fat percentage SDS and LBM SDS were calculated according to sex- and height-matched reference values of the Dutch population.[33,34]

Anthropometrics

Standing height was measured with a Harpenden Stadiometer (Holtain Ltd., Crosswell, UK). Weight was assessed on an accurate scale (Servo Balance KA-20-150S). HeightSDS and BMISDS were calculated with Growth Analyser 3.0 (available at www.growthanalyser.org), according to age- and sex-matched reference values from the Dutch population.[35]

Scoliosis

At start and subsequently each year, standardized posteroanterior X-rays were taken in standing position. Scoliosis is defined as a spinal curve with a Cobb angle of more than 10° on a posteroanterior radiograph. The Cobb angle is the angle between the two steepest vertebrae, i.e. the upper border of the upper vertebra in the curve and the lower border of the lower vertebra. As previously reported,[36,37] Cobb angles were measured independently by two observers with minimal intra- and inter-observer variance (intraclass correlation coefficient=0.998 and 0.97, respectively).

Assay

Serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, California). The intra-assay coefficient of variation (CV) was 4% and the inter-assay CV was 6%. IGF-I SDS was calculated according to age- and sex-matched reference values of the Dutch population.[38]

Data analysis

Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) and with SAS 9.1 (SAS Institute Inc., Cary, North Carolina). All data had a normal distribution, based on a p-value above 0.05 with Levene's test for normal distribution. Therefore, all data were expressed as means with standard deviations (SD). To correct for multiple testing,

the changes over time and differences between GH-treated children and randomized controls were analyzed using repeated measures of variance.[39] Repeated measurements analysis was also performed to investigate the effects of different variables on BMD during study. Additional analyses were performed correcting for the effect of BMISDS on BMD parameters. Effects are presented as β with 95% confidence intervals (C.I.). SD-scores were compared with -2, 0, and +2 SDS using Student's one sample t-test. P-values less than 0.05 were considered statistically significant.

Results

Baseline

Clinical data

Forty-six prepubertal children with PWS (21 boys, 25 girls) were included in our randomized controlled GH trial (Table 1). At start of study, the mean (SD) age was 7.1 years (2.2). The genotype was specified in 40 children: 18 (45%) had a deletion of chromosome 15q11-q13, 17 (43%) a maternal uniparental disomy, and 5 (13%) an imprinting center defect. Positive methylation test was demonstrated in the remaining 6 patients, but the underlying genetic defect was not identified. Children had short stature, with a baseline height significantly below -1 SDS ($p<0.0001$). Baseline BMI was high, but still significantly lower than 2 SDS ($p<0.0001$). Body composition showed an increased fat mass and decreased lean body mass. Twenty-two children had a baseline IGF-I SDS below -2 SDS.

Bone mineral density

Bones in the total body consist for 80% of cortical bone, whereas the lumbar spine mainly consists of trabecular bone. Therefore, BMD was divided into total body and lumbar spine BMD (BMD_{TB} and BMD_{LS}). At baseline, the mean ± SD BMD_{TB} was -0.23 SDS ± 1.1, which did not significantly differ from 0 SDS (Table 2, Figure 1). The mean ± SD baseline BMD_{LS} was -0.44 SDS ± 1.2. Baseline BMD_{LS} SDS was significantly lower than 0 SDS ($p=0.01$), but still significantly higher than -2 SDS ($p<0.0001$). BMD_{LS} SDS correlated significantly with age ($r=0.38$, $p=0.01$, Figure 1). In children with PWS, BMD should be corrected for height by calculating the lumbar spine bone mineral apparent density ($BMAD_{LS}$). The mean ± SD baseline $BMAD_{LS}$ was 0.40 SDS ± 1.1, which was even significantly higher than 0 SDS ($p=0.02$), but still significantly lower than 2 SDS ($p<0.0001$).

Table 1. Data at baseline and during GH treatment in prepubertal children with PWS.

		Baseline	6 months	12 months	24 months	6 months vs. start[b]	12 months vs. start[b]	24 months vs. start[b]
N	GH-treated	27	27	27	23			
	Controls	19	19	19	18			
Age	GH-treated	7.37 ± 2.3	7.87 ± 2.3	8.37 ± 2.3	9.61 ± 2.4			
	Controls	6.81 ± 2.0	7.32 ± 2.1	7.82 ± 2.1	8.88 ± 2.1			
	P-value[a]	0.41	0.41	0.41	0.31			
HeightSDS	GH-treated	-1.95 ± 1.5	-1.46 ± 1.5	-1.07 ± 1.3	-0.46 ± 1.1	**<0.0001**	**<0.0001**	**<0.0001**
	Controls	-2.35 ± 1.1	-2.38 ± 1.1	-2.38 ± 1.2	-2.51 ± 1.2	0.61	0.74	0.36
	P-value[a]	0.32	**0.02**	**0.001**	**<0.0001**			
BMISDS	GH-treated	1.28 ± 1.2	0.82 ± 1.3	0.74 ± 1.2	0.85 ± 1.3	**<0.0001**	**<0.0001**	**0.001**
	Controls	1.37 ± 0.7	1.39 ± 0.7	1.47 ± 0.6	1.41 ± 0.6	0.84	0.44	0.82
	P-value[a]	0.74	0.07	**0.02**	0.08			
LBM SDS	GH-treated	-2.44 ± 1.8	-1.79 ± 1.3	-1.90 ± 1.1	-2.30 ± 1.3	**<0.0001**	**0.004**	0.37
	Controls	-1.70 ± 2.2	-2.02 ± 2.1	-2.19 ± 1.7	-2.74 ± 1.6	**0.045**	**0.03**	**0.0002**
	P-value[a]	0.22	0.64	0.56	0.33			
Fat% SDS	GH-treated	2.80 ± 0.4	2.09 ± 0.7	1.93 ± 0.7	2.05 ± 0.6	**<0.0001**	**<0.0001**	**<0.0001**
	Controls	2.81 ± 0.6	2.81 ± 0.5	2.89 ± 0.5	2.89 ± 0.4	0.90	0.56	0.63
	P-value[a]	0.98	**<0.0001**	**<0.0001**	**<0.0001**			
IGF-I SDS	GH-treated	-1.89 ± 1.1	1.81 ± 0.9	2.49 ± 1.3	2.26 ± 1.0	**<0.0001**	**<0.0001**	**<0.0001**
	Controls	-1.87 ± 1.0	-1.51 ± 1.3	-1.63 ± 2.0	-1.58 ± 1.4	0.38	0.30	0.41
	P-value[a]	0.97	**<0.0001**	**<0.0001**	**<0.0001**			

Data presented as mean (SD). [a] P-values for differences between GH-treated children and randomized controls, [b] p-values for results compared to baseline (both with repeated measurements analysis). BMI, body mass index; LBM, lean body mass; Fat%, fat mass expressed as percentage of total body mass; IGF-I, insulin-like growth factor type I.

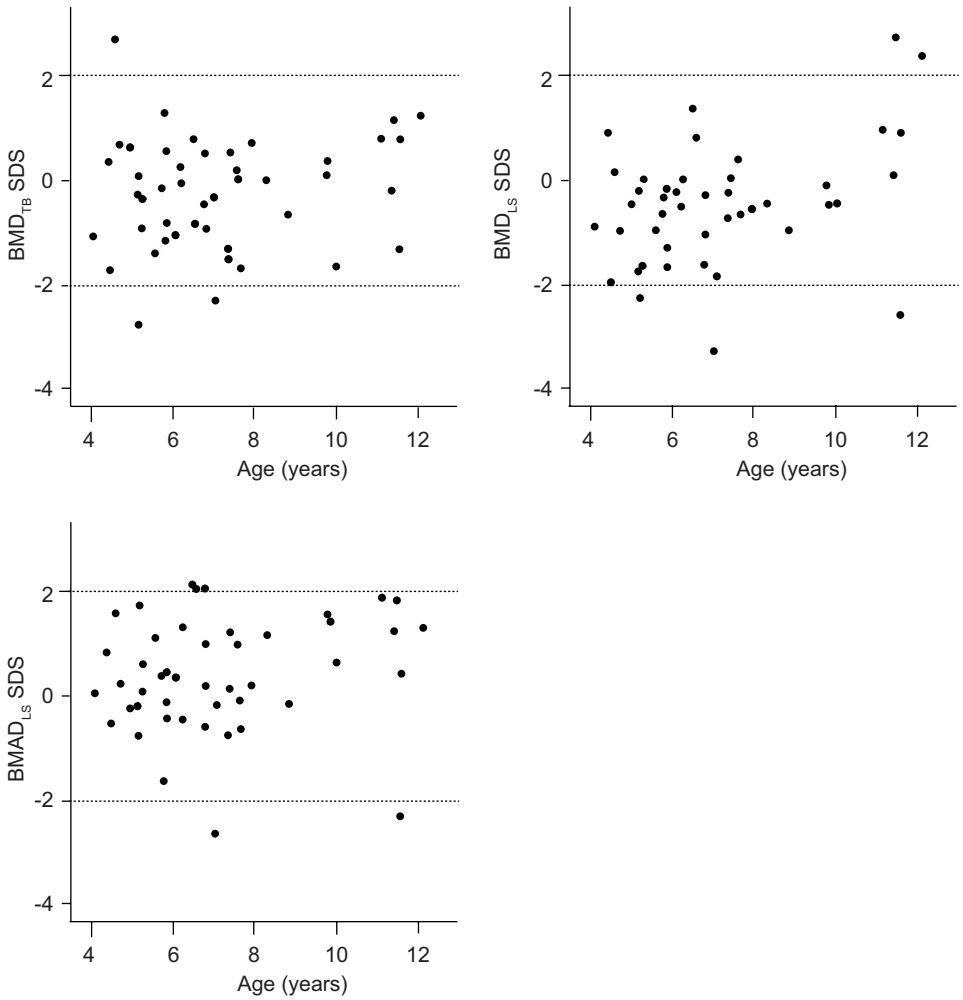

Figure 1. Scatterplots depicting baseline results on the y-axes and age on the x-axes. All circles represent values at baseline. BMD_{TB} SDS, total body bone mineral density SDS; BMD_{LS} SDS, lumbar spine bone mineral density SDS; and $BMAD_{LS}$ SDS, lumbar spine bone mineral apparent density (volumetric BMD_{LS}) SDS.

BMISDS was associated with BMD_{TB} SDS [β (95% C.I.)=0.35 (0.06-0.65), p=0.02], BMD_{LS} SDS [β (95% C.I.)=0.73 (0.46-0.99), p<0.0001], and $BMAD_{LS}$ SDS [β (95% C.I.)=0.33 (0.02-0.64), p=0.04]. The normal BMD in prepubertal children could be related to the high-normal BMISDS. We therefore calculated the BMD parameters after correction for BMISDS. Corrected for BMISDS, the BMD_{TB} SDS and BMD_{LS} SDS were within normal

Table 2. Bone mineral density in 46 prepubertal children with PWS.

		Start	6 months	12 months	24 months	6 months vs. start[b]	12 months vs. start[b]	24 months vs. start[b]	24 months vs. start[c]
BMD_{TB} SDS	GH-treated	-0.37 ± 1.1	-0.71 ± 1.1	-0.73 ± 1.2	-0.49 ± 1.1	**<0.0001**	**0.001**	0.23	0.70
	Controls	-0.04 ± 1.0	-0.07 ± 1.1	-0.05 ± 0.9	-0.15 ± 0.9	0.72	0.96	0.51	0.47
	P-value[a]	0.31	**0.04**	**0.03**	0.23				
$Area_{TB}$ (dm^2)	GH-treated	8.92 ± 3.5	9.92 ± 3.8	11.15 ± 4.1	12.86 ± 3.8	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Controls	8.33 ± 2.4	8.72 ± 2.4	9.09 ± 2.4	10.10 ± 2.6	**0.007**	**0.0003**	**<0.0001**	**<0.0001**
	P-value[a]	0.35	0.13	0.05	**0.009**				
BMD_{LS} SDS	GH-treated	-0.41 ± 1.3	-0.52 ± 1.3	-0.19 ± 1.1	-0.08 ± 1.0	0.28	0.11	0.07	**0.009**
	Controls	-0.49 ± 0.9	-0.26 ± 0.9	-0.27 ± 1.1	-0.34 ± 1.2	0.05	0.18	0.51	0.55
	P-value[a]	0.83	0.43	0.82	0.46				
$Area_{LS}$ (dm^2)	GH-treated	11.07 ± 3.0	12.10 ± 3.4	12.93 ± 3.7	14.91 ± 4.0	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Controls	10.67 ± 2.9	10.87 ± 3.1	11.29 ± 3.1	12.29 ± 3.2	0.31	**0.02**	**<0.0001**	**<0.0001**
	P-value[a]	0.49	0.14	0.11	**0.01**				
$BMAD_{LS}$ SDS	GH-treated	0.39 ± 1.2	0.23 ± 1.4	0.35 ± 1.1	0.37 ± 1.2	0.27	0.82	0.85	0.64
	Controls	0.40 ± 1.0	0.66 ± 1.2	0.48 ± 1.2	0.67 ± 1.4	0.14	0.73	0.38	0.39
	P-value[a]	0.97	0.24	0.70	0.57				
BMCSDS	GH-treated	-2.06 ± 1.6	-1.78 ± 1.5	-1.41 ± 1.4	-0.97 ± 1.3	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
	Controls	-2.03 ± 0.8	-1.97 ± 0.8	-1.92 ± 0.7	-1.83 ± 0.8	0.44	0.29	0.08	0.09
	P-value[a]	0.93	0.46	0.17	**0.03**				

Data presented as mean (SD). [a] P-values for differences between GH-treated children and randomized controls; [b] p-values for results compared to baseline, [c] corrected for BMISDS (all with repeated measurements analysis). BMD_{TB} SDS, total body bone mineral density SDS; BMD_{LS} SDS, lumbar spine bone mineral density SDS; and $BMAD_{LS}$ SDS, lumbar spine bone mineral apparent density (volumetric BMD_{LS}) SDS; BMCSDS, bone mineral content SDS.

range with a mean ± SD of -0.70 ± 1.0 and -1.40 ± 0.9 SDS, respectively. The $BMAD_{LS}$ was -0.04 ± 1.1 SDS when corrected for BMISDS and did not significantly differ from 0 SDS (p=0.88). This indicates that prepubertal children with PWS have a normal BMD, independently from BMISDS.

BMD_{TB} SDS, BMD_{LS} SDS, and $BMAD_{LS}$ SDS did not significantly differ between boys and girls, nor between children with different genotypes. Furthermore, BMD_{TB} SDS, BMD_{LS} SDS, and $BMAD_{LS}$ SDS did not significantly differ between children with an IGF-I level below -2 SDS and those with a level above -2 SDS and did not correlate with IGF-I levels and IGF- I SDS.

Twenty children (44%) had scoliosis at start of study, defined as a Cobb's angle of 10° or above. The mean ± SD baseline Cobb angle was 22.6 ± 12.5 degrees. BMD_{TB} SDS, BMD_{LS} SDS, and $BMAD_{LS}$ SDS did not significantly differ between children with scoliosis and those without.

Growth hormone treatment

Effects on height, BMI, body composition, and IGF-I levels

HeightSDS increased in GH-treated children and was significantly higher than in randomized controls at 6, 12 and 24 months of study (Table 1). In contrast to the control group, BMISDS decreased in GH-treated children during the first year and was still significantly lower than baseline after 24 months of study. LBMSDS significantly increased during the first year of GH treatment, but returned to baseline values thereafter. In contrast, the LBMSDS of the control group declined continuously during 24 months of study. Fat percentage SDS significantly decreased during GH treatment, but did not significantly change in the control group. Also, IGF-I SDS significantly increased in GH-treated children, but did not change in randomized controls.

Effects of GH treatment on BMD_{TB}

BMD_{TB} SDS fluctuated in GH-treated children, whereas it did not significantly change in randomized controls (Table 2 and Figure 2). In the GH-treated group, BMD_{TB} SDS significantly decreased during the first six months of treatment ($p<0.0001$), but did not significantly change during the second six months of treatment. BMD_{TB} SDS increased during the second year of GH treatment (p=0.04 compared to randomized controls). After 24 months of study, BMD_{TB} SDS did not significantly differ between GH-treated children and randomized controls.

The fluctuation in BMD_{TB} SDS may be the result of a disturbed equilibrium between bone expansion and mineral acquisition. Concurrently with the temporary decrease in BMD_{TB} SDS during the first six months, the total body bone area increased significantly more in GH-treated children than in randomized controls (p=0.001). Although the increase in total

body bone area during the second six months and the second year also was significantly greater in GH-treated children compared to randomized controls (p=0.02 and p=0.001, respectively), the BMD_{TB} SDS did not significantly differ between GH-treated children and randomized controls after 24 months of study. This indicates that the equilibrium between bone expansion and mineral acquisition had restored.

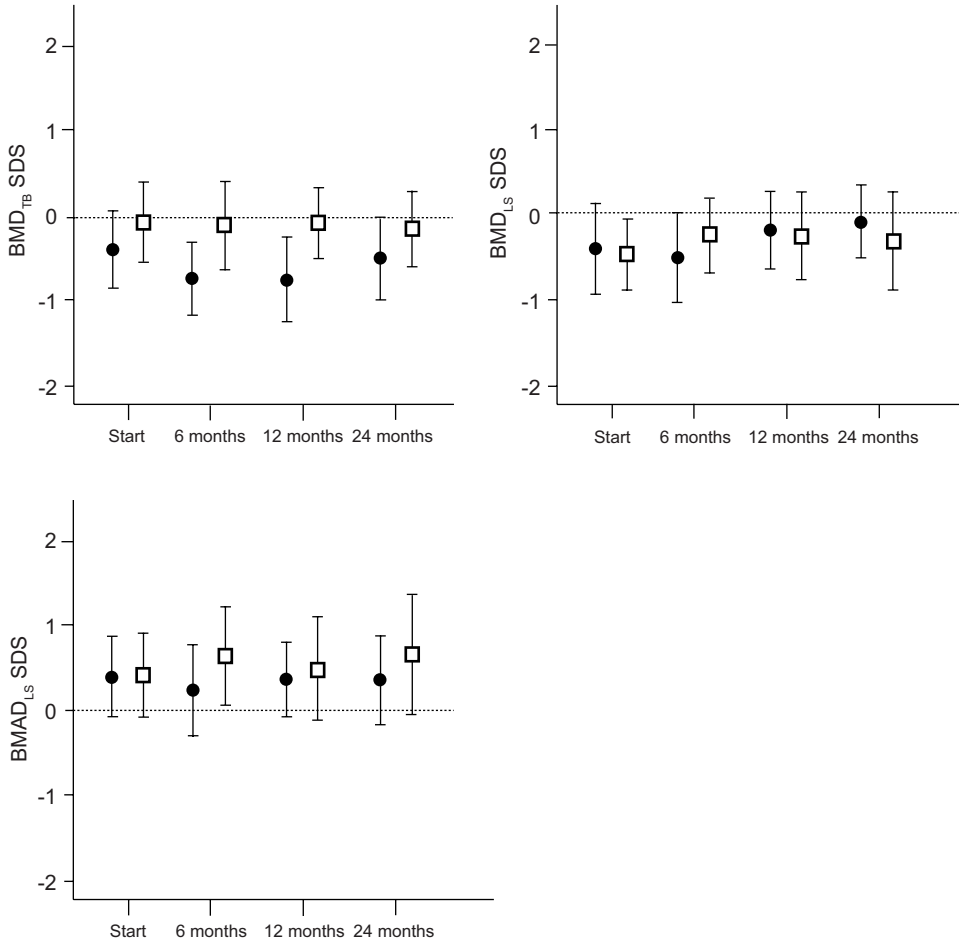

Figure 2. Results depicted as means with error bars representing 95% confidence intervals. BMD_{TB} SDS, total body bone mineral density SDS; BMD_{LS} SDS, lumbar spine bone mineral density SDS; and $BMAD_{LS}$ SDS, lumbar spine bone mineral apparent density (volumetric BMD_{LS}) SDS. Circles, GH-treated group; Squares, control group.

Results in Table 2 were similar when corrected for BMISDS.
Repeated measurements analysis showed a significant positive association between IGF-I SDS and BMD_{TB} SDS at 24 months of study [β (95% C.I.)=0.17 (0.06-0.29), p=0.004]. LBM and fat mass corrected for age and gender were both significantly associated with BMD_{TB} SDS [β=0.05 (0.01-0.09), p=0.01; and β=0.05 (0.02-0.07), p=0.0001, respectively].

Effects of GH treatment on BMD_{LS}

BMD_{LS} SDS did not significantly change during study, both in GH-treated children and in randomized controls (Table 2 and Figure 2). Thus, BMD_{LS} SDS did not significantly differ between GH-treated children and randomized controls after 24 months of study, nor did the change in BMD_{LS} SDS.
The lumbar spine mainly consists of trabecular bone, which has a higher rate of bone turnover than cortical bone.[25] The equilibrium between bone formation and bone expansion is therefore earlier restored in trabecular bone. We found no significant fluctuation in BMD_{LS} SDS during 24 months of study, while the increase in lumbar spine bone area was significantly greater in GH-treated children than in randomized controls.
BMISDS was significantly associated with BMD_{LS} SDS during 24 months of study [β (95% C.I.)=0.36 (0.19-0.53), p<0.0001]. After correction for BMISDS, the BMD_{LS} SDS at 24 months of study was significantly greater compared to baseline in GH-treated children, but not in randomized controls (p=0.009 and p=0.55, respectively). However, corrected for BMISDS, there was no significant difference in BMD_{LS} SDS at 24 months of study between GH-treated children and randomized controls (p=0.19).
IGF-I SDS was significantly associated with BMD_{LS} SDS at 24 months of study [β (95% C.I.)=0.19 (0.05-0.35), p=0.01]. LBM and fat mass corrected for age and gender were both significantly associated with BMD_{LS} SDS [β=0.08 (0.03-0.12), p=0.002; and β=0.05 (0.02-0.08), p=0.002, respectively].

Effects of GH treatment on $BMAD_{LS}$

$BMAD_{LS}$ is the BMD corrected for height. $BMAD_{LS}$ SDS did not significantly change during study, both in GH-treated children and in randomized controls (Table 2 and Figure 2). Therefore, $BMAD_{LS}$ SDS did not significantly differ between GH-treated children and randomized controls after 24 months of study. Results in Table 2 were similar when corrected for BMISDS.
IGF-I SDS was not associated with $BMAD_{LS}$ SDS during 24 months of study (p=0.62). Furthermore, LBM and fat mass corrected for age and gender were not significantly associated with $BMAD_{LS}$ SDS.

Discussion

Our study shows that BMD_{TB}, BMD_{LS}, and $BMAD_{LS}$ are normal in prepubertal children with PWS. $BMAD_{LS}$ SDS, which corrects for short stature, was even significantly above zero SDS, indicating that BMD is not decreased in prepubertal children with PWS.

The normal $BMAD_{LS}$ SDS is in contrast to results from cross-sectional studies in adults, showing osteoporosis in a high percentage of them.[18-20] These studies, however, did not correct for the short stature of persons with PWS. It is on the other hand likely that adults with PWS do have a decreased $BMAD_{LS}$ as the prevalence of osteoporosis in older subjects with PWS is up to 90% and associated complications are frequently reported.[18-20] Presumably, there is a change from a normal $BMAD_{LS}$ SDS in prepubertal children towards a low $BMAD_{LS}$ SDS in adults. We postulate that this decrease results from delayed and abnormal pubertal development due to hypogonadotropic hypogonadism in many patients with PWS, which will lead to a lack of increase in BMD and a lower peak bone mass. As hypogonadotropic hypogonadism more often affects men than women with PWS, it would be interesting to investigate whether the prevalence of a decreased BMD in adults with PWS varies with gender. Unfortunately, no data are available regarding gender-specific BMD in adults with PWS. In prepubertal children with PWS, our results show no effect of gender on BMD.

The mean BMISDS in our group of children was within normal range, but was higher than 0 SDS. Although BMISDS was significantly associated with BMD parameters, $BMAD_{LS}$ did not significantly differ from 0 SDS after correction for BMISDS. This indicates that prepubertal children with PWS have a normal BMD, independently from BMISDS. We did not measure DHEAS levels in our patients. Other studies have demonstrated high levels of DHEAS in children with PWS,[40,41] which may contribute to the normal BMD demonstrated in our patients.[42]

We evaluated the effects of GH treatment on BMD. BMD_{TB} SDS significantly decreased during the first six months of treatment, whereas the total body bone area increased significantly more in GH-treated children than in randomized controls. This finding suggests that the rate of bone expansion is higher than the rate of mineral acquisition. This has also been suggested in studies of other patient populations.[27,28] In contrast to bones in the total body which consist for 80% of cortical bone, the lumbar spine mainly consists of trabecular bone which has a higher rate of bone turnover.[25] Therefore, the equilibrium between bone expansion and mineral acquisition is earlier restored, which most likely explains why no significant decrease in BMD_{LS} SDS was found during the initial phase of GH treatment despite a significantly higher increase of lumbar spine bone area in GH-treated children. The decrease in BMD_{TB} SDS in our study was temporary and we found no significant difference in BMD_{TB} SDS between GH-treated children and randomized controls after 24 months of study. The fluctuation in BMD_{TB} SDS during GH treatment was not described by

Carrel et al. and Myers et al.,[12,23] who studied a group of infants and toddlers with PWS. Their data suggested a normal BMD_{TB} and no effect of GH treatment on BMD_{TB} in young children with PWS. However, as measurements were not performed at six months of study, a fluctuation in BMD_{TB} could not be detected. Additionally, our results suggest that long-term GH treatment might increase BMD_{TB} SDS as well as BMD_{LS}, suggested by the significant positive association with IGF-I SDS at 24 months of study. We have evaluated $BMAD_{LS}$ as the best presentation of true BMD in children with PWS.[29] Our study showed that $BMAD_{LS}$ SDS did not significantly change during GH treatment and was not significantly associated with IGF-I SDS. Children with GH deficiency have a low BMD which normalizes during GH treatment. In contrast, baseline BMD is not decreased in prepubertal children with PWS. Therefore, it is not unexpected to find no effect of GH treatment on BMD in this patient population. As $BMAD_{LS}$ SDS did not significantly change during the study, we conclude that GH treatment has no significant effect on BMD in prepubertal children with PWS during 24 months of study. Future research evaluating the effect of GH treatment on BMD in children with PWS and hypogonadotropic hypogonadism is warranted.

In line with other reports,[43,44] LBM and fat mass corrected for age and gender were significantly associated with BMD_{TB} SDS and BMD_{LS} SDS. In contrast to reports on adolescents with idiopathic scoliosis,[45] we found no significant difference in BMD between children with scoliosis and those without. Thus, our study shows that the high prevalence of scoliosis in children with PWS[36,37,46] is not caused by a decreased BMD.

In conclusion, our study shows that prepubertal children with PWS have a normal BMD_{TB} SDS, BMD_{LS} SDS, and BMD corrected for short stature ($BMAD_{LS}$ SDS). BMD_{TB} SDS temporarily decreased during the first six months of GH treatment. After 24 months of study, BMD_{TB} SDS, BMD_{LS} SDS, and $BMAD_{LS}$ SDS did not significantly differ between GH-treated children and randomized controls.

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Chapter 8

Cardiovascular and metabolic risk profile and acylation stimulating protein levels in children with Prader-Willi syndrome and effects of growth hormone treatment

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Abstract

Context: Reports on the cardiovascular and metabolic risk profile in children with PWS and the effects of GH treatment are scarce. Acylation stimulating protein (ASP) stimulates glucose uptake and triglyceride storage in adipose tissue.

Objectives: To study the metabolic and cardiovascular risk profile and ASP levels and to investigate the effects of GH treatment.

Design: Randomized controlled trial: infants and prepubertal children received GH (1 mg/m^2·day) or were followed as controls for 12 and 24 months, respectively.

Patients: Eighty-five children with PWS; mean ± SD age of 4.9 ± 3.0 years.

Main outcome measures: Fat% with DXA, blood pressure, fasting insulin and glucose levels, serum lipids, and ASP levels.

Results: Mean ± SD fat% was 28.4 ± 6.2 in infants and 36.9 ± 8.5 in prepubertal children. Fat%SDS was elevated in 95% of prepubertal children. In addition to high fat%, 63% of infants and 73% of prepubertal children demonstrated at least one cardiovascular risk factor. The metabolic syndrome was demonstrated in 5% of all children; at least one component was present in 40%. Mean ± SD baseline ASP was 107 ± 45 nmol/l (normal<58 nmol/l) and correlated with fat mass and TG levels. GH improved fat%SDS and the HDLc/LDLc ratio ($p<0.0001$ and $p=0.04$). GH had no effect on ASP levels.

Conclusions: Many children with PWS have dyslipidemia and high ASP levels. GH improves fat% and HDLc/LDLc, but not ASP. High ASP levels may prevent complete normalization of fat%SDS during GH treatment, but may contribute in keeping glucose and insulin levels within normal range.

Introduction

Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia, hypogonadism, scoliosis, psychomotor delay, and temper tantrums.[1-8] PWS results from the lack of expression of the paternally derived chromosome 15q11-q13 caused either by a deletion, uniparental disomy, imprinting center defect, or a balanced translocation.[3,6] Hypothalamic dysfunction may be responsible for many features of PWS.[9-11]

The prevalence of cardiovascular disease is increased in adult patients with PWS, resulting in increased mortality.[12-15] Microcirculatory dysfunction may be present at an early age.[16] Children with PWS have an unfavorable body composition, with a high fat percentage (fat%), even in underweight infants.[6,17-21] A high fat% is considered a risk factor for cardiovascular disease and diabetes mellitus type 2. However, there is limited knowledge about the presence of other cardiovascular and metabolic risk factors in young children with PWS. Dyslipidemia may be present, also before puberty.[22,23] In contrast, insulin resistance is less than expected based on the degree of obesity, with higher adiponectin levels compared to obese controls but still lower than lean controls.[24-26] Growth hormone (GH) treatment has a beneficial effect on body composition by decreasing fat%, but reports about the effects on serum lipids are very scarce.[22,23]

Adipose tissue is a metabolically active organ producing numerous proteins, enzymes, and hormones. Acylation stimulating protein (ASP) is a hormone generated through activation of the alternative complement pathway from the precursor protein C3 and is produced by adipocytes.[27] ASP stimulates free fatty acid incorporation into adipose tissue by increasing triglyceride synthesis and storage and increases glucose uptake through enhanced translocation of glucose transporters to the plasma membrane surface.[28-32] These effects are both additive and independent of those of insulin.[30-32] Furthermore, ASP reduces triglyceride lipolysis in adipocytes by inhibiting hormone-sensitive lipase.[33] By controlling the storage of triglycerides, ASP is an important factor in keeping lipid levels within normal range.

In obesity, insulin resistance, dyslipidemia, and cardiovascular disease, ASP and serum triglyceride levels are both increased, suggestive of ASP resistance.[34-38] In vitro, this is demonstrated by reduced specific binding and response to ASP of cells from subjects with high ASP levels.[39,40] Thus, ASP is an important marker for abnormal lipid metabolism and accumulation of fat tissue, whereas high ASP levels are associated with increased risk factors for cardiovascular disease.[27,36,38]

There is a paucity of data regarding the cardiovascular and metabolic risk factors in young children with PWS and the effect of GH treatment on these risk factors. In view of the abnormal body composition in young children with PWS, we hypothesized that these children may have an unfavorable cardiovascular and metabolic risk profile, even at an early age. Based on the lipolytic effect of GH, we hypothesized that GH might have favorable

effects on these risk factors. The increased fat mass in patients with PWS[6,17-21] may result in high ASP levels. Given its critical role in triglyceride storage into adipose tissue, high ASP levels may in turn contribute to the sustainment of an increased fat mass. The high fat mass in children with PWS does not completely normalize during GH treatment.[17-21] We hypothesized that in children with PWS high ASP levels may be involved in preventing complete normalization of fat mass during GH treatment. We therefore expected no effect of GH on ASP levels. We assessed the cardiovascular and metabolic risk profiles and ASP levels in prepubertal children with PWS and investigated the effects of GH treatment on these factors in a randomized controlled trial.

Methods

Patients

The study population comprised of 85 children with a genetically confirmed diagnosis of PWS by a positive methylation test. The study group was divided in an infant and a prepubertal group. The infant group consisted of 35 children (21 boys and 14 girls) aged 6 months to 3 years. The prepubertal group consisted of 50 children: 26 girls aged 3.5 to 12 years with Tanner breast stage <2 and 24 boys aged 3.5 to 14 years with Tanner genital stage <2 and a testicular volume <4 ml.[41] None of the children had onset of puberty during 24 months of study. Caloric intake and activity level of all participants were standardized three months prior to inclusion. All children were naïve to GH treatment at start of study. The study protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center / Sophia Children's Hospital in Rotterdam, The Netherlands. Written informed consent was obtained from parents and from children over 12 years of age. Assent was obtained from children between 4 and 12 years of age.

Design

The primary aim of our study was to assess the cardiovascular and metabolic risk profile and ASP levels in children with PWS. The secondary aim was to investigate the effects of GH treatment on cardiovascular and metabolic risk factors and ASP levels.

Cardiovascular risk factors were defined as a systolic or diastolic blood pressure >2 SDS or dyslipidemia (TC, LDLc, TG, or Lp(a) levels above normal range; HDLc levels below normal range). Metabolic risk factors were defined as a BMISDS >2 SDS; systolic or diastolic blood pressure >2 SDS; triglyceride level ≥1.7 mmol/l; HDLc level <1.03 mmol/l; glucose ≥5.6 mmol/l; based on the criteria for children by Weiss et al.[42] and De Ferranti et al.[43] and revised by the International Diabetes Federation.[44] The metabolic syndrome was defined as the presence of at least three metabolic risk factors. Fat% and ASP levels were described separately.

Infants and prepubertal children were randomly allocated to either a GH-treated or a control group for one and two years, respectively. Biosynthetic GH (1.0 mg/m^2·day, Genotropin, Pfizer, New York) was administered subcutaneously once daily at bedtime. The first four weeks of GH treatment, children received 0.5 mg/m^2·day to prevent fluid retention. Three-monthly, children were seen by the PWS research team of the Dutch Growth Research Foundation in collaboration with local pediatric endocrinologists and pediatricians throughout The Netherlands. At each visit, the GH dose was adjusted to the calculated body surface area. All anthropometric measurements were performed in the Erasmus University Medical Center Rotterdam / Sophia Children's Hospital, at start and at 6, 12, and 24 months of study. Assays were performed in a central laboratory.

Anthropometrics and blood pressure

Standing height was measured with a Harpenden Stadiometer and supine length with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was assessed on an accurate scale (Servo Balance KA-20-150S). SD-scores (SDS) for height and body mass index (BMI) were calculated with Growth Analyser 3.0 (available at www.growthanalyser.org), according to age- and sex-matched reference values from the Dutch population.[45]

Systolic and diastolic blood pressure were measured while in a sitting position. The mean of two measurements was used for analysis. Because height is an important determinant of blood pressure in childhood, blood pressure SDS was calculated according to height- and sex-matched reference values.[46] None of the children received antihypertensive therapy.

Dual energy x-ray absorptiometry

Fat% and LBM were measured by dual energy x-ray absorptiometry (type Lunar Prodigy, GE Healthcare, Chalfont St. Giles, UK). Quality assurance was performed daily. The coefficients of variation for fat tissue and lean mass were 0.41% to 0.88% and 1.57% to 4.49%, respectively. Fat mass was expressed as percentage of total body mass. Fat%SDS and LBMSDS were calculated for children ≥87 cm, according to height- and sex-matched reference values from the Dutch population.[47,48]

Assays

Fasting blood samples were collected for measurement of serum insulin-like growth factor I (IGF-I), insulin, glucose, total cholesterol (TC), high- and low-density lipoprotein cholesterol (HDLc and LDLc), triglycerides (TG), lipoprotein a [Lp(a)], ASP, and C3 levels.

Serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, California), with an intra- and interassay CV of 4% and 6%, respectively. IGF-I SDS was calculated.[49] Serum TC, HDLc, LDLc, TG, and Lp(a) levels were determined on an Abbott

Architect analyzer (type ci8200, Abbott Diagnostics, Abbott Park, IL), with an intra and interassay CV <5%. Serum insulin levels were measured by immunoradiometric assay (Medgenix, Biosource Europe, Nivelles, Belgium) with an intra- and interassay CV of 2% to 4.7% and 4.2% to 11.3%. Serum glucose levels were assessed on an Abbott Architect Clinical Chemistry Analyzer (Abbott Diagnostics, Abbott Park, IL), with an intra- and interassay CV of 0.7% and 0.8%.

Plasma ASP was measured using a sandwich ELISA immunoassay method as previously described in detail [35], with an intra- and interassay CV of <4% and <8%, respectively. C3 was measured by immunonephelometry.

Elevated levels were defined as: TC >5.0 mmol/l; LDLc ≥3.2 mmol/l; TG ≥1.7 mmol/l; Lp(a) ≥300 mg/l; glucose ≥5.6 mmol/l; insulin ≥15 mU/l; ASP >58 nmol/l; and C3 >1.45 g/l. Reduced HDLc was defined as levels <1.03 mmol/l.

Homeostatic model assessment of insulin resistance (HOMA-IR) was performed using the model HOMA-IR=(fasting insulin x fasting glucose)/22.5.[50] The atherogenic index of plasma (AIP) was calculated with the model AIP=Log(TG/HDLc).[51] A higher HOMA-IR and higher AIP are associated with a higher risk for metabolic and cardiovascular disease.[50, 51]

Data analysis

Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) and with SAS 9.1 (SAS Institute Inc., Cary, North Carolina). Data were normally distributed according to Levene's test and were therefore expressed as mean ± standard deviation (SD). Two analyses were performed: one for prepubertal children during 24 months and one for the total group during 12 months of study. Differences at baseline were calculated with Student's t-tests and chi-square tests. The changes over time were corrected for age and gender and were analyzed using repeated measures of variance in order to correct for multiple testing.[52] Effects are presented as β with 95% confidence intervals (C.I.). Spearman's correlation coefficients were calculated. P-values less than 0.05 were considered statistically significant.

Results

The total group consisted of 85 children with a mean ± SD age of 4.9 ± 3.0 years. Thirty-six children (48%) had a deletion of chromosome 15q11-q13, 34 (45%) a uniparental disomy, and 5 (7%) an imprinting center defect. Positive methylation test was demonstrated in the remaining 10 patients, but the underlying genetic defect was not identified. In the infant group (Table 1), the baseline heightSDS was low-normal, with 49% having a height below -2 SDS. The mean ± SD baseline BMISDS was 0.13 ± 1.7. Nine infants (26%) had a baseline IGF-I SDS below -2 SDS.

In the prepubertal group (Table 1), 30 children (60%) had a baseline height below -2 SDS. The mean ± SD baseline BMISDS was 1.33 ± 1.0. Twenty-one children (42%) had an IGF-I level below -2 SDS.

Baseline

Infant group (0-3 years)

The mean ± SD baseline fat% was 28.41 ± 6.2. Mean ± baseline systolic and diastolic blood pressure SDS was normal (Table 2); only one infant had an elevated systolic blood pressure.

TC levels were elevated in 26% of infants, whereas 33% had elevated LDLc levels and 11% had reduced HDLc levels. Lp(a) levels were elevated in 31% of infants. In 63% of infants at least one of the following factors was present: systolic or diastolic blood pressure > 2 SDS; elevated serum TC, LDLc, TG, or Lp(a) levels; or reduced HDLc levels; indicating an unfavorable cardiovascular profile in addition to a high fat%. In 33% of infants, at least two of these factors were present.

Fasting glucose levels were elevated in two children with a fat% of 31% and 35% (Tables 2 and 3). Serum insulin levels were normal in all infants. BMI was above 2 SDS in 5 children (Table 3). None of the infants met the criteria for metabolic syndrome in children.[42-44] However, 43% of infants had at least one component of the metabolic syndrome.

ASP levels were elevated in 68% of children (Table 2) and were significantly higher than the normal value of 58 nmol/l (p=0.002). C3 levels were elevated in 46% of infants.

Table 1. Baseline characteristics and effects of GH treatment.

		GH-treated				Controls				
		Baseline	6 months	12 months	24 months	Baseline	6 months	12 months	24 months	P-value[a]
Infants	Age	2.1 ± 0.8	2.6 ± 0.8	3.1 ± 0.8		2.2 ± 1.0	2.7 ± 1.1	3.2 ± 1.0		
N = 35	HeightSDS	-1.64 ±1.1	-1.01 ± 1.1	-0.44 ± 1.1		-2.18 ± 1.4	-2.19 ± 1.4	-2.25 ± 1.4		**<0.0001**
	BMISDS	-0.14 ± 1.9	0.24 ± 1.9	0.43 ± 1.6		0.46 ± 1.6	0.69 ± 1.8	1.00 ± 1.9		0.80
	IGF-I SDS	-1.40 ± 0.9	1.49 ± 2.0	2.93 ± 1.3		-1.61 ± 1.2	-1.46 ± 0.9	-1.46 ± 1.1		**<0.0001**
Prepubertal children	Age	7.2 ± 2.4	7.7 ± 2.4	8.2 ± 2.4	9.3 ± 2.5	6.3 ± 2.3	6.8 ± 2.3	7.3 ± 2.3	8.3 ± 2.3	
N= 50	HeightSDS	-1.99 ± 1.5	-1.49 ± 1.5	-1.08 ± 1.3	-0.56 ± 1.2	-2.46 ± 1.0	-2.46 ± 1.1	-2.43 ± 1.1	-2.46 ± 1.2	**<0.0001**
	BMISDS	1.30 ± 1.2	0.85 ± 1.2	0.76 ± 1.2	0.84 ± 1.3	1.36 ± 0.8	1.42 ± 0.7	1.38 ± 0.7	1.40 ± 0.5	**<0.0001**
	IGF-I SDS	-1.87 ± 1.1	1.83 ± 0.9	2.51 ± 1.0	2.52 ± 0.8	-1.99 ± 1.0	-1.69 ± 1.2	-1.99 ± 1.3	-1.70 ± 1.1	**<0.0001**

Results at baseline and during study in infants and prepubertal children, allocated to either a GH-treated group or a control group. Data are expressed as mean ± SD. BMI, body mass index; IGF-I, insulin-like growth factor I; SDS, SD-score according to age- and sex-matched reference values from the Dutch population.[45,49]

[a] P-value for differences between GH-treated children and randomized controls during 12 or 24 months of study (repeated measurements analysis).

Prepubertal group (3-12/14 years)

The mean ± SD fat% in prepubertal children was 36.88 ± 8.5, which corresponded with 2.81 ± 0.5 SDS (Table 2). The fat%SDS was significantly higher than 2 SDS ($p<0.0001$). Notably, the fat%SDS was elevated in 95% of prepubertal children, who had a significantly higher fat% than infants ($p<0.0001$). The mean ± SD systolic blood pressure SDS was normal, but 12% of prepubertal children had an elevated systolic blood pressure. Diastolic blood pressure was normal in all children. TC levels were elevated in 35% of prepubertal children, whereas 46% had elevated LDLc levels and 20% had reduced HDLc levels. Lp(a) levels were elevated 11%. In 73% of prepubertal children at least one of the following factors was present: systolic or diastolic blood pressure >2 SDS; elevated serum TC, LDLc, TG, or Lp(a) levels; or reduced HDLc levels; indicating an unfavorable cardiovascular profile in addition to an elevated fat%SDS. In 49% of prepubertal children, at least two of these factors were present.

Fasting glucose levels were elevated in one child, whereas insulin levels were elevated in another child, both with a fat% of 3.1 SDS (Tables 2 and 3). Prepubertal children had significantly higher insulin levels than infants ($p=0.001$). Although fat%SDS was elevated in 95% of prepubertal children, BMISDS was elevated in 24% (Table 3). Therefore, three children met the criteria for metabolic syndrome listed in Table 3. These three children, aged 3.9, 7.6 and 7.6 years, had a fat% of 3.0 to 3.1 SDS and a BMISDS of 2.6 to 3.7 SDS. Notably, 41% of prepubertal children demonstrated at least one component of the metabolic syndrome. When an elevated fat%SDS was included as a criterion instead of BMISDS, 95% demonstrated at least one metabolic risk factor, whereas 43% demonstrated at least two metabolic risk factors.

ASP levels were significantly higher than normal ($p<0.0001$), with 94% of prepubertal children having elevated ASP levels (Table 2). C3 levels were elevated in 44% of prepubertal children.

Table 2. Baseline cardiovascular and metabolic parameters and ASP levels in children with PWS.

	Infants			Prepubertal children				
	N	Mean ± SD	Elevated (%)	N	Mean ± SD	Elevated (%)	P-value[a]	P-value[b]
Fat%	33	28.4 ± 6.2		50	36.9 ± 8.5		**<0.0001**	
Fat%SDS[*]				41	2.81 ± 0.5	39 (95)		
SBPSDS	17	0.41 ± 1.0	1 (6)	50	0.59 ± 1.0	6 (12)	0.51	0.44
DBPSDS	17	0.46 ± 1.0	0 (0)	50	0.04 ± 1.0	0 (0)	0.14	0.44
TC (mmol/l)	27	4.49 ± 0.6	7 (26)	46	4.85 ± 0.9	16 (35)	0.07	0.20
HDLc (mmol/l)[†]	27	1.28 ± 0.2	3 (11)[†]	46	1.29 ± 0.3	9 (20)[†]	0.82	0.62
LDLc (mmol/l)	27	2.84 ± 0.6	9 (33)	46	3.15 ± 0.7	21 (46)	0.06	0.35
TG (mmol/l)	27	0.84 ± 0.3	0 (0)	46	0.89 ± 0.4	4 (9)	0.55	0.48
Lp(a) (mg/l)	26	174 ± 162	8 (31)	45	128 ± 135	5 (11)	0.21	0.42
Glucose (mmol/l)	32	4.53 ± 1.2	2 (6)	46	4.49 ± 0.6	1 (2)	0.84	0.09
Insulin (mU/l)	28	4.49 ± 2.0	0 (0)	46	6.69 ± 3.7	1 (2)	**0.001**	0.41
ASP (nmol/l)	22	90 ± 42	15 (68)	33	117 ± 44	31 (94)	**0.03**	**0.01**
C3 (g/l)	22	1.41 ± 0.3	10 (46)	32	1.50 ± 0.4	14 (44)	0.41	0.36

Overview of baseline cardiovascular and metabolic risk profile and ASP levels in children with PWS, separately depicted for the infant group and the prepubertal group. Levels are expressed as mean ± SD. N, number of children; elevated (%), the number and percentage of children with elevated levels, except for HDLc (number of children with decreased levels). Fat%SDS, SD-score of body fat percentage; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol (elevated when >5.0 mmol/l); HDLc, high density lipoprotein cholesterol (reduced when <1.03 mmol/l); LDLc, low density lipoprotein cholesterol (elevated when ≥3.2 mmol/l); TG, triglycerides (elevated when ≥1.7 mmol/l); Lp(a), lipoprotein a (elevated when ≥300 mg/l); glucose levels were considered elevated when ≥5.6 mmol/l; insulin levels were considered elevated when ≥15 mU/l; ASP, acylation stimulating protein (elevated when >58 nmol/l); C3, complement factor C3 (elevated when >1.45 g/l).

[*] Reference values for the calculation of fat%SDS were not available for children <87 cm; [†] Presented are the number of children with decreased HDLc levels, defined as <1.03 mmol/l.

[a] P-value for differences in levels between infants and prepubertal children (Student's t-test). [b] P-value for difference in number of children with abnormal levels between infants and prepubertal children (chi-square test).

Table 3. The metabolic syndrome in children with PWS.

	Total		Infants		Prepubertal children	
	Total	N (%)	Total	N (%)	Total	N (%)
BMI > 2 SDS	85	17 (20)	35	5 (14)	50	12 (24)
SBP > 2 SDS	67	7 (10)	17	1 (6)	50	6 (12)
DBP > 2 SDS	67	0 (0)	17	0 (0)	50	0 (0)
TG ≥ 1.7 mmol/l	73	4 (6)	27	0 (0)	46	4 (9)
HDLc < 1.03 mmol/l	73	12 (16)	27	3 (11)	46	9 (20)
Glucose ≥ 5.6 mmol/l	78	3 (4)	32	2 (6)	46	1 (2)
≥ 3 criteria present	60	3 (5)	14	0 (0)	46	3 (7)
≥ 1 criteria present	60	25 (42)	14	6 (43)	46	19 (41)

Overview of the number of children fulfilling criteria for the metabolic syndrome. Total, the total number of children of whom data were available. N (%), number of children fulfilling the criterion for metabolic syndrome. BMI, body mass index; SDS, SD-score; SBP, systolic blood pressure; DBP, diastolic blood pressure; TG, serum triglyceride level; HDLc, serum high density lipoprotein cholesterol level. The metabolic syndrome was defined as the presence of three or more risk markers.[42-44]

Effects of growth hormone treatment

HeightSDS and IGF-I SDS were significantly higher in GH-treated infants and prepubertal children compared to randomized controls (all p<0.0001, Table 1). BMISDS did not significantly differ between GH-treated infants and controls. In prepubertal children, GH treatment resulted in a significant decrease in BMISDS (p<0.0001).

In prepubertal children, GH treatment induced a significant decline in fat%SDS during 24 months of study, corrected for age and gender [β (95% C.I.)=-0.85 SDS (-1.1 to -0.7), p<0.0001; Table 4]. GH treatment did not result in a significant change in systolic and diastolic blood pressure SDS. Serum lipids and AIP did not significantly change, but the HDLc to LDLc ratio significantly improved in response to GH [β (95% C.I.)=0.05 (0.0 to 0.1), p=0.04]. GH had no significant effect on fasting glucose and insulin levels and HOMA-IR, nor on ASP and C3 levels.

When results were analyzed for the total group of 85 children that followed a randomized controlled design for a shorter duration of 12 months, we found similar results. GH treatment significantly improved fat mass, fat% and the HDLc to LDLc ratio (p<0.0001, p<0.0001, and p=0.049).

Table 4. The cardiovascular and metabolic risk profile and ASP levels in prepubertal children with PWS.

	Prepubertal children								
	GH-treated				Controls				
	Baseline	6 months	12 months	24 months	Baseline	6 months	12 months	24 months	P-value
Fat%SDS*	2.80 ± 0.4	2.09 ± 0.7	1.94 ± 0.7	2.01 ± 0.6	2.82 ± 0.6	2.83 ± 0.6	2.84 ± 0.6	2.88 ± 0.5	**<0.0001***
SBPSDS	0.64 ± 1.0	0.94 ± 0.6	0.59 ± 0.8	0.49 ± 0.8	0.53 ± 1.1	0.82 ± 0.9	0.47 ± 0.8	0.56 ± 0.8	0.87
DBPSDS	0.17 ± 0.9	0.15 ± 0.8	0.21 ± 0.9	0.34 ± 0.6	-0.12 ± 1.1	0.06 ± 0.7	0.33 ± 0.8	0.27 ± 0.7	0.46
TC (mmol/l)	4.88 ± 0.8	4.69 ±0.8	4.55 ± 0.7	4.58 ± 0.9	4.80 ± 0.9	4.31 ± 1.2	4.79 ± 1.6	4.89 ± 1.0	0.56
HDLc (mmol/l)	1.33 ± 0.3	1.20 ± 0.3	1.21 ± 0.3	1.23 ± 0.6	1.25 ± 0.3	1.26 ± 0.7	1.07 ± 0.2	1.24 ± 0.3	0.58
LDLc (mmol/l)	3.15 ± 0.7	2.98 ± 0.6	2.88 ± 0.5	3.02 ± 0.8	3.16 ± 0.8	2.92 ± 0.9	3.35 ± 1.5	3.26 ± 0.9	0.19
HDLc/LDLc†	0.44 ± 0.1	0.41 ± 0.1	0.43 ± 0.1	0.48 ± 0.5	0.41 ± 0.1	0.58 ± 0.8	0.35 ± 0.1	0.40 ± 0.1	**0.04†**
TG (mmol/l)	0.91 ± 0.4	1.15 ± 0.8	0.99 ± 0.4	1.12 ± 0.7	0.87 ± 0.4	0.81 ± 0.3	0.84 ± 0.5	0.96 ± 0.6	0.40
Lp(a) (mg/l)	131 ± 137	161 ± 151	178 ± 232	143 ± 176	124 ± 137	156 ± 153	137 ± 156	88 ± 104	0.70
AIP	-0.18 ± 0.2	-0.07 ± 0.3	-0.10 ± 0.2	-0.05 ± 0.3	-0.18 ± 0.3	-0.15 ± 0.1	-0.15 ± 0.3	-0.14 ± 0.2	0.34
Glucose (mmol/l)	4.47 ± 0.8	4.73 ± 0.5	4.60 ± 0.4	4.66 ± 0.4	4.50 ± 0.2	4.43 ± 0.6	4.52 ± 0.5	4.60 ± 0.5	0.16
Insulin (mU/l)	6.57 ± 3.7	7.23 ± 4.1	8.15 ± 4.7	8.72 ± 5.5	6.85 ± 3.7	6.16 ± 2.2	5.85 ±3.1	5.89 ± 3.8	0.45
HOMA-IR	1.34 ± 0.9	1.50 ± 0.8	1.68 ± 1.0	1.82 ± 1.2	1.38 ± 0.7	1.21 ± 0.4	1.21 ± 0.7	1.22 ± 0.8	0.35
ASP (nmol/l)	124 ± 48	102 ± 42	94 ± 58	89 ± 31	107 ± 36	74 ± 32	91 ± 15	95 ± 42	0.93
C3 (g/l)	1.49 ± 0.4	1.54 ± 0.2	1.43 ± 0.3	1.45 ± 0.5	1.51 ± 0.6	1.61 ± 0.5	1.50 ± 0.3	1.53 ± 0.4	0.86

Overview of the cardiovascular and metabolic risk profile and ASP levels in the prepubertal group, divided by the GH-treated and control group. Fat%SDS, SD-score of body fat percentage; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostatic model assessment of insulin resistance; TC, total cholesterol; HDLc, high density lipoprotein cholesterol; LDLc, low density lipoprotein cholesterol; HDLc/LDLc, ratio of HDLc to LDLc; TG, triglycerides; Lp(a), lipoprotein a; AIP, atherogenic index of plasma; ASP, acylation stimulating protein; C3, complement factor C3.

[a] P-values for differences between GH-treated children and randomized controls during 24 months of study, corrected for age and gender (repeated measurements analysis).

* GH had a negative effect on fat%SDS, corrected for age and gender [β (95% C.I.)=-0.85 SDS (-1.1 to -0.7), p<0.0001]. † GH had a positive effect on the HDLc to LDLc ratio, corrected for age and gender [β (95% C.I.)=0.05 (0.0 to 0.1), p=0.04].

Correlations

In the total group at baseline, fat mass correlated significantly with systolic blood pressure SDS, glucose and insulin levels, HOMA-IR, and ASP levels (r=0.35, p=0.005; r=0.25, p=0.03; r=0.48, p<0.0001; r=0.48, p<0.0001; and r=0.46; p=0.001). At 12 months of study, fat mass correlated significantly with ASP levels (r=0.42, p=0.004) and with systolic and diastolic blood pressure, when corrected for age and gender (r=0.32, p=0.02; and r=0.29, p=0.03, respectively). Systolic blood pressure correlated with diastolic blood pressure (r=0.43, p<0.0001). TC levels correlated inversely with the HDLc to LDLc ratio (r=-0.42, p=0.001) and positively with TG levels (r=0.25, p=0.046), indicating that those with higher TC levels had a more unfavorable lipid profile. A more favorable HDLc to LDLc ratio was associated with a more favorable atherogenic index in plasma (r=-0.29, p=0.02). ASP levels correlated inversely with TG levels (r=-0.32, p=0.03). IGF-I SDS did not correlate with fat mass, but did correlate with fat% (r=-0.33, p=0.005).

Discussion

Our study shows that many young children with PWS have a high fat% and dyslipidemia, whereas blood pressure and glucose homeostasis are normal in nearly all patients. Furthermore, our results demonstrate two-fold higher ASP levels, which is in concordance with the high fat%, but also coincided with normal TG levels. GH-treated children showed significant improvement of heightSDS, BMISDS, and IGF-I SDS during 12 and 24 months of treatment compared to randomized controls. In addition, GH reduced fat mass and fat%SDS and increased the HDLc to LDLc ratio. GH treatment had no effect on glucose homeostasis, serum lipids, and plasma ASP and C3 levels.

Our results demonstrate that 95% of children in the prepubertal group had a baseline fat% above 2 SDS. Notably, fat mass corrected for age and gender was significantly correlated with systolic and diastolic blood pressure, even in this young group of children with PWS. In 63% of infants and 73% of prepubertal children, at least one of the following factors was present: systolic or diastolic blood pressure >2 SDS; elevated serum TC, LDLc, TG, or Lp(a) levels; or reduced HDLc levels; indicating an unfavorable cardiovascular profile in addition to the high fat%. The high prevalence of cardiovascular risk factors demonstrated in our study, is in line with two reports in smaller groups of children with PWS.[22,23] In contrast to these studies, our results do not show a beneficial effect of GH treatment on HDLc, and LDLc. However, in line with Carrel et al.,[22] we did find a significant beneficial effect of GH on the HDLc to LDLc ratio. Thus, GH treatment may have a favorable effect on the cardiovascular risk profile by reducing fat%SDS and increasing the HDLc to LDLc ratio.

About 40% of infants and prepubertal children demonstrated at least one component of the metabolic syndrome.[42-44] Notably, 7% of children in the prepubertal group had three or more components and could therefore be diagnosed with the metabolic syndrome. The elevated glucose and insulin levels were found in children with a high fat%SDS or BMISDS. This underlines the importance of monitoring insulin and glucose levels in children with PWS, preventing excessive food intake as this may result in obesity when uncontrolled. GH treatment, corrected for age and gender, had no significant effect on serum insulin and glucose levels and HOMA-IR, which is in line with other reports.[24,26,53]

Fat% and insulin levels were significantly higher in prepubertal children than in infants. However, serum lipids did not significantly differ between both groups, indicating that the unfavorable lipid profile is most likely present from infancy onwards, although this should be confirmed in studies investigating neonates with PWS. Future studies may investigate the need for and effectiveness of early pharmacological intervention in normalizing serum lipids in children with PWS.

Our study demonstrated high ASP levels in young children with PWS, coinciding with an increased fat mass, but normal TG levels. ASP is produced by adipose tissue and stimulates free fatty acid incorporation into adipose tissue by increasing TG synthesis and storage and by inhibiting hormone-sensitive lipase mediated TG lipolysis.[27-33] This is shown by the positive correlation of ASP with fat mass and the negative correlation with TG levels, as demonstrated in our study. Thus, ASP is produced by adipose tissue, but is also involved in the accumulation of adipose tissue. High ASP levels are suggestive of ASP resistance, also shown in subjects with obesity, insulin resistance, dyslipidemia and cardiovascular disease.[34-38] In subjects with ASP resistance, however, high ASP levels coincide with high TG levels and may show a positive correlation with TG levels. In contrast, most of our children with PWS had normal TG levels, which correlated inversely with ASP, indicating an effective function of ASP in TG storage. Thus, high ASP levels in PWS seem to contribute in keeping TG levels within normal range. Furthermore, ASP increases glucose uptake through enhanced translocation of glucose transporters to the plasma membrane surface.[28-32] We found normal glucose and insulin levels in nearly all children, despite high fat%. High ASP levels without ASP resistance results in increased TG storage as well as in increased glucose uptake. High ASP levels may therefore not only contribute to the high fat%, but may also keep glucose and insulin levels within normal range, as has also been suggested for adiponectin.[24-26]

GH treatment does not result in complete normalization of fat mass in children with PWS,[17-21] which suggests that GH insufficiency is not the only factor involved in the mechanism leading to abnormal body composition in PWS. ASP levels were high in most children with PWS and were not significantly different between GH-treated children and randomized controls. Furthermore, ASP levels correlated positively with fat mass and inversely with TG levels.

This indicates that high ASP levels may contribute to the sustainment of high fat%, even during GH treatment. Thus, the high ASP levels do not change in response GH treatment and may play a role in preventing complete normalization of fat mass during GH in children with PWS.

In conclusion, the majority of young children with PWS have a high fat% and dyslipidemia, indicating an unfavorable cardiovascular and metabolic profile. Systolic and diastolic blood pressure were normal in most children, as well as insulin and glucose levels. As ASP stimulates glucose uptake and TG synthesis and storage into adipose tissue, the high ASP levels in children with PWS may contribute to the high fat%. The high ASP levels may also keep glucose and insulin levels within normal range. GH improved fat% and the HDLc to LDLc ratio, but had no effect on blood pressure, glucose homeostasis, and serum lipids. The high ASP levels did not change in response to GH and may therefore contribute to the lack of complete normalization of fat% during GH treatment in patients with PWS.

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Chapter 9

Efficacy and safety of long-term continuous growth hormone treatment in children with Prader-Willi syndrome

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Submitted

Abstract

Background: Children with Prader-Willi syndrome have abnormal body composition and impaired growth. Short-term growth hormone (GH) treatment has beneficial effects.

Objectives: To investigate effects of long-term continuous GH treatment on body composition, growth, bone maturation, and safety parameters.

Setting: A multicenter prospective trial.

Design: Fifty-five children with a mean ± SD age of 5.9 ± 3.2 years were followed during 4 years of continuous GH treatment (1 mg/m^2·day). Data were annually obtained in one center: fat percentage (fat%) and lean body mass (LBM) by dual-energy x-ray absorptiometry, height, weight, head circumference, bone age, blood pressure, and fasting IGF-I, IGFBP-3, glucose, insulin, HbA1c, total cholesterol, HDL, and LDL. SD-scores were calculated according to Dutch and PWS reference values (SDS and SDS_{PWS}).

Results: Fat%SDS was significantly lower after 4 years of GH treatment ($p<0.0001$). LBMSDS significantly increased during the first year ($p=0.02$), but returned to baseline values the second year and remained unchanged thereafter. Mean ± SD height normalized from -2.27 ± 1.2 SDS to –0.24 ± 1.2 SDS ($p<0.0001$). Head circumference SDS increased from -0.79 ± 1.0 at start to 0.07 ± 1.1 SDS after 4 years. $BMISDS_{PWS}$ significantly decreased. Mean ± SD IGF-I and the IGF-I/IGFBP-3 ratio significantly increased to 2.08 ± 1.1 and 2.32 ± 0.9 SDS, respectively. GH treatment had no adverse effects on bone maturation, blood pressure, glucose homeostasis, and serum lipids.

Conclusions: Our study in children with PWS shows that long-term continuous GH treatment (1 mg/m^2·day) improves body composition, by decreasing fat%SDS and stabilizing LBMSDS, and head circumference SDS and normalizes heightSDS without adverse effects. Thus, long-term continuous GH treatment is an effective and safe therapy for children with PWS.

Introduction

Prader-Willi syndrome (PWS) is characterized by hypotonia, short stature, hyperphagia, hypogonadism, scoliosis, psychomotor delay, and temper tantrums.[1-7] PWS results from the lack of expression of the paternally derived chromosome 15q11-q13, caused by deletion, uniparental disomy, imprinting center defect, or balanced translocation.[3,6] Hypothalamic dysfunction may be responsible for many features of PWS.[8-10] Patients with PWS have symptoms resembling growth hormone (GH) deficiency, such as increased fat mass, decreased lean body mass, short stature, small hands and feet, a decreased spontaneous and provoked GH secretion, low insulin-like growth factor I (IGF-I) levels, and a positive response to GH treatment.[6,7,11-18] Short-term GH treatment administered for 1 or 2 years improves, but does not normalize, body composition by decreasing fat percentage (fat%) and stabilizing lean body mass (LBM).[12,13,15,19-25] Furthermore, it increases height-for-age[11-14,19-22,26], improves psychomotor development in the very young, and has psychological and behavioral benefits.[13,24,25,27] GH does not increase the risk for the onset and progression of scoliosis during 2 years of treatment and has no adverse effects on glucose homeostasis.[28, 29]

Although GH has been proven beneficial for children with PWS during 1 or 2 years of treatment, reports on long-term efficacy and safety are very limited.[29-32] Some studies showed a normalization of adult height when GH treatment was started before onset of puberty.[30,31,33,34] Carrel et al.[32] showed a dose-dependent effect on height, body composition and resting energy expenditure. In that study, short-term metabolic effects of GH treatment were optimal with a dose of at least 1 mg/m^2·day. However, the decreased fat% was not sustained with 1 mg/m^2·day after 2 years. LBM persisted to increase after 2 years of GH treatment, but SD-scores for both fat% and LBM were not calculated. It is therefore unknown whether the increase in lean body mass was merely the result of the increase in height or reflected an increase in lean body mass SD-score.

Thus, although GH treatment seems beneficial for children with PWS, results on long-term GH treatment in a large group of prepubertal children, continuously treated for 4 consecutive years with a standard dose of 1 mg/m^2·day, have not been reported. We therefore performed a multicenter prospective follow-up study evaluating efficacy and safety of long-term continuous standard-dose GH treatment in children with PWS, started before onset of puberty.

Methods

Patients

Patients were initially recruited for a randomized controlled GH trial.[22,28] Depending on age and randomization, children started GH treatment either directly, or after 1 or 2 years. All children had a genetically confirmed diagnosis of PWS by positive methylation test and were naïve to GH treatment at time of inclusion. In the present study, we describe a total group of 55 children, who were prepubertal at start of GH treatment and who were prospectively followed for 4 consecutive years. Puberty was defined in girls as Tanner breast stage ≥2 and in boys as Tanner genital stage ≥2 and a testicular volume ≥4 ml.[35] During 4 years of study, nine girls and five boys had either spontaneous or induced onset of puberty at a mean ± SD age of 11.3 ± 1.3 years and 14.4 ± 1.3 years, respectively. One child died after 1 year, most likely due to central adrenal insufficiency during an upper respiratory tract infection.[36] Additionally, one child stopped participation, but not GH treatment, after 3 years. Data of these patients were included in the analyses.

Change in anthropometric results may be influenced by absent or delayed puberty, especially when expressed as change in SDS according to reference values for healthy Dutch children. We therefore also performed a subgroup analysis in 37 children who remained prepubertal during the total study period and who were aged less than 11 years (girls) or less than 12 years (boys) at the end of the study.

Design

The primary objective of our study was to investigate the long-term effect of GH treatment on body composition. The secondary objectives were to assess efficacy of GH treatment by studying the effect on height, BMI, head circumference, anthropometric data, and bone age and to assess safety of GH treatment by studying the effect on blood pressure, fasting serum insulin-like growth factor I (IGF-I), IGF binding protein 3 (IGFBP-3), glucose homeostasis and serum lipids. Children participating in our multicenter prospective follow-up study were continuously treated with somatropin 1 mg/m^2·day (Genotropin, Pfizer Inc., New York) for 4 consecutive years. Somatropin was administered subcutaneously once daily at bedtime. The first four weeks of GH treatment, children received 0.5 mg/m^2·day to prevent fluid retention. Three-monthly, children were seen by the PWS research team of the Dutch Growth Research Foundation in collaboration with pediatric endocrinologists and pediatricians. At each visit, the GH dose was adjusted to the calculated body surface area. In addition, children annually visited the Erasmus University Medical Center Rotterdam / Sophia Children's Hospital, Rotterdam, The Netherlands, where the following data were obtained: fat mass, lean body mass (LBM), height, weight, head circumference, length of the hand, foot, and tibia, arm span, bone age, blood pressure, and fasting blood levels of

insulin-like growth factor I (IGF-I), IGF binding protein 3 (IGFBP-3), glucose, insulin, HbA1c, total cholesterol, and high and low density lipoproteins cholesterol (HDL and LDL). The study protocol was approved by the Medical Ethics Committees of the Erasmus University Medical Center / Sophia Children's Hospital in Rotterdam, The Netherlands, and of each collaborating center. Written informed consent was obtained from parents and from children over 12 years of age. Assent was obtained from children under 12 years of age.

Dual energy x-ray absorptiometry

Fat mass and lean body mass (LBM) were measured in all children by dual-energy x-ray absorptiometry (type Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Quality assurance was performed daily. The coefficient of variation for fat tissue and lean mass was 0.41% to 0.88% and 1.57% to 4.49%, respectively.[37] Fat mass was expressed as percentage of total body mass (fat%). Fat%SDS and LBMSDS were calculated, according to reference values for gender and height of the Dutch population for children with a height above 87 cm.[38,39]

Anthropometry

Standing height was measured with a Harpenden Stadiometer and supine length with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was assessed on an accurate scale (Servo Balance KA-20-150S). In children above 3 years at start of study, length of the left hand, foot and tibia, and arm span were measured according to Cameron using a Harpenden anthropometer.[40] The mean of three measurements was used for analysis. All measurements were obtained by three observers (R.F.A.d.L.v.W., D.A.M.F., and research nurse). Target height (TH) was calculated.[41] Birth weight and length were retrieved from patient records or reported by parents. Standard deviation scores (SDS) were calculated with Growth Analyser 3.0 (available at www.growthanalyser.org), according to age- and sex-matched reference values of the Dutch population and of a population with PWS.[41-43]

Assessment of bone maturation

Radiographs of the left hand and wrist were taken annually. Bone age was assessed by two observers (R.F.A.d.L.v.W. and E.P.C.S.) using the method of Greulich and Pyle.[44] The mean of measurements was taken for analysis. Inter-observer variability was minimal [mean (95% confidence interval) difference = -0.11 years (-0.2 to 0.0); intraclass correlation coefficient = 0.98, $p<0.0001$]. The "bone age to calendar age" ratio (BA/CA) and the "change in bone age to change in calendar age" ratio ($\Delta BA/\Delta CA$) were calculated.

Blood pressure

Systolic and diastolic blood pressure were measured while in a sitting position. The mean of two measurements was used for analysis. Because height is an important determinant of blood pressure in childhood, blood pressure was expressed as SDS adjusted for height and gender.[45] None of the children were receiving antihypertensive therapy.

Assays

Fasting blood samples were collected for measurement of IGF-I, IGFBP-3, glucose, insulin, HbA1c, total cholesterol, HDL and LDL. Samples were investigated in one central laboratory.

The first 2 years of study, serum IGF-I and IGFBP-3 levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, California). The intra- and interassay coefficient of variation (CV) were 4% and 6%, respectively. After 2 years, IGF-I and IGFBP-3 were measured with the Immulite 2000 (Siemens Healthcare Diagnostics, Deerfield, Illinois) with an interassay CV of 6.5% and 8%, respectively. The first 2 years of the study, serum glucose levels were assessed on an Abbott Architect Clinical Chemistry Analyzer (Abbott Laboratories, Irving, Texas), with an intra- and interassay CV of 0.7% and 0.8%. After two years, glucose levels were measured with the Hitachi 917 (Hitachi Device Development Center, Tokyo, Japan), detecting glucose levels between 0 and 42 mmol/liter. The first 2 years, serum insulin levels were measured by immunoradiometric assay (Medgenix, Biosource Europe, Nivelles, Belgium) with an intra- and interassay CV of 2% to 4.7% and 4.2% to 11.3%, respectively. Thereafter, insulin levels were measured with the Immulite 2000 (Siemens Healthcare Diagnostics, Deerfield, Illinois) with an interassay CV of 4.4%. The first 2 years, HbA1c levels were measured using an automatic HPLC analyzer (DIAMAT; Bio-Rad Laboratories Inc., Edgemont, California) with an intra- and interassay CV of 1.3% and 7.0%. Thereafter, HbA1c levels were measured using an automatic HPLC analyzer (HA-8160, A. Menarini Diagnostics International, Florence, Italy) with an intra- and interassay CV less than 3%. Serum total cholesterol, HDL, and LDL were determined enzymatically using a homogeneous assay on a Hitachi 917.

SD-scores were calculated for IGF-I, IGFBP-3, and the IGF-I/IGFBP-3 ratio, according to age- and sex-matched reference values from the Dutch population.[46] To allow comparison between two assays, IGFBP-3 SDS and IGF-I/IGFBP-3 SDS measured with the second assay were converted to match samples measured with the first assay by using the model: $Y = \mu_1 + SD_1 [(X - \mu_2) / SD_2]$, in which Y is the converted value of a sample with an original value X; μ_1 and μ_2 are the group means of the values of the first and second laboratory, respectively; and SD_1 and SD_2 are the standard deviations of the values of the first and second laboratory, respectively.[47] After conversion, results did not significantly differ between

assays. Homeostatic model assessment of insulin resistance (HOMA-IR) was performed using the model HOMA-IR=(fasting insulin x fasting glucose)/22.5.[48]

Data analysis

Statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, Illinois, USA) and with SAS 9.1 (SAS Institute Inc., Cary, North Carolina). Data are expressed as mean ± standard deviation (SD). To correct for multiple testing, the changes over time were analyzed using repeated measures of variance.[49] Repeated measurements analysis was also performed to investigate the effects of different parameters on various outcomes. Effects are presented as β with 95% confidence intervals (C.I.). SD-scores were compared with -2, 0, and +2 SDS using repeated measurements analysis and Student's one sample t-test. P-values less than 0.05 were considered statistically significant. Efficacy and safety of GH treatment was studied in the total group of children. Subgroup analyses were performed investigating the effects of GH treatment on anthropometric results in a prepubertal group.

Results

The total group consisted of 55 children, all prepubertal at start of study, with a mean ± SD age of 5.9 ± 3.2 years. Twenty-six (51%) had a deletion of chromosome 15q11-q13, 21 (41%) an uniparental disomy and 4 (8%) an imprinting center defect. Positive methylation test was demonstrated in the remaining 4 patients, but the underlying genetic defect was not identified. Birth weight SDS and birth length SDS were low, but still within normal range (Table 1). Body composition was unfavorable, as baseline fat%SDS was significantly higher than 2 SDS and LBMSDS was significantly lower than 0 SDS (both $p<0.0001$). Baseline heightSDS was significantly lower than 0 SDS ($p<0.0001$), but was normal according to PWS reference values ($heightSDS_{PWS}$). Notably, the distance to THSDS was significantly greater than 2 SDS ($p=0.003$). BMISDS was normal compared to Dutch and PWS reference values. Mean IGF-I SDS and IGFBP-3 SDS were significantly lower than 0 SDS.

Table 1. Baseline characteristics.

	Total group	Prepubertal group
N (m/f)	55 (29/26)	37 (23/14)
Age	5.9 ± 3.2	4.1 ± 1.9
Birth weight SDS	-1.20 ± 1.1[b]	-1.12 ± 1.1[c]
Birth length SDS	-1.47 ± 2.1[a]	-1.01 ± 1.5[a]
Fat%SDS	2.89 ± 0.6[a]	3.08 ± 0.6[a]
LBMSDS	-1.94 ± 2.5[a]	-1.92 ± 3.1[c]
HeightSDS	-2.27 ± 1.2[a]	-2.29 ± 1.3[a]
$HeightSDS_{PWS}$	-0.09 ± 1.0	-0.06 ± 0.9
Distance to THSDS	2.51 ± 1.2[a]	2.60 ± 1.2[a]
BMISDS	0.92 ± 1.6[a]	0.64 ± 1.8[d]
$BMISDS_{PWS}$	-0.43 ± 0.9[b]	-0.43 ± 0.8[c]
IGF-I SDS	-1.97 ± 1.1[a]	-1.90 ± 1.0[a]
IGFBP-3 SDS	-2.11 ± 2.9[a]	-2.71 ± 1.1[c]

Results at baseline of the total and prepubertal group. Data are expressed as mean ± SD. SDS, SD score according to age- and sex-matched Dutch reference values[41]; SDS_{PWS}, standard deviation score according to age and sex-matched PWS reference values[43]; TH, target height; BMI, body mass index; IGF-I, insulin-like growth factor type I; IGFBP-3, IGF binding protein type 3.
[a] p≤0.0001, [b] p≤0.001, [c] p≤0.01, [d] p≤0.05, compared to 0 SDS.

Efficacy of growth hormone treatment

Body composition

Mean ± SD baseline fat% was 2.89 ± 0.6 SDS. Fat%SDS decreased significantly during the first year of GH treatment to 2.14 ± 0.7 SDS (p<0.0001, Figure 1) and did not significantly change thereafter. After 4 years of treatment, fat%SDS was still significantly lower than at baseline (p<0.0001), but remained higher than 2 SDS (p=0.02). LBMSDS significantly increased during the first year of GH treatment (p=0.02), but returned to baseline values during the second year and did not significantly change thereafter. After 4 years of treatment, LBMSDS was not significantly different from baseline. Thus, GH treatment stabilized LBMSDS, but LBMSDS did not normalize as it was significantly lower than -2 SDS at 4 years of study (p=0.04). Results were similar when a subgroup analysis was performed in children that remained prepubertal throughout the study.

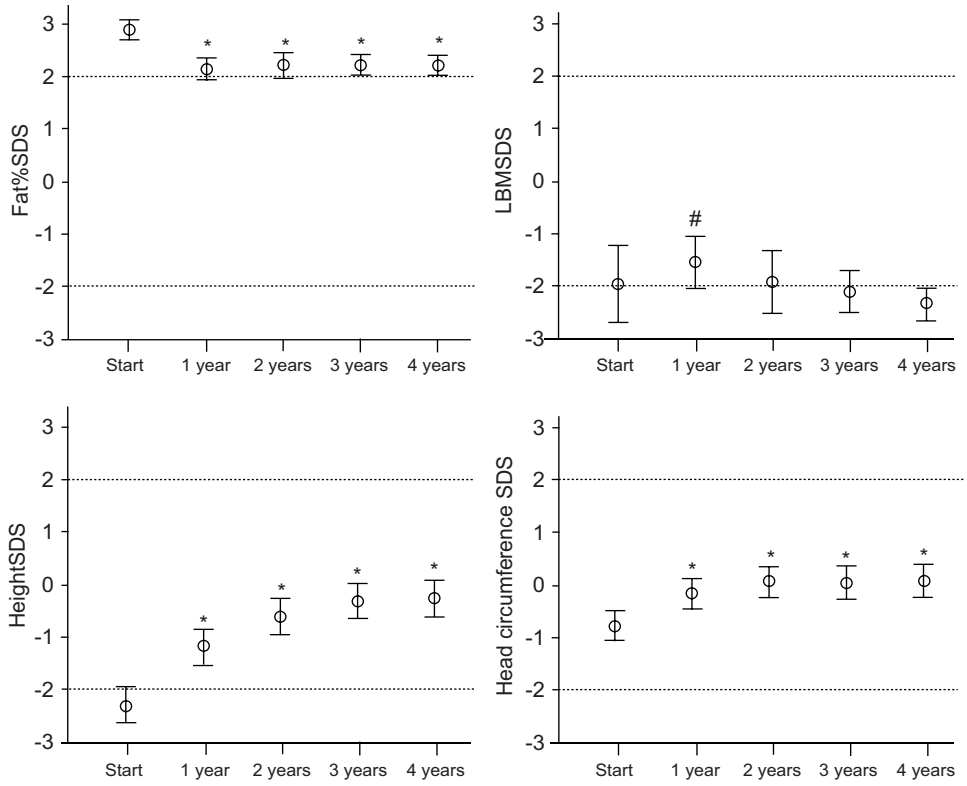

Figure 1. Body composition and growth in the total group of children with PWS during 4 years of continuous GH treatment. Fat percentage SDS (fat%SDS) and lean body mass SDS (LBMSDS) were calculated sex- and height-matched reference values from the Dutch population.[38, 39] HeightSDS and head circumference SDS according to sex- and age-matched reference values from the Dutch population.[41,42] Error bars represent means with 95% confidence intervals. *: p<0.0001, #: p<0.05, compared to baseline.

Anthropometry

HeightSDS significantly increased during the first 3 years of GH treatment (Table 2 and Figure 1), but did not change during the fourth year of treatment. HeightSDS was not significantly different from 0 after 3 and 4 years of treatment (p=0.08 and p=0.16). However, distance to target heightSDS (THSDS) remained significantly greater than 0 (p<0.0001).

Baseline head circumference was significantly lower than 0 SDS (p<0.0001, Table 2 and Figure 1). Head circumference SDS significantly increased during the first 2 years of GH treatment (p<0.0001 and p=0.002) and did no longer significantly differ from 0 SDS after 1 year of treatment.

Table 2. Anthropometric values during GH treatment.

		Start	1 year	2 years	3 years	4 years
Total group	HeightSDS	-2.27 ± 1.2	-1.16 ± 1.2[a]	-0.60 ± 1.2[a]	-0.29 ± 1.2[a]	-0.24 ± 1.2[a]
	$HeightSDS_{PWS}$	-0.09 ± 1.0	0.68 ± 1.0[a]	1.16 ± 1.0[a]	1.50 ± 0.9[a]	1.65 ± 1.0[a]
	Distance to THSDS	2.51 ± 1.2	1.47 ± 1.1[a]	0.93 ± 1.0[a]	0.63 ± 1.0[a]	0.55 ± 1.1[a]
	BMISDS	0.92 ± 1.6	0.85 ± 1.4	1.14 ± 1.2[d]	1.22 ± 1.2[d]	1.29 ± 1.1[d]
	$BMISDS_{PWS}$	-0.43 ± 0.9	-0.75 ± 0.9[a]	-0.70 ± 0.9[b]	-0.71 ± 1.0[b]	-0.69 ± 1.0[b]
	HCSDS	-0.79 ± 1.0	-0.18 ± 1.0[a]	0.06 ± 1.1[a]	0.03 ± 1.1[a]	0.07 ± 1.1[a]
	SH/H ratio	0.44 ± 1.4	0.64 ± 1.4	-0.01 ± 1.3[c]	0.25 ± 1.4	-0.10 ± 1.1[c]
	Hand length SDS	-2.26 ± 1.3	-1.59 ± 1.0[a]	-1.84 ± 1.1[c]	-1.78 ± 0.8[b]	-1.92 ± 1.1[c]
	Foot length SDS	-2.23 ± 1.6	-1.37 ± 1.6[a]	-1.29 ± 1.6[a]	-1.23 ± 1.3[a]	-1.24 ± 1.4[a]
	Tibia length SDS	-1.44 ± 1.5	-0.93 ± 1.3[c]	-0.76 ± 1.4[b]	-0.86 ± 1.1[b]	-0.97 ± 2.2[c]
	Arm span SDS	-1.78 ± 1.4	-1.19 ± 1.3[a]	-1.07 ± 1.2[a]	-0.97 ± 0.8[a]	-0.96 ± 1.0[a]
	BA/CA*	0.83 ± 0.3	0.90 ± 0.2[b]	0.95 ± 0.2[b]	1.00 ± 0.2[c]	1.02 ± 0.1[d]
	ΔBA/ΔCA*		1.16 ± 0.4	1.21 ± 0.5	1.21 ± 0.5	1.19 ± 0.4
Prepubertal group	HeightSDS	-2.29 ± 1.3	-1.08 ± 1.3[a]	-0.38 ± 1.2[a]	-0.05 ± 1.2[a]	0.11 ± 1.1[a]
	$HeightSDS_{PWS}$	-0.06 ± 0.9	0.77 ± 0.9[a]	1.26 ± 1.0[a]	1.54 ± 1.0[a]	1.69 ± 1.0[a]
	Distance to THSDS	2.60 ± 1.2	1.39 ± 1.1[a]	0.72 ± 1.0[a]	0.39 ± 1.0[a]	0.20 ± 0.9[a]
	BMISDS	0.64 ± 1.8	0.78 ± 1.5	1.15 ± 1.3[c]	1.26 ± 1.3[c]	1.29 ± 1.2[b]
	$BMISDS_{PWS}$	-0.43 ± 0.8	-0.68 ± 0.9[b]	-0.65 ± 1.0[d]	-0.68 ± 1.0[c]	-0.70 ± 1.0[b]
	HCSDS	-0.87 ± 1.1	-0.11 ± 1.0[a]	0.22 ± 1.1[a]	0.24 ± 1.1[a]	0.25 ± 1.1[a]
	SH/H ratio	0.79 ± 1.5	0.92 ± 1.5	0.14 ± 1.4[c]	0.48 ± 1.5	0.08 ± 1.1[c]
	Hand length SDS	-2.17 ± 1.2	-1.39 ± 1.0[b]	-1.61 ± 1.2[d]	-1.57 ± 0.9[c]	-1.52 ± 0.8[b]
	Foot length SDS	-2.25 ± 1.8	-1.26 ± 1.9[a]	-1.18 ± 2.0[a]	-1.17 ± 1.6[a]	-1.22 ± 1.8[a]
	Tibia length SDS	-1.44 ± 1.3	-0.89 ± 1.4[a]	-0.60 ± 1.5[a]	-0.76 ± 1.0[a]	-0.53 ± 1.2[a]
	Arm span SDS	-1.62 ± 1.5	-0.90 ± 1.4[a]	-0.76 ± 1.4[a]	-0.75 ± 0.8[a]	-0.61 ± 0.8[a]
	BA/CA*	0.73 ± 0.3	0.85 ± 0.2[a]	0.93 ± 0.2[a]	1.00 ± 0.2[a]	1.04 ± 0.2[a]
	ΔBA/ΔCA*		1.28 ± 0.4	1.30 ± 0.5	1.34 ± 0.4	1.30 ± 0.4

Overview of anthropometric results in the total group and prepubertal group. Data expressed as mean ± SD. SDS, SD score according to age- and sex-matched Dutch reference values[41, 42]; SDS_{PWS}, standard deviation score according to age and sex-matched PWS reference values[43]; THSDS, target heightSDS; BMISDS, body mass index SDS. BA, bone age; CA, calendar age; ΔBA/ΔCA, ratio of change in bone age to change in calendar age, also presented for children above 7 yrs at start of study. [a] p≤0.0001, [b] p≤0.001, [c] p≤0.01, and [d] p<0.05, all compared to baseline. * Not significant after correction for age.

BMISDS significantly increased from the second year of study onward (Table 2). However, this increase is part of the natural development of patients with PWS. When adjusting for this natural development by using PWS reference values, $BMISDS_{PWS}$ significantly decreased during the first year of GH and remained significantly lower than baseline during 4 years of GH treatment.

The sitting height to height ratio was significantly lower after 4 years of treatment than at baseline and did not significantly differ from 0 SDS. Baseline hand, foot, and tibia length SDS, and arm span SDS significantly increased during GH treatment, but remained significantly below 0 SDS.

We performed a subgroup analysis in children who remained prepubertal during study and were less than 11 years (girls) or 12 years (boys) of age after 4 years of study. The mean ± SD age at start of study was 4.1 ± 1.9 years. In general, results in the prepubertal group were similar, but changes were more pronounced. In this group, heightSDS significantly increased during each year of treatment ($p<0.0001$, $p<0.0001$, $p<0.0001$, and $p=0.0009$, for each time interval, respectively). Distance to THSDS was significantly greater than 0 after 3 years ($p=0.03$), but had completely normalized after 4 years of GH treatment ($p=0.23$, Figure 1). Tibia length SDS did no longer significantly differ from 0 after 4 years of GH treatment ($p=0.06$).

Bone maturation

The baseline BA/CA ratio was significantly lower than 1 ($p<0.0001$, Table 2). Age had a significant effect on the BA/CA and the ΔBA/ΔCA ratio ($p=0.0006$ and $p<0.0001$). At start of study, bone age was delayed in children under 5 year. Figure 2 shows a shift from a delayed bone maturation in children under 5 years of age toward a normal bone maturation in those above 7 years. Thus, bone age caught up with calendar age in children between 5 to 7 years. This is also shown by the lower mean BA/CA ratio in the prepubertal group compared to the total group. When adjusting for the effect of age, GH treatment had no significant effect on the ΔBA/ΔCA ratio ($p=0.91$ and $p=0.95$ in the total and prepubertal group).

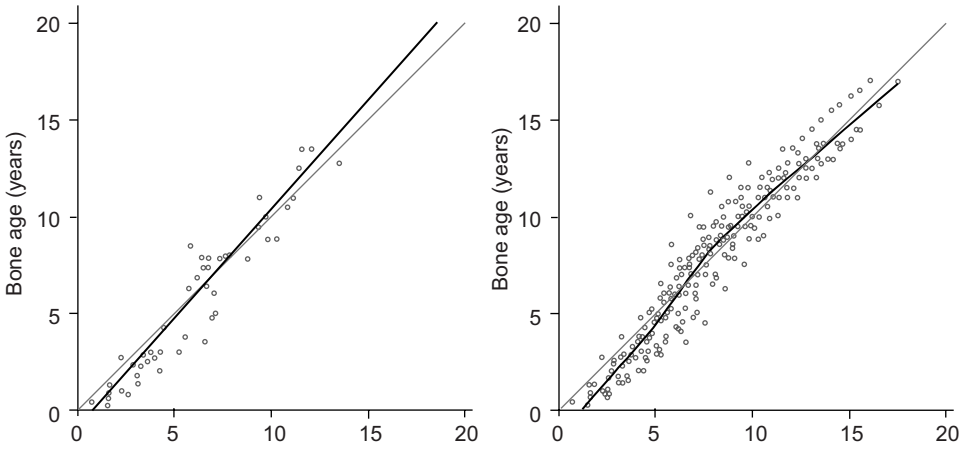

Figure 2. Overview of calendar age and corresponding bone age in the total group of children at start and during the entire study period. Circles represent individual values. The 45° line represents perfect bone maturation. Values under the 45° line represent delayed bone maturation and values above this line an advanced bone maturation. The fitted line is depicted in black.

Safety of growth hormone treatment

IGF-I and IGFBP-3

IGF-I SDS significantly increased during the first year of treatment to a mean (95% C.I.) of 2.45 SDS (2.1-2.8) (Table 3). Thereafter, IGF-I levels did not significantly change, but levels tended to be lower after 4 years of study than after 1 year with a mean (95% C.I.) of 2.1 SDS (1.8-2.4) ($p=0.06$). After 4 years of GH treatment, IGF-I SDS was significantly higher than at baseline ($p<0.0001$), but was not significantly higher than 2 SDS ($p=0.61$). IGFBP-3 also significantly increased during the first year of GH treatment and was still significantly higher after 4 years of treatment than at baseline. The IGF-I/IGFBP-3 ratio SDS significantly increased, which might indicate that more unbound IGF-I was present in the systemic circulation.

Glucose homeostasis

Fasting glucose and insulin levels, and HOMA-IR were significantly associated with age [β (95% C.I.)=0.03 (0.01-0.05), $p=0.008$; $\beta=0.94$ (0.62-1.26), $p<0.0001$; and $\beta=0.21$ (0.14-0.28), $p<0.0001$, respectively]. Effects of GH treatment on these variables were therefore

adjusted for age. GH treatment had no significant effect on glucose and insulin levels and HOMA-IR (p=0.65, p=0.89, and p=0.92, Table 3), or on HbA1c (p=0.79). IGF-I SDS had also no significant effect on fasting glucose and insulin levels, HOMA-IR or HbA1c levels (p=0.27, p=0.16, p=0.18, p=0.56). Fat mass showed a positive association with fasting insulin levels and HOMA-IR [β=0.28 (0.17-0.38), p<0.0001 and β=0.06 (0.04-0.08), p<0.0001].

Table 3. Safety parameters during 4 years of GH treatment.

	Start	1 year	2 years	3 years	4 years
IGF-I SDS	-1.97 ± 1.1	2.45 ± 1.3[a]	2.43 ± 1.0[a]	2.37 ± 1.2[a]	2.08 ± 1.1[a]
IGFBP-3 SDS	-2.11 ± 2.9	0.05 ± 1.5[a]	0.67 ± 0.9[a]	0.73 ± 1.2[a]	0.58 ± 1.2[a]
IGF-I/BP-3 ratio SDS	-0.78 ± 1.7	2.68 ± 1.0[a]	2.56 ± 0.9[a]	2.66 ± 1.0[a]	2.32 ± 0.9[a]
Glucose (mmol/L)	4.53 ± 0.5	4.49 ± 0.5	4.58 ± 0.4	4.64 ± 0.3	4.63 ± 0.4
Insulin (mU/L)	8.75 ± 6.2	10.75 ± 6.5	11.23 ± 8.0	10.96 ± 6.4	12.02 ± 7.7
HOMA-IR	1.83 ± 1.4	2.22 ± 1.4	2.44 ± 1.8	2.29 ± 1.4	2.49 ± 1.7
HbA1c (%)	5.31 ± 0.8	5.37 ± 0.4	5.30 ± 0.4	5.28 ± 0.3	5.21 ± 0.2
Total cholesterol (mmol/L)	4.56 ± 0.8	4.71 ± 1.0	4.57 ± 0.7	4.24 ± 0.6	4.31 ± 0.5
HDL (mmol/L)	1.32 ± 0.8	1.17 ± 0.2	1.20 ± 0.5	1.40 ± 0.3	1.38 ± 0.3
LDL (mmol/L)	2.92 ± 0.7	3.11 ± 0.9	2.95 ± 0.7	2.59 ± 0.6[d]	2.55 ± 0.4[c]
Systolic BPSDS	0.59 ± 0.9	0.59 ± 0.7	0.48 ± 0.7	0.46 ± 0.8	0.46 ± 0.9
Diastolic BPSDS	0.28 ± 0.8	0.48 ± 0.9	0.44 ± 0.6	0.47 ± 0.8	0.45 ± 0.6

Overview of IGF-I, IGFBP-3, bioavailability of IGF-I, glucose homeostasis, serum lipids and blood pressure in the total study population during 4 years of GH treatment. Data expressed as mean ± SD. SDS, SD score according to age- and sex-matched Dutch reference values[50] or according to height-matched reference values [blood pressure[45]]; IGF-I, insulin-like growth factor type I; IGFBP-3, IGF binding protein type 3. HOMA-IR, homeostatic model assessment of insulin resistance; HbA1c, the A1c fraction of hemoglobulin; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; BPSDS, blood pressure SDS.
[a] p≤0.0001, [b] p≤0.001, [c] p≤0.01, and [d] p<0.05, all compared to baseline.

Serum lipids and blood pressure

Serum total cholesterol, HDL, and LDL levels were not significantly associated with age. Therefore, effects of GH treatment were not adjusted for age. Total cholesterol and HDL cholesterol did not significantly change during GH treatment (Table 3). LDL cholesterol was significantly lower after 3 and 4 years of treatment. IGF-I SDS showed a positive association with HDL [β (95% C.I.)=0.07 (0.01-0.13), p=0.03], but not with total cholesterol and LDL. Fat mass was not significantly associated with any of the serum lipids.

Baseline systolic and diastolic blood pressure SDS were normal and did not change during 4 years of GH treatment.

Individual cases

One boy deceased after 1 year of study during a mild upper respiratory tract infection.[36] As serum cortisol one hour after death was undetectable, central adrenal insufficiency was suspected to be the cause of death. During a polysomnography seven months prior to his demise, the central apnea index was only 0.9 per hour, which is considered very low for children with PWS.[51] The death of this boy was considered not GH-related.

There were no cases of non-insulin dependent diabetes mellitus in our study population, but one girl developed diabetes mellitus type I after 45 months of GH treatment. BMISDS was normal (0.77) and was decreasing at the time of diagnosis, whereas gain in height stunted. After induction of insulin therapy, glucose levels and weight normalized, and height improved.

In one boy, height increased during 1 year of GH treatment from 1.23 SDS to 2.84 SDS with an increase of serum IGF-I levels to 4.70 SDS. He was not excluded from GH treatment as baseline body composition was unfavorable with an IGF-I level of -1.49 SDS. GH dose was first reduced to 0.5 mg/m^2·day and later to 0.33 mg/m^2·day. Finally, GH treatment was discontinued after five years. After reduction of the GH dose and discontinuation of GH treatment, his body composition worsened. An excessive increase in gain in heightSDS and IGF-I SDS during GH treatment occurred in three more cases. All three boys had a low heightSDS at start of treatment. They were not included in the current analyses, as they had not completed 4 years of GH treatment. In all cases a reduction of GH dosage sufficed to decrease IGF-I to levels between 2 and 3 SDS and to normalize heightSDS. GH treatment was therefore not discontinued.

Discussion

Our multicenter prospective follow-up study in children with PWS shows that 4 years of continuous GH treatment with a standard dose (1 mg/m^2·day) had a significant favorable effect on body composition, heightSDS, $BMISDS_{PWS}$, head circumference SDS, without significant adverse effects on safety parameters.

Fat%SDS was significantly lower after 4 years of GH treatment than at baseline. LBMSDS significantly decreased during the first year of GH treatment, but returned to baseline values the second year and did not significantly change thereafter. Thus, GH resulted in a significant reduction of fat%SDS and a stabilization of LBMSDS. This is in contrast to the continuous decrease of LBMSDS in untreated children with PWS.[22] Notably, although body composition markedly improved during treatment, long-term GH did not result in a complete normalization. This has previously been shown by Carrel et al.,[32] but children participating in that study were not continuously treated with a standard dose. The fact that long-term

GH treatment did not normalize body composition suggests that the unfavorable fat% and LBM are not only the result of GH insufficiency, but are also the result of the genetic defect causing the syndrome. Most importantly, our study shows that the beneficial effects of short-term GH treatment on body composition[11-14,19-26,52] persist during 4 years of treatment.

At start of treatment, heightSDS was low according to Dutch references, but normal according to PWS references. HeightSDS significantly increased during GH treatment. The distance to THSDS did, however, not completely normalize in the total group. This effect may be due to delayed or absent puberty in some children resulting in a lack of pubertal growth spurt. When height is expressed as SDS, a steady gain in height during the time that healthy children have a pubertal growth spurt results in a paradoxical decrease of heightSDS in those with a delayed puberty. We therefore performed a subgroup analysis in a group that remained prepubertal for 4 years. In this group, distance to THSDS completely normalized after 4 years of GH treatment. Thus, our results indicate that 4 years of GH treatment result in a complete normalization of heightSDS and distance to THSDS in prepubertal children. Bone maturation was not significantly affected by GH treatment. There was a shift from a delayed bone maturation in children below 5 years of age toward a normal bone maturation in those above 7 years. This catch-up in bone maturation might be related to adrenarche, typically occurring early in many children with PWS.[34,53] As bone age did not accelerate in response to GH treatment, our data suggest that a normal adult height can be reached when long-term GH treatment is started several years before puberty.

In the general population, a lower head circumference SDS is associated with lower intelligence, whereas brain growth during infancy and early childhood is important in determining cognitive function.[54-56] In our study, head circumference SDS improved during GH treatment. Improvement of mental development and psychosocial functioning during short-term GH treatment in patients with PWS has previously been described.[13,24,25,27] The improvement of head circumference during long-term continuous GH treatment may have persistent beneficial effects on mental development and psychosocial functioning, but this needs to be investigated in future studies.

It has previously been suggested that when the size of hands and feet normalize during GH treatment, this would indicate that these features are the result of GH deficiency rather than due to the genetic defect.[31] Our results, however, show that hand and foot length, as well as arm span do not completely normalize during GH treatment. Tibia length only normalized in the prepubertal group, thus when GH treatment was started at a young age. Our results show that the main catch-up growth of hands and feet occurs during the first year with little change thereafter. Catch-up in tibia length SDS and arm span SDS may continue in the second year of treatment reaching a plateau thereafter. These findings suggest that both GH deficiency and the genetic defect are responsible for the smaller hands, feet, tibia, and arm span in children with PWS.

Mean IGF-I levels rapidly increased during GH treatment, whereas IGFBP-3 increased more gradually. IGFBP-3 is the major carrier protein of IGF-I and binds 70-95% of IGF-I as a binary complex or as a ternary complex together with the acid labile subunit (ALS). Only a minor fraction of IGF-I circulates in its unbound form, which is considered the biologically active form.[57] We found an increase in the IGF-I/IGFBP-3 ratio, suggesting an increase in free IGF-I levels. Notably, there were a few cases of children with exceptional increases of heightSDS and IGF-I SDS during GH treatment. One may consider individualizing the GH dosage in order to keep IGF-I within reasonable limits. However, Carrel et al.[32] reported that metabolic effects of GH treatment in children with PWS are optimal with a dose of at least 1 mg/m^2·day, whereas a dose of 0.3 mg/m^2·day had no effect on LBM and fat%. Our limited data on reduction of GH dosage or discontinuation of treatment also suggest a worsening of body composition. Thus, GH dosage may be individualized, but caution should be taken as lower IGF-I levels might result in a less favorable body composition with higher fat%.[32] Our results show that IGF-I levels between 2 and 3 SDS result in sustained improvement of body composition, head circumference, and normalization of heightSDS without adverse effects.

As insulin levels and HOMA-IR increase with age in untreated prepubertal children with PWS,[58] we adjusted for age in our analysis of glucose homeostasis. GH treatment adjusted for age had no significant effect on fasting glucose and insulin levels, HOMA-IR, and HbA1c levels in our group of 55 children. This is in line with Lindgren et al. describing normal glucose homeostasis during five years of GH treatment in 16 children without rapid weight gain.[29] Furthermore, we had no cases of non-insulin dependent diabetes mellitus. Our findings suggest that the high prevalence of non-insulin dependent diabetes mellitus in adult patients with PWS[59] is likely due to increased fat mass. Indeed, our results show that a significant positive association of fat mass with both fasting insulin levels and HOMA-IR is already present in childhood. These results underline the importance of achieving normal BMI and reducing fat mass as part of the primary aims in the comprehensive care of patients with PWS. In this respect, it is interesting to note that BMISDS according to PWS reference values significantly decreased during GH treatment. Thus, GH treatment might have favorable effects on insulin resistance as it decreases fat mass and slows down weight gain over the years.

GH treatment had no significant effect on total cholesterol and HDL cholesterol, but LDL cholesterol was significantly lower after 4 years of GH treatment. We therefore conclude that GH treatment had no adverse effect on serum lipids. Furthermore, systolic and diastolic blood pressure SDS did not significantly change during 4 years of GH treatment.

In conclusion, our study shows that long-term GH treatment improves body composition by decreasing fat%SDS and stabilizing LBMSDS. Furthermore it normalizes heightSDS and improves head circumference SDS $BMISDS_{PWS}$. In addition, hand, foot, and tibia

length SDS, and arm span SDS all improved. Measurements of IGF-I and IGFBP-3 during treatment are important. Children with an exceptional increase of heightSDS and IGF-I SDS need adjustment of GH dose to attain an IGF-I level between 2 and 3 SDS. No adverse effects on blood pressure, glucose homeostasis, and serum lipids were observed. Thus, our study shows that long-term continuous standard-dose GH treatment is an effective and safe therapy for children with PWS.

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Chapter 10

General discussion and conclusions, clinical implications, and recommendations for future research

This doctoral dissertation describes studies embedded in the Dutch national growth hormone trial for children with Prader-Willi syndrome (PWS), coordinated by the Dutch Growth Research Foundation and performed to improve the knowledge of PWS and care for patients with PWS. The study population consisted of children with PWS, referred to our center by pediatricians and pediatric endocrinologists throughout The Netherlands. Patients were initially recruited for the multicenter randomized controlled GH trial for the duration of one or two years for infants and prepubertal children, respectively. After the randomized controlled trial, all children continued growth hormone treatment in a multicenter follow-up study (the Dutch Cohort Study). In this dissertation we present the results of 4 years continuous GH treatment. This chapter discusses the findings of our studies in view of the current literature. Subsequently, clinical implications of our results are presented and recommendations for future research are provided.

10.1 Sudden death in children with Prader-Willi syndrome

10.1.1 High prevalence of central adrenal insufficiency

The annual mortality rate in patients with PWS is very high (3% under 30 years).[1] The sudden deaths in children often share a similar pattern: a sudden deterioration during a mild or moderate infection, which is often – but not always – an upper respiratory tract infection. Furthermore, a reduced adrenal size has been found during autopsies in children.[2, 3] Adrenal size may be associated with the cause and duration of the various disease states leading to adrenal insufficiency.[4] These findings, coinciding with the presence of hypothalamic dysregulation in patients with PWS, prompted us to hypothesize that patients with PWS suffer from central adrenal insufficiency during stress. We performed a cross-sectional study, investigating the ACTH response during a metyrapone test in 25 children. Strikingly, ACTH levels failed to increase sufficiently in 60% of patients (15/25), indicating central adrenal insufficiency (CAI). Morning cortisol levels and diurnal cortisol rhythm were assessed on a different day in all children who underwent a metyrapone test and demonstrated no abnormalities. We therefore conclude that CAI becomes apparent only during stressful conditions. The inadequate ACTH response during stress most likely contributes to the high rate of sudden death in children with PWS. In addition to CAI, the condition of acutely ill patients with PWS is further compromised by an increase in the number of sleep apneas during upper respiratory tract infections (URTIs)[5] and a vague clinical presentation, as they often have an increased pain threshold and do not vomit or develop fever. Based on our findings, a new guideline advices treatment with a hydrocortison stress dose during illness or surgery, unless CAI has recently been ruled out with a metyrapone test.

Four tests are widely used to investigate the integrity of the HPA-axis: the standard-dose and low-dose ACTH tests, the insulin tolerance test (ITT) and the overnight single dose metyrapone test (metyrapone test). ACTH tests are the most commonly used tests. However, the ACTH tests are not be suitable for the diagnosis of partial CAI, which is the case in patients with PWS. ACTH tests stimulate the adrenal cortex to produce cortisol. In full-blown CAI, the adrenal cortex is chronically understimulated, resulting in adrenal atrophy. Therefore, the cortisol response during an ACTH test is blunted. In partial CAI, however, which occurs only during stress, the diurnal cortisol rhythms are normal, thereby preventing adrenal atrophy. ACTH stimulation, even low-dose ACTH (1 µg, which is still supraphysiological), may stimulate the adrenal cortex enough to produce cortisol levels above the cut-off level for this test. Thus, ACTH tests are appropriate for general screening, but are unsatisfactory for diagnosing CAI, because partial ACTH deficiency may not always result in atrophy of the adrenal cortex.[6-10] Sensitivity and specificity have been reported to be 60-80% compared to the ITT and metyrapone test.[11,12] The ITT, although highly sensitive and specific, requires close medical supervision and can cause life threatening shock.[9,10,13-18] The metyrapone test is of similar or even superior quality in assessing CAI compared to the ITT and the risk for an Addisonian crisis is much lower than with the ITT.[9,13,17,19-21] The metyrapone test is considered a safe and reliable procedure for the assessment of CAI.[14,17,19,20] Due to the suspicion of partial central adrenal insufficiency and the high sensitivity and specificity of this test, we chose to perform metyrapone tests in our patients with PWS. Such tests should be performed in the pediatric intensive care unit, under continuous monitoring. Our study demonstrated low blood pressures in the early morning hours, which supports the need for admission during the metyrapone test. Notably, no metyrapone test had to be discontinued.

In line with literature, patients with CAI had slightly higher cortisol levels after the metyrapone test than those without CAI.[22,23] These differences could be interpreted as a reduced cortisol inhibition and consequent ACTH response. However, there was no significant difference between the two groups in the decline of cortisol levels from 2330 h to 0400 h, in the maximally suppressed cortisol levels (0400 h), nor in the rise of cortisol levels thereafter. Thus, cortisol production was equally inhibited by metyrapone in both groups. A significant difference in ACTH levels was already present at 0400 h and lasted throughout the metyrapone test. The interpretation of our results are therefore considered valid, demonstrating a high prevalence of CAI in patients with PWS.

During the metyrapone test, blood pressure was low during early morning hours. Notably, most sudden deaths in children with PWS occur during early morning hours.[24] The decrease in blood pressure was demonstrated despite the absence of illness or shock, as all metyrapone tests were performed during healthy condition. The low blood pressures during the metyrapone test occurred in all children and correlated significantly with serum cortisol

levels. Thus, the early morning hours might be the critical period during which PWS patients with CAI are at risk of sudden demise.

Our study showed no abnormalities in morning cortisol levels and normal cortisol diurnal rhythm. In an invited comment, Butler et al. recently reported morning cortisol levels of 63 patients with PWS between 0800 h and 1100 h.[25] In contrast to our study, low morning cortisol levels were found in 4 children, all ≤3 years of age. In our metyrapone study we additionally investigated the diurnal salivary cortisol rhythm, but the youngest child included in our study was 3.7 years old. Therefore, we have no data available on morning cortisol levels in children with PWS ≤3 years of age. Butler et al. suggested that the transition from the infant phase to the hyperphagic obese phase in PWS coincides with hypothalamic changes, possibly explaining the difference in morning cortisol secretion between patients ≤3 years and those >3 years of age. Off note, the peak morning cortisol level usually occurs within 30 minutes after wake-up, but morning cortisol levels rapidly decrease after 0800 h. Perhaps, the low morning cortisol levels demonstrated in the study of Butler et al. reflect the time of measurement rather than a hypoadrenal state. Nonetheless, Butler et al. concluded that cortisol levels in patients with PWS support changes in routine care, as advised in the new guideline based on our findings.

All of our patients who underwent a metyrapone test were treated with GH. We do not believe that this influenced the outcomes of our studies. Data on relationships between the GH-IGF-I system and the HPA-axis are contradictory.[26,27] The prevalence of CAI is not increased in GH-treated children with isolated GH deficiency, Turner syndrome, or other disorders.[26] Moreover, the similar death rate of PWS patients with and without GH treatment,[24,28] suggests an intrinsic rather than an extrinsic cause of CAI. Tauber et al.[28] presented an interesting review of 64 cases of sudden death. In this report, a clustering of sudden deaths was found during the first 9 months of GH treatment. However, this finding may be biased due to selection: one would earlier consider GH treatment of patients in less healthy condition, aiming to support them by improving body composition and endurance. Importantly, when patients are followed three-monthly in clinical trials, sudden deaths are earlier noticed and better reported than of patients not participating in studies. This increases the a priori chance of noticing sudden death during the initial phase of GH treatment. Nonetheless, patients on GH treatment should be regularly evaluated for fluid retention and enlarged tonsils. In order to prevent fluid retention, children participating in our studies received a GH dose of 0.5 mg/m^2·day, which was increased after 4 weeks to 1.0 mg/m^2·day.

In conclusion, there is a high prevalence of central adrenal insufficiency during stress, which may contribute to the high mortality rate in patients with PWS. Based on our findings, a hydrocortisone stress dose should be administered to patients with PWS during stress, unless CAI has recently been ruled out with a metyrapone test. Appendix A provides an

example of a cortisone treatment regimen that could be applied for patients with PWS during stress, in particular during illness or surgery.

10.1.2 Central adrenal insufficiency is associated with sleep-related breathing disorders in children with Prader-Willi syndrome

Patients with PWS often have sleep-related breathing disorders (SRBDs), consisting of obstructive and central sleep apneas. Sleep apneas are the result of a reduction in upper airway diameter, hypoventilation, decreased pulmonary function, and a decreased ventilatory and arousal response during hypercapnia.[5,29-33] The number of central and obstructive sleep apneas increase during illness.[5,29] Sleep apneas have been suggested to play a role in the sudden death of patients with PWS.[5,24,28,29,34] We therefore investigated the relationship between central adrenal insufficiency and sleep apneas. We evaluated sleep-related breathing during a metyrapone test and studied the difference in change of sleep-related breathing in response to metyrapone between children later diagnosed with CAI and those not. Sleep-related breathing deteriorated in response to metyrapone-induced stress in all children, both with and without CAI. Interestingly, the deterioration was significantly worse in children with CAI. In addition, children with CAI already had a significantly higher central apnea index compared to those without, several months prior to the metyrapone test.

As patients with PWS suffer from hypothalamic dysregulations[34-37], CAI is likely to result from an inappropriate CRH secretion by the hypothalamus. Low CRH levels have been demonstrated to decrease EEG frequency, causing deeper sleep and less wakefulness.[38] A deeper stage of sleep is associated with a higher arousal threshold.[39] Possibly, children with CAI may be more prone to have central sleep apneas due to lower CRH levels resulting in deeper sleep and an increased arousal threshold. This may also account for the higher increase in oxygen-desaturation-index in response to metyrapone in children with CAI. On the other hand, CAI during stress and a high central apnea index during health may also be considered two independent symptoms, both resulting from a more severe degree of hypothalamic dysfunction.

Our group investigated sleep-related breathing in a cohort of 53 children with PWS, before and 6 months after initiation of GH treatment.[5] GH treatment had no significant effect on sleep-related breathing. Miller et al.[29] investigated sleep-related breathing before and 6 weeks after initiation of GH. In 20 children, 6 had worsening of sleep-related breathing. However, all noteworthy changes occurred in those with an URTI. In contrast to Miller et al.[29], we interpret these as demonstrating that GH treatment has no effect on sleep-related breathing, similar to results from our study.[5] Notably, Lindgren et al.[32] reported an increase in inspiratory drive in 9 children 6-9 months after start of GH treatment compared to baseline, which was demonstrated by an improved ventilatory response to low pCO_2 levels. Thus, we

conclude that GH has no adverse effect on sleep-related breathing in children with PWS. In conclusion, children with PWS and CAI during stress have a significantly higher central apnea index during healthy condition and a higher increase in central apnea index during stress. The combination of CAI and compromised sleep-related breathing will aggravate the deterioration of the condition of an acutely ill patient with PWS. This combination might well be the prelude of a fatal cascade during illness.

10.1.3 A new hypothesis on sudden death in Prader-Willi syndrome

Based on our findings, we present a new hypothesis on sudden death in children with PWS, depicted in Figure 1.

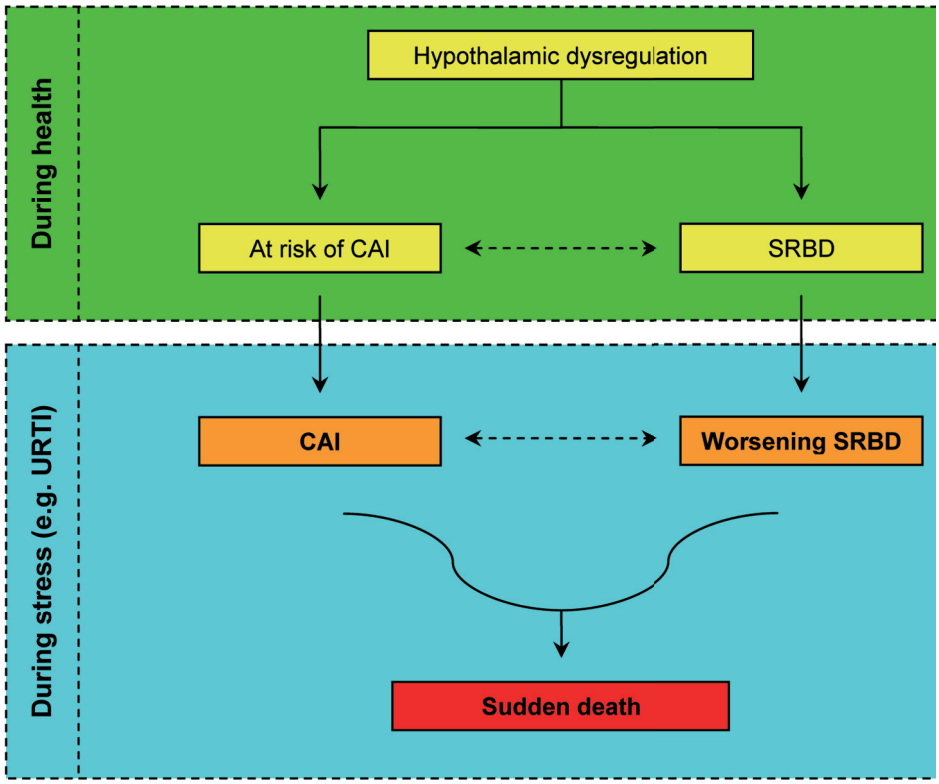

Figure 1. New hypothesis on the mechanism of sudden death in patients with PWS. CAI, central adrenal insufficiency; SRBD, sleep-related breathing disorder; URTI, upper respiratory tract infection

We postulate the following pathway leading to sudden death in patients with PWS (Figure 1):

- Due to a dysregulated hypothalamus, children are at risk for developing CAI during stress. In addition, this may coincide with sleep-apneas. Children at risk of CAI during stress have a higher number of central sleep apneas, as shown in our study.
- During stress, CAI occurs and sleep-related breathing may worsen, resulting in a deterioration of the patient's condition. The worsening of sleep-related breathing in response to stress is more severe in children with CAI than in those without, as demonstrated in our study. Importantly, the deterioration of the condition of a patient with PWS may be masked due to absence of fever and vomiting, and a decreased pain threshold.
- In children with an upper respiratory tract infection (URTI), sleep-related breathing can be further compromised by enlarged tonsils, causing an increase in the number and severity of obstructive sleep apneas. Children suffering an upper respiratory tract infection are therefore at higher risk of sudden death than those with other infections, which would explain why most - but not all - sudden deaths occur during URTI.
- As CAI during stress and sleep-related breathing disorders (SRBD) could be considered two distinct features, they may also result in sudden death independently from each other. However, it seems logical to assume that a combination of CAI and SRBD yields a higher risk of mortality.

10.2 Scoliosis and effects of growth hormone treatment in children with Prader-Willi syndrome

10.2.1 Scoliosis

Information on scoliosis in PWS was limited and varied greatly.[40-44] The prevalence was estimated to be between 15% and 86%.[40-44] As prospective data concerning prevalence of scoliosis in a large group of children with PWS not treated with GH were not available, we investigated the prevalence of scoliosis and the effects of age, gender, body mass index and lean body mass before start of GH treatment. Our study demonstrated a prevalence of 30% in children under 10 years, increasing to 80% in children above 10 years of age. Notably, in the total group, 50% of children had a Cobb's angle above 20° and therefore required referral to an orthopedic surgeon. We found two types of scoliosis, long C-curve type scoliosis (LCS) and one type resembling idiopathic scoliosis (IS). LCS was mainly found in young children, whereas children above 10 years only showed IS-type. Children with LCS had a significantly lower truncal lean body mass to body surface area ratio, compared to those with IS, suggestive of a relationship between truncal hypotonia and LCS. Thus, the

decreasing prevalence of LCS with increasing age, may be associated with an increase in truncal lean body mass. This suggests that the change in predominance is partly caused by regression of LCS-type, resulting from an increase in truncal muscle strength.
Odent et al.[45] assessed the prevalence of scoliosis in a group of 145 children with PWS. That study found an overall prevalence of about 40%, which is in line with our findings. Moreover, they had a larger adolescent population, demonstrating that nearly 70% of adolescents had scoliosis at skeletal maturity.
In our study, nearly 50% of adolescents diagnosed with scoliosis required conventional or surgical intervention. Accadbled et al.[46] reported the outcome of surgical intervention in 16 patients demonstrating major complications in 9 and minor complications in 6 patients. Thus, surgical intervention was uncomplicated in only one patient. Four of the major complications consisted of progressing kyphosis requiring reoperation, complicated with permanent spinal cord injury in three patients. In addition, skin picking and autism-like repetitive upper body movements may further complicate recovery. This underlines the importance of scoliosis surgery being performed by a skilled orthopedic surgeon experienced in spinal surgery in patients with PWS. Comprehensive multidisciplinary care becomes even more important during times of invasive treatment. For example, physicians, parents, and patients should take into account the need for change of diet during a prolonged period of immobilization, such as during recovery of spinal surgery.
In conclusion, the prevalence of scoliosis in children with PWS without GH treatment is 30% under 10 years and 70-80% above 10 years. Young children mainly have LCS, whereas adolescents only have IS. There is a strong association between the types of scoliosis and age and truncal hypotonia. The number of children with PWS with moderate or severe scoliosis is alarmingly high, resulting in many children requiring treatment.

10.2.2 Effects of growth hormone treatment on scoliosis

Accelerated growth, either spontaneous or during GH treatment, has been associated with the onset of scoliosis and scoliotic curve progression.[47-52] Scoliosis was therefore often considered a contraindication for GH treatment. However, beneficial effects of GH treatment, such as increasing lean body mass, may counteract potential adverse effects on spinal growth. It was therefore unknown whether abstinence or withdrawal of GH treatment in children with scoliosis should also be advised for those with PWS and the need for controlled data was emphasized.[34,45,53] In the randomized controlled GH trial we investigated the effects of GH treatment on onset and progression of scoliosis in 91 children with PWS. Our study demonstrated no significant difference between GH-treated children and randomized controls with regard to onset of scoliosis, curve progression, and start of treatment of scoliosis. GH treatment, gain in heightSDS, gain in truncal LBM (used as a proxy for gain in height), and IGF-I SDS were not associated with an increased risk of onset

of scoliosis or curve progression, both before and after correction for confounders.
Some authors have described an association between increased GH levels and a higher rate of curve progression in children without PWS.[50-52] In contrast to these reports, our data show that a higher baseline IGF-I SDS was associated with a lower severity of scoliosis, suggesting a protective effect of higher IGF-I SDS in children with PWS. Because IGF-I SDS was also positively associated with the trunkLBM:BSA ratio, the protective effect may be due to a higher trunkLBM.
In conclusion, GH treatment has no adverse effect on onset of scoliosis and curve progression in children with PWS. Because of the high prevalence of scoliosis and the potential associated morbidities in patients with PWS, it is recommended to perform frequent physical examinations and yearly radiographic examination, independently from GH treatment.

10.3 Bone mineral density and effects of growth hormone treatment in children with Prader-Willi syndrome

10.3.1 Bone mineral density

Although childhood is the critical period for bone accumulation, reports on BMD in children with PWS were very scarce.[54-56] Moreover, corrections for short stature in the analysis of BMD were never performed. We evaluated total body and lumbar spine BMD (BMD_{TB} and BMD_{LS}), and lumbar spine bone mineral apparent density ($BMAD_{LS}$), thereby adjusting for height. Our results show that BMD_{TB}, BMD_{LS}, and $BMAD_{LS}$ are normal in prepubertal children with PWS. $BMAD_{LS}$ SDS was even significantly above zero SDS, indicating that BMD, corrected for short stature, is not decreased in prepubertal children with PWS. The normal $BMAD_{LS}$ SDS is in contrast to results from cross-sectional studies in adults, showing osteoporosis in a high percentage of them.[57-59] These studies, however, did not correct for the short stature of persons with PWS. It is, on the other hand, likely that adults with PWS do have a decreased $BMAD_{LS}$ as the prevalence of osteoporosis in older subjects with PWS is up to 90% and associated complications are frequently reported.[57-59] Presumably, there is a change from a normal $BMAD_{LS}$ SDS in prepubertal children toward a low $BMAD_{LS}$ SDS in adults. We postulate that this decrease results from delayed and abnormal pubertal development due to hypogonadotropic hypogonadism in many patients with PWS, which will lead to a lack of increase in BMD and a lower peak bone mass.
In our study BMI was significantly higher than 0 SDS, although still within the normal range. As BMISDS was significantly associated with all BMD parameters, the high-normal BMISDS could contribute to the normal BMD found in our study. However, only 1 of 13 patients with a BMI below 1 SDS had a reduced $BMAD_{LS}$, indicating that BMD is normal in most prepubertal

children with PWS, independently from BMISDS. We have not measured DHEAS levels in our patients. Other studies have demonstrated high levels of DHEAS in children with PWS[60, 61], which may contribute to the normal BMD demonstrated in our patients.[62]
Thus, BMD is normal in prepubertal children with PWS and the low BMD in adult patients is most likely the result from hypogonadal hypogonadism, preventing the attainment of normal peak bone mass during normal pubertal development.

10.3.2 Effects of growth hormone treatment on bone mineral density

In patients with GH deficiency, BMD is decreased but normalizes during GH treatment.[63,64] However, GH and IGF-I secretion are usually more affected in GH-deficient children than in those with PWS.[65] The effects of GH treatment on BMD in children with PWS were unknown. We therefore investigated the effects of GH treatment on bone mineral density in the randomized controlled GH trial.
After start of GH treatment, BMD_{TB} SDS decreased during the first 6 months, did not change during the second 6 months, and increased thereafter. The decrease during the initial phase of GH treatment is most likely due to a higher rate of bone expansion compared to mineral acquisition and is also found in other study populations.[63,64] In contrast to bones in the total body which consist for 80% of cortical bone, the lumbar spine mainly consists of trabecular bone which has a higher rate of bone turnover.[66] Therefore, the equilibrium between bone expansion and mineral acquisition is earlier restored, and this most likely explains why no significant decrease in BMD_{LS} SDS was found during the initial phase of GH treatment. The fluctuation in BMD_{TB} SDS during the first 6 months of GH treatment was not previously described, as measurements in other studies were only performed annually.[54,55] These studies suggested no effect of GH treatment on BMD_{TB}, which is in line with our results. However, our results also show a significant positive association of BMD_{TB} and BMD_{LS} SDS with IGF-I SDS at 24 months of study, suggesting that long-term GH treatment might increase BMD_{TB} and BMD_{LS} SDS. However, our study showed that $BMAD_{LS}$ did not significantly change during GH treatment and was not significantly associated with IGF-I SDS. Unlike children with GH deficiency, baseline BMD is not decreased in children with PWS. Therefore, it is not unexpected to find no effect of GH treatment on BMD in children with PWS, in contrast to results in patients with GH deficiency showing normalization of BMD.[63,64] As $BMAD_{LS}$ SDS, which is considered the best presentation of BMD in children with short stature,[67] did not significantly change during our study, we conclude that GH treatment has no significant effect on BMD in prepubertal children with PWS.

10.4 Cardiovascular and metabolic risk profile and ASP levels in children with Prader-Willi syndrome and effects of growth hormone treatment

10.4.1 Cardiovascular and metabolic risk profile and effects of growth hormone treatment

There was a paucity of knowledge regarding the cardiovascular and metabolic risk profile in young children with PWS. Also, reports about effects of GH treatment on cardiovascular and metabolic risk factors were scarce.[68,69]

Our study shows that 95% of children in the prepubertal group had a baseline fat% above 2 SDS. Notably, fat mass corrected for age and gender was significantly correlated with systolic and diastolic blood pressure, even in our young group of children with PWS. In 63% of infants and 73% of prepubertal children, at least one of the following factors was present: systolic or diastolic blood pressure >2 SDS; elevated serum total cholesterol, LDLc, triglyceride, or Lp(a) levels; or reduced HDLc levels; indicating an unfavorable cardiovascular profile in addition to the high fat%.

In the prepubertal group, 7% of children were diagnosed with the metabolic syndrome, defined as the presence of at least three of the following components: BMI or systolic or diastolic blood pressure >2 SDS; triglyceride level ≥1.7 mmol/l; HDLc level <1.03 mmol/l; glucose level ≥5.6 mmol/l. Notably, about 40% of infants and prepubertal children demonstrated at least one component of the metabolic syndrome. The elevated glucose and insulin levels were found in children with a high fat%SDS or BMISDS. Systolic and diastolic blood pressure were normal in nearly all children.

GH treatment improved the HDLc to LDLc ratio, which is in line with Carrel et al.[68] However, in contrast to literature,[68,69] our results do not show a beneficial effect of GH treatment on serum lipids. Furthermore, our study demonstrated no effect of GH on systolic and diastolic blood pressure, serum lipids, and glucose homeostasis.

Importantly, our results show that serum lipids did not significantly differ between the infant and prepubertal group, indicating that the unfavorable lipid profiles are most likely present from infancy onwards, although this should be confirmed in studies investigating neonates with PWS.

Although about 40% of children demonstrated at least one component of the metabolic syndrome, nearly all children had normal glucose and insulin levels, despite the severely elevated fat%. Increased adiponectin has been suggested to play a role in preventing insulin resistance, also by our group.[70-72]

In conclusion, our study demonstrates a high fat% and dyslipidemia in many young children with PWS, indicating an unfavorable cardiovascular and metabolic risk profile. GH

treatment significantly reduced fat% and improved the HDLc to LDLc ratio during 24 months of study.

10.4.2 Acylation stimulating protein and effects of growth hormone treatment

ASP stimulates free fatty acid incorporation into adipose tissue by increasing triglyceride synthesis and storage and increases glucose uptake through enhanced translocation of glucose transporters to the plasma membrane surface.[73-79] Furthermore, ASP reduces triglyceride lipolysis in adipocytes by inhibiting hormone-sensitive lipase.[78,79] By controlling the storage of triglycerides, ASP is an important factor in keeping lipid levels within normal range. The high fat% in children with PWS, even in underweight infants, and the role of ASP levels in triglyceride storage prompted us to assess ASP levels in children with PWS and to investigate the effects of GH treatment.

Our study demonstrated high ASP levels in young children with PWS, coinciding with an increased fat mass, but normal triglyceride levels. High ASP levels are suggestive of ASP resistance, also shown in subjects with obesity, insulin resistance, dyslipidemia and cardiovascular disease.[79-84] In subjects with ASP resistance, however, high ASP levels coincide with high triglyceride levels and may show a positive correlation with triglyceride levels. In contrast, most of our children with PWS had normal triglyceride levels, which correlated inversely with ASP, indicating an effective function of ASP in triglyceride storage. Thus, high ASP levels in PWS seem to contribute in keeping triglyceride levels within normal range. Furthermore, ASP increases glucose uptake through enhanced translocation of glucose transporters to the plasma membrane surface.[73-77] We found normal glucose and insulin levels in nearly all children, despite high fat%. High ASP levels without ASP resistance results in increased triglyceride storage as well as in increased glucose uptake. The high ASP levels in PWS may therefore not only contribute to the high fat%, but may also keep glucose and insulin levels within normal range, as has also been suggested for adiponectin.[70-72]

GH treatment does not result in complete normalization of fat mass in children with PWS,[85-89] which suggest that GH insufficiency is not the only factor involved in the mechanism leading to abnormal body composition in PWS. ASP levels were high in most children with PWS and were not significantly different between GH-treated children and randomized controls. Furthermore, ASP levels correlated positively with fat mass and inversely with triglyceride levels. This indicates that high ASP levels may contribute to the sustainment of high fat%, even during GH treatment. Thus, the high ASP levels do not change in response GH treatment and may play a role in preventing complete normalization of fat mass during GH in children with PWS.

In conclusion, ASP levels are high in children with PWS. As ASP stimulates triglyceride synthesis and storage into adipose tissue and does not decline in response to GH, elevated ASP levels may contribute to the high fat% and may possibly play a role in the lack of complete normalization of body composition during GH treatment in children with PWS. As ASP also stimulates glucose uptake, high ASP levels may play a role in keeping glucose and insulin levels within normal range.

10.5 Genetics

Previously reported prevalences of chromosomal abnormalities were 70-75% for deletion, 20-25% for UPD, 2-5% for ICD and less than 1% for chromosomal translocation.[34,90-97] These prevalences were based on studies which included patients diagnosed according to clinical criteria and may therefore be subject to selection bias. To date, recommendations advice to exclude the diagnosis of PWS in any neonate with hypotonia, feeding difficulties, low reflexes, and/or hypoplasia of the external genitalia.[34,98-100] Our large cohort of children with PWS show a much higher prevalence of UPD and therefore a lower prevalence of deletions (both about 45%). The higher prevalence of UPD may be due to a trend of increased maternal age at time of conception and enhanced diagnostic methods.[101-103] In earlier years, the use of clinical criteria for diagnosing PWS may have led to a lower prevalence of UPD, as this diagnosis is more easily missed due to the more discrete phenotypic appearance.[34,91] We found a slightly higher prevalence of ICD (9%), but the prevalence of chromosomal translocation (1%) was similar to previous reports.[91,96,97] The changed prevalence of genetic subtypes has also been demonstrated in the United Kingdom.[104] Thus, the current prevalences of genetic subtypes are 45% for deletion, 45% for UPD, 9% for ICD and 1% for Robertsonian translocation.

10.6 Efficacy and safety of long-term growth hormone treatment in children with Prader-Willi syndrome

Data of the randomized controlled GH trial demonstrated that body composition, height, and anthropometric outcomes improved in children with short-term GH treatment, compared to randomized controls. This is in line with other studies demonstrating beneficial effects during 1 or 2 years of treatment.[54,68,88,105-113] Although short-term GH has been proven beneficial, reports on effects of long-term GH treatment in children with PWS were scarce.[89,114-116] We therefore performed a 4-year multicenter prospective follow-up study (the Dutch Cohort Study) to evaluate efficacy and safety of long-term continuous GH treatment in a large

group of children with PWS, with onset of treatment before puberty. The various outcomes of this study are described below.

10.6.1 Body composition

Our randomized controlled study showed a significant beneficial effect of GH treatment on fat%, compared to controls. The Dutch Cohort Study demonstrated that fat%SDS was still significantly lower after 4 years of GH treatment than at baseline. LBMSDS significantly decreased during the first year of GH treatment, but returned to baseline values during the second year and did not significantly change during the two years thereafter. In contrast, the results from our randomized controlled study showed that LBMSDS continuously decreased in untreated children with PWS. We therefore conclude that 4 years of continuous GH treatment with a standard dose (1 mg/m^2·day) has a significant favorable effect on body composition, with a significant reduction of fat%SDS and a stabilization of LBMSDS.

Figures 2 and 3 depict the effects of GH treatment on body composition and height in two patients with PWS. The remarkable changes during GH treatment, as well as the striking difference between the untreated 3-year old boy in Figure 2 (left) and the GH-treated boy of the same age in Figure 3 (right), endorse the view that GH treatment should be initiated at an early age in children with PWS. This is also supported by studies showing significant beneficial effects on psychomotor development in young children with PWS.[54,110,117]

Although body composition markedly improved during treatment, long-term GH did not result in a complete normalization. This has previously been shown by Carrel et al.,[89] but children participating in that study were not continuously treated with a standard dose. The fact that long-term GH treatment did not completely normalize body composition suggests that the unfavorable fat% and LBM are not only the result of GH insufficiency. Other factors, such as genetic programming and elevated ASP levels, most likely contribute to the abnormal body composition, as well.

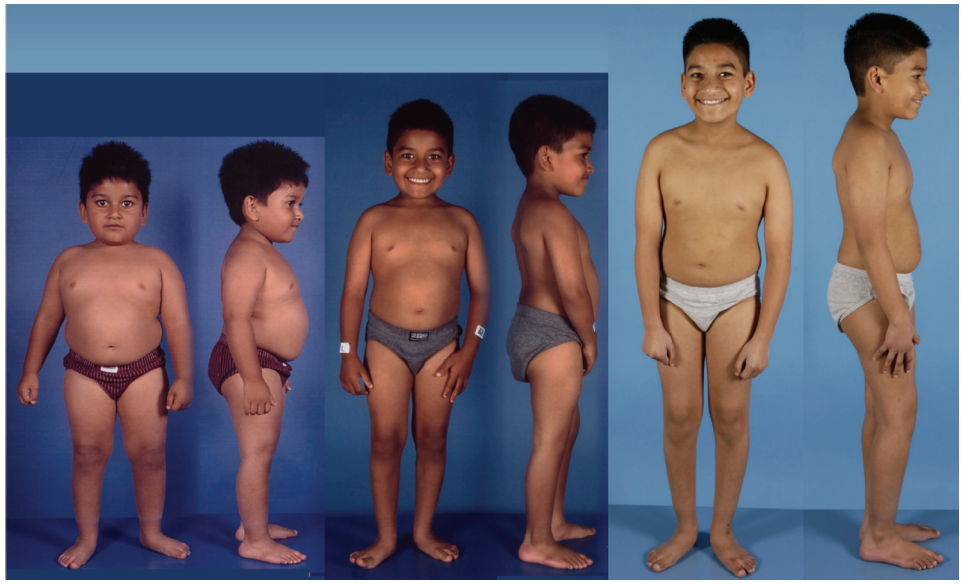

Figure 2. Changes during growth hormone treatment in a boy with PWS.
Left, without GH treatment (3 years of age); middle, after 1 year of GH treatment (5 years of age); right, after 5.4 years of GH treatment (age 9.4 years). Photos are depicted with permission of parents and the child.

Figure 3. Changes during growth hormone treatment in a boy with PWS.
Left, without GH treatment (6 weeks of age); middle, without GH treatment (10 months of age); right, after 1.6 years of GH treatment (age 3.2 years). Photos are depicted with permission of parents and the child.

10.6.2 Height and bone maturation

Our randomized controlled trial showed that GH treatment has a significant beneficial effect on heightSDS, which is in line with previous studies.[54,86,88,105-113] Our data from the Dutch Cohort study show that the beneficial effect of GH on height persists during long-term treatment. In our study, heightSDS had completely normalized after 4 years of GH treatment, indicating that a normal heightSDS can be attained when GH treatment is started several years before onset of puberty (Figure 4). This is in line with two previous reports in a small group of patients with PWS, suggesting that a normal adult height may be obtained in patients with PWS if GH treatment is initiated before puberty.[114,116]

Bone maturation was not significantly affected by GH treatment. There was a shift from a delayed bone maturation in children below 5 years of age toward a normal bone maturation in those above 7 years. This catch-up in bone maturation might be related to adrenarche, typically occurring early in many children with PWS.[61,118] As bone age did not accelerate in response to GH treatment, our data suggest that a normal adult height can be reached when long-term GH treatment is started several years before puberty.

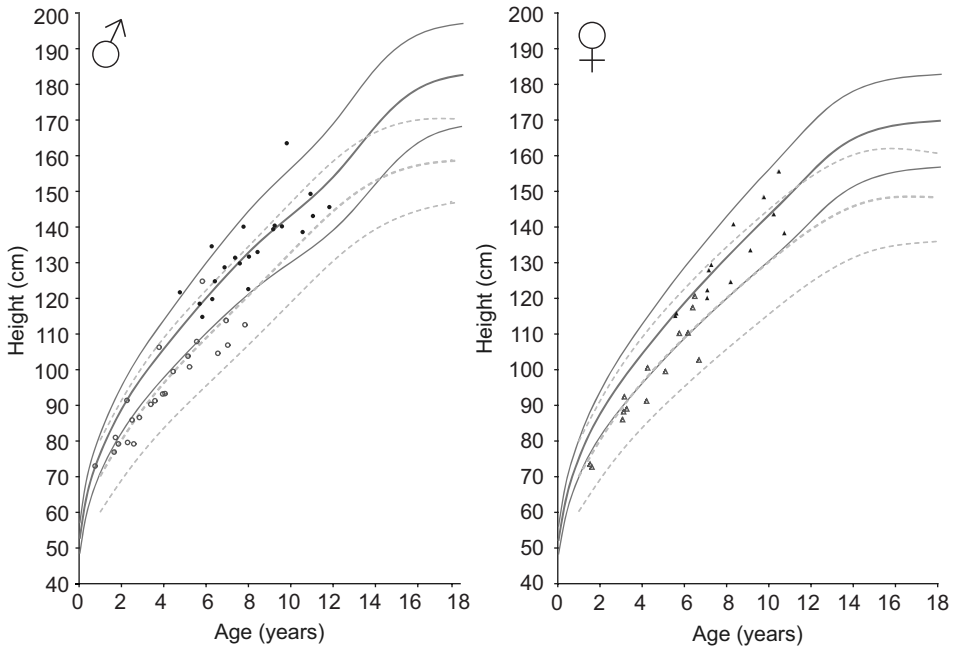

Figure 4. Growth charts depicting the change in heightSDS in boys and girls after 4 years of GH treatment. Lines represent -2, 0, and 2 SDS for Dutch and PWS (dotted) reference values. Open and closed symbols represent values at start and after 4 years of GH treatment, respectively. Height in boys is depicted in circles, whereas height in girls is depicted in triangles.

10.6.3 Head circumference

Head circumference SDS improved significantly during short-term GH treatment. This effect persisted during long-term GH treatment. In the general population, a lower head circumference SDS is associated with lower intelligence, whereas brain growth during infancy and early childhood is important in determining cognitive function.[119-121] Improvement of mental development and psychosocial functioning during short-term GH treatment in patients with PWS has previously been described.[105,122] Our group reported a median (iqr) increase in mental development quotient of 9.3% (-5.3 to 13.3) in infants after 12 months of GH treatment versus -2.9% (-8.1 to 4.9) in controls ($p<0.05$).[122] There are no studies available regarding long-term effects of GH on psychomotor development, intelligence, or psychosocial functioning, but we hypothesize that GH may have a favorable effect on these outcomes, resulting from sustained improvement of head circumference SDS as demonstrated by our study.

10.6.4 Hands, feet, tibiae, and arm span

Long-term GH treatment improved, but did not completely normalize SDS of the hand and foot length and arm span. Tibia length only normalized in the prepubertal group, thus when GH treatment was started at an early age. The main catch-up growth of hands and feet occurred during the first year with little change thereafter. Catch-up in tibia length SDS and arm span SDS continued in the second year of treatment and reached a plateau thereafter. It has previously been suggested that when these outcomes normalize during GH treatment, this would indicate that these features are the result of GH deficiency rather than due to the genetic defect.[116] Our findings suggest that both GH deficiency and the genetic defect might explain the shorter hands, feet, tibiae, and arm span in children with PWS.

10.6.5 IGF-I and IGFBP-3 levels, glucose homeostasis, blood pressure, and serum lipids

In the Dutch Cohort Study, mean IGF-I levels rapidly increased during GH treatment, whereas IGFBP-3 levels increased more gradually. IGFBP-3 is the major carrier protein of IGF-I and binds 70-95% of IGF-I as a binary complex or as a ternary complex together with the acid labile subunit (ALS). Only a minor fraction of IGF-I circulates in its unbound form, which is considered the biologically active form.[123] We found an increase in the IGF-I/IGFBP-3 ratio during GH treatment, suggesting an increase in free IGF-I levels. Carrel et al.[89] reported that metabolic effects of GH treatment in children with PWS are optimal with a dose of at least 1 mg/m^2·day, whereas a dose of 0.3 mg/m^2·day had no effect on LBM and fat%. Our results show that the improvement of body composition and head circumference and normalization of heightSDS is sustained with IGF-I levels between 2 and 3 SDS, without adverse effects during 4 years of GH treatment. Thus, it is reassuring that the beneficial effects of high IGF-I

levels outweigh the potential unfavorable effects during at least 4 years of study. However, research of longer-term effects of high IGF-I levels is required for definite conclusions.

Notably, there were a few children with exceptional increases of heightSDS and IGF-I SDS during GH treatment. One may consider individualizing the GH dosage in order to attain IGF-I levels between 2 and 3 SDS. However, the limited data on reduction of GH dosage or discontinuation of treatment also suggest a worsening of body composition.[89] Thus, GH dosage may be individualized, but caution should be taken as lower IGF-I levels might result in a less favorable body composition with higher fat%.[89]

One major concern with high IGF-I levels is insulin resistance. Our study showed an increase in insulin resistance with age, but demonstrated that GH treatment had no effect on fasting glucose and insulin levels, HOMA-IR, and HbA1c levels in our group of 55 children, when adjusted for age and gender. This is in line with Lindgren et al. describing normal glucose homeostasis during five years of GH treatment in 16 children without rapid weight gain.[114] We had no patients with non-insulin dependent diabetes mellitus. The outcomes of our study suggest that the high prevalence of non-insulin dependent diabetes mellitus in adult patients with PWS[40] is most likely due to increased fat mass. Our data show that a significant positive association of fat mass with both fasting insulin levels and HOMA-IR is already present in childhood. These results underline the importance of achieving normal BMI and reducing fat mass as part of the primary aims in the comprehensive care of patients with PWS. In this respect, it is interesting to note that BMISDS according to PWS reference values significantly decreased during GH treatment. Thus, GH treatment might have favorable effects on insulin resistance as it decreases fat mass and slows down weight gain over the years. In addition, our research group found high adiponectin levels in prepubertal children with PWS, which further increased during GH treatment. This is reassuring with regard to the development of insulin resistance.

Blood pressure SDS did not significantly differ between GH-treated children and randomized controls. Furthermore, blood pressure SDS did not significantly change during long-term GH treatment. GH treatment had no significant effect on total cholesterol and HDL cholesterol, but LDL cholesterol was significantly lower after 4 years of GH treatment. We therefore conclude that GH treatment had no adverse effect on serum lipids.

In conclusion, GH treatment with 1.0 mg/m^2·day results in IGF-I levels between 2 and 3 SDS, leading to sustained improvement of body composition, head circumference, and normalization of heightSDS without adverse effects on glucose homeostasis, blood pressure, and serum lipids during 4 years of study.

10.7 Main conclusions of the thesis and clinical implications

10.7.1 Central adrenal insufficiency and sleep-related breathing disorders

There is a high prevalence of central adrenal insufficiency during stress, which may contribute to the high mortality rate in patients with PWS. Based on our findings, a new guideline was made advicing treatment with a hydrocortison stress dose during illness or surgery, unless CAI has recently been ruled out with a metyrapone test.

Children with CAI during stress have a significantly higher central apnea index during healthy condition and a higher increase in central apnea index in response to metyrapone-induced stress. The combination of CAI and compromised sleep-related breathing may aggravate the deterioration of the condition of an acutely ill patient with PWS. This combination might well be the prelude of a fatal cascade during illness. A new hypothesis regarding the pathway leading to sudden death is presented.

10.7.2 Scoliosis and effects of growth hormone treatment

The prevalence of scoliosis in children with PWS without GH treatment is 30% under 10 years and 70-80% above 10 years. Two types of scoliosis are distinguished: long C-curve type scoliosis (LCS) and scoliosis resembling idiopathic scoliosis (IS). There is a strong association between the types of scoliosis and age and truncal hypotonia. The number of children with PWS with moderate or severe scoliosis is alarmingly high, resulting in many children requiring treatment. Surgical intervention for scoliosis should be performed by a skilled orthopedic surgeon experienced in spinal surgery in patients with PWS and familiar with the clinical manifestations of the syndrome affecting post-operative recovery.

GH has no adverse effects on onset and progression of scoliosis in children with PWS. Scoliosis should therefore no longer be considered a contraindication for GH treatment in these children. Because of the high prevalence of scoliosis and the potential associated morbidities in patients with PWS, it is recommended to perform frequent physical examinations and yearly radiographic examination, independently from GH treatment.

10.7.3 Bone mineral density and effects of growth hormone treatment

Bone mineral density is normal in prepubertal children with PWS. The transition of normal bone mineral density in prepubertal children toward low bone mineral density in adults may be related to hypogonadal hypogonadism, preventing the attainment of normal peak bone mass during normal pubertal development. GH treatment had no effect on BMD, except for a temporary decrease of total body BMDSDS during the first 6 months of treatment.

10.7.4 Cardiovascular and metabolic risk profile, ASP levels, and effects of GH treatment

The majority of prepubertal children with PWS have a high fat% and abnormal serum lipids, indicating an unfavorable cardiovascular and metabolic profile. Systolic and diastolic blood pressure were normal in most children, as well as insulin and glucose levels. ASP stimulates glucose uptake and triglyceride synthesis and storage into adipose tissue. ASP levels are elevated in prepubertal children with PWS, possibly contributing to the high fat%. The high ASP levels may also keep glucose and insulin levels within normal range.

GH improved fat% and the HDLc to LDLc ratio, but had no effect on blood pressure, serum lipids, and glucose homeostasis. The high ASP levels did not change in response to GH and may therefore contribute to the lack of normalization of fat% during GH treatment in patients with PWS.

10.7.5 Safety and efficacy of long-term GH treatment

Four years of GH treatment with a dose of 1 mg/m^2·day results in a sustained improvement of body composition and head circumference and a normalization of heightSDS, when started several years before puberty. Long-term GH treatment reduced fat% and stabilized LBMSDS, but body composition did not completely normalize. GH increased the SDS of hand, foot, and tibia length, as well as arm span.

IGF-I levels rapidly increased during GH treatment, whereas IGFBP-3 increased more gradually. IGF-I levels between 2 and 3 SDS were attained, without adverse effects on glucose homeostasis, blood pressure, and serum lipids during 4 years of study.

10.8 Recommendations for future research

Based on our research, new insights and recommendations have been provided. But in concordance with this growing knowledge, new questions and dilemmas appeared, revealing possible directions for future research.

We have demonstrated a high prevalence of central adrenal insufficiency in children with PWS, apparent only during stressful conditions, and found an association between CAI and sleep-related breathing. Future research should focus on confirming the high prevalence of CAI, in children as well as in adults. In addition, it would be interesting to investigate if CAI is caused by pituitary or, more likely, hypothalamic insufficiency. Also, the possibility of using other screening methods, such as ACTH tests, should be explored. Based on our findings, it would be interesting to investigate whether correct implementation of hydrocortisone treatment during illness reduces the annual mortality rate. Also, more research needs to be

performed studying the relationship between central adrenal insufficiency and sleep-related breathing in larger numbers of patients.

We assessed prevalences of scoliosis in children with PWS and evaluated the effects of GH treatment on onset and progression of scoliosis. Future research should focus on optimizing scoliosis treatment regimens, divided by type of scoliosis. For non-severely progressing LCS type scoliosis, one may focus on increasing truncal lean body mass rather than applying orthopedic treatments. IS type scoliosis probably warrant earlier treatment, but the complication rate of spinal surgery in patients with PWS is high. In this respect, the option of upper body immobilization during post-operative recovery should be explored in order to prevent skin-picking and instrument-related complications and to minimize the need for reoperation.

Our study demonstrated normal bone mineral density in prepubertal children with PWS, which was not affected by GH treatment. We hypothesized that the decreased BMD in adult patients with PWS is the result of hypogonadotropic hypogonadism. Future research should aim to investigate this hypothesis.

The elevated ASP levels may be confirmed, not only in children, but also in adults. Perhaps future research could render a means to decrease ASP levels, thereby possibly providing new treatment options for the obesity of patients with PWS. Furthermore, we found a high prevalence of cardiovascular and metabolic risk factors in children with PWS. Future research may explore the efficacy of treatment of these risk factors at an early age.

We concluded that the improvement of body composition and head circumference and normalization of heightSDS is sustained with IGF-I levels between 2 and 3 SDS, without adverse effects during 4 years of GH treatment. Future research may focus on individualizing the GH dose and assessing the efficacy and particularly the safety of high IGF-I levels during a prolonged follow-up period. Also, long-term effects of GH on psychosocial functioning and intelligence warrant investigation. Thus, it is important to carefully follow GH-treated children with PWS until adult height.

Currently, GH treatment is discontinued when adult height is attained. Studies investigating the effects of GH treatment during several years after attainment of adult height and during adulthood are warranted.

Children with PWS present as a heterogeneous group of patients, in part due to the genotype-phenotype correlations. Our study demonstrated new prevalences of the different chromosomal defects causing PWS. More research investigating the genotype-phenotype correlations is warranted and could possibly provide distinct means of treatment and care.

Children with PWS require comprehensive multidisciplinary care. GH treatment alone is not enough to relieve symptoms of PWS, but should be combined with a strict diet and physical therapy and/or training. The effectiveness of different physical therapeutic programs should be investigated. We have conducted research in a group of mainly young children

with PWS, belonging to a new generation with an early diagnosis allowing early treatment. From clinical practice, it is evident that this new generation thrives much better, through the early implementation of the combined efforts of physicians, allied health professionals, parents, and patients. These children need to be carefully followed in order to optimize treatment abilities specific to this new patient population, with the ultimate goal of promoting health and increasing quality of life.

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Appendix A. Example of a cortisone treatment regimen for patients with PWS during stress.

Name: Physician: Date:	**This is an example which should always be filled in and approved by a physician.**

Severity of stress	Your son/daughter is able to take medication	Your son/daughter is not able to take medication
Mild stress Not well Listless *Temperature < 38 °C*	Do not administer medication	
Moderate stress Flu Infection Vaccination Anesthesia (also dental) Important exam *Vomiting, diarrhea* *Temperature 38-39 °C*	**Method A: Moderate dose of hydrocortisone** 3-4 times daily 10 mg/m^2·day (daily 30-40 mg/m^2) Duration of administration should be as short as possible. Sometimes only 1 dose may be sufficient. If illness resolves within 1-2 days, medication can be discontinued. If illness does not resolve within 1-2 days: consult the pediatrician to discuss the need for examination and the method and the time of discontinuation of treatment (i.e. whether a withdrawal regimen should be applied).	Method D and E should be applied for emergency care when your child vomits or is not able to take medication. Medication in the form of tablets should be continued when possible. **Method D: Administer suppository of hydrocortison for emergency care** < 2 years: 1 suppository of hydrocortisone 50 mg 2-10 years: 1 suppository of hydrocortisone 100 mg > 10 years: 2 suppositories of hydrocortisone 100 mg When vomiting resolves thereafter, switch directly to tablets (method B). When vomiting does not resolve within 1 hour, contact the pediatrician or pediatric endocrinologist and method E should be applied (by a physician or nurse). **OR** **Method E: Administration of injection of dexamethasone (oradexone, ampoule 5 mg/ml) by physician or nurse** < 6 years: one time ½ ampoule of oradexone (2.5 mg), intramuscular ≥ 6 years: one time 1 ampoule of oradexone (2.5 mg), intramuscular If improvement within 1.5 hours: switch to tablets (method B). If no improvement within 1.5 hours: again consult the pediatrician or pediatric endocrinologist.
Severe stress Severely ill Surgery, anesthesia Emergency *Temperature > 39 °C*	**Method B: High dose of hydrocortison** 3-4 times daily 20-25 mg/m2·day (daily 60-75 mg/m^2) **OR** **Method C: Tablet of oradexone instead of normal medication** < 10 years: 2 times daily ½ tablet of oradexone 0,5 mg ≥ 10 years: 2 times daily 1 tablet of oradexone 0,5 mg Consult the pediatrician to discuss the need for examination and the method and the time of discontinuation (i.e. whether a withdrawal regimen should be applied)	

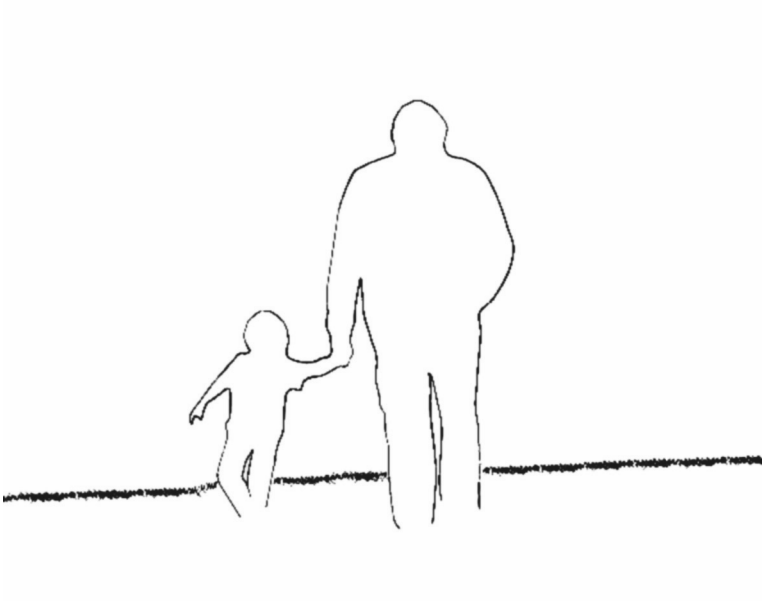
Time	**Morning**	**Afternoon**	**Evening**	**Night**
Method A	mg	mg	mg	mg
Method B	mg	mg	mg	mg
Method C	mg	mg	mg	mg
Method D	Suppository hydrocortisone … 50 mg /100 mg *(encircle)*			
Method E	Dexamethasone (orexone 5 mg) ½ ampoule / whole ampoule *(encircle)* intramuscular			

Important phone numbers:

Important: Patients with PWS often do not develop fever and do not vomit, despite the presence of illness.

Chapter 11

Summary



This doctoral dissertation provides a detailed account of the various studies performed to improve the knowledge of PWS and care for patients with PWS. Studies were embedded in the Dutch national randomized controlled GH trial. General aspects of children with PWS were studied, such as the prevalence of central adrenal insufficiency, sleep-related breathing disorders, scoliosis, bone mineral density, and cardiovascular risk factors including ASP levels. Furthermore, we evaluated the efficacy and safety of short- and long-term growth hormone treatment by investigating the effects of GH on body composition, height, head circumference and other anthropometric outcomes, bone maturation, scoliosis, bone mineral density, blood pressure, glucose homeostasis, and serum lipids. This chapter summarizes these studies and their most important outcomes.

Chapter 2

High prevalence of central adrenal insufficiency in patients with Prader-Willi syndrome

The annual death rate in patients with PWS is very high (3% under 30 years). The sudden deaths in children often share a similar pattern: a sudden deterioration during a mild or moderate infection, which is often – but not always – an upper respiratory tract infection. Furthermore, a reduced adrenal size has been found during autopsies in children. These findings, coinciding with the presence of hypothalamic dysregulation in PWS, prompted us to investigate the prevalence of central adrenal insufficiency during stress. We admitted 25 randomly selected patients, with a median (iqr) age of 9.7 years (6.8-13.6), in a Pediatric Intensive Care Unit to undergo a metyrapone test. At 2330 h, metyrapone was administered. At 0400, 0600, and 0730 h, ACTH, 11-deoxycortisol, cortisol, and glucose levels were measured. The cut-off level for an adequate ACTH response is >33 pmol/l at 0730 h. Diurnal salivary cortisol profiles were assessed on a different day at wake-up, 30 min after wake-up, at 1400 h, and at 2000 h. All patients received GH treatment and were naïve to glucocorticoid therapy. All tests were performed during healthy condition.

Strikingly, 15 patients (60%) had CAI, defined as ACTH levels failing to increase sufficiently during a metyrapone test. The difference in ACTH response between children later diagnosed with CAI and those not was already significant at 0400 h and 0600 h. Age, gender, genotype, and BMISDS did not significantly differ between patients with and without CAI. Morning salivary cortisol levels and diurnal profiles were normal in all children, suggesting that CAI becomes apparent only during stressful conditions. The high percentage of CAI in PWS patients might explain the high rate of sudden death in these patients, particularly during infection-related stress. Based on our data, one should consider treatment with hydrocortisone during acute illness in PWS patients unless CAI has recently been ruled out with a metyrapone test.

Chapter 3

The relationship between central adrenal insufficiency and sleep-related breathing disorders in children with Prader-Willi syndrome

Next to the high prevalence of CAI in children with PWS (Chapter 2), sleep apneas have also been suggested to play a role in sudden deaths. We therefore investigated the relationship between CAI and sleep-related breathing disorders. In 20 children who underwent a metyrapone test, sleep-related breathing was evaluated by polysomnography prior to the metyrapone test, as part of the protocol of the randomized controlled GH trial. In addition, we could record sleep-related breathing in 10 children with PWS during their metyrapone test. The median (iqr) age was 8.4 years (6.5-10.2) at time of the metyrapone test. After metyrapone administration, the median (iqr) central apnea index (number/hour) increased significantly, from 2.2 (0.4-4.7) to 5.2 (1.5-7.9). The increase tended to be higher in children with CAI [2.8 (2.0-3.9) vs. 1.0 (-0.2 to 2.6), p=0.09]. During polysomnography prior to the metyrapone test, sleep-related breathing was worse in children with CAI, as they had a significantly higher central apnea index and tended to have a lower minimum oxygen saturation compared to those without CAI (p=0.03 and p=0.07).

In conclusion, our study showed that the central apnea index increased significantly after metyrapone administration, particularly in children diagnosed with CAI during stress. In addition, children with CAI had a higher central apnea index compared to those without, several months prior to the metyrapone test. Based on our findings described in Chapter 3, we launched a new hypothesis on the most common mechanism of sudden death in children with PWS in the General Discussion in Chapter 10.

Chapter 4

Scoliosis in Prader-Willi syndrome: Effects of age, gender, body mass index, lean body mass, and genotype

The reported prevalence of scoliosis varied greatly. We therefore assessed the prevalence of scoliosis in a group of 96 children with PWS in a cross-sectional study design. None of the children had been treated with growth hormone. Radiographs were taken, length and weight were measured, BMI standard deviation scores and body surface areas were calculated, and dual energy x-ray absorptiometry (DXA) was performed, measuring lean body mass and truncal lean body mass. The median (iqr) age was 4.8 (2.1-7.5) years. The prevalence of scoliosis was 30% in children under 10 years to 80% in children above 10 years. Fifty percent of children had a Cobb's angle above 20°, requiring referral to an orthopedic surgeon, whereas 13% had undergone treatment for scoliosis (brace or surgery). Two types of scoliosis were identified: 1) Long C-curve type scoliosis, mainly occurring in young children with a low truncal lean body mass to body surface area ratio; and 2)

Scoliosis resembling idiopathic scoliosis (IS), mainly occurring in older children with less hypotonia of the truncal musculature.
Based on our findings, we recommend frequent physical examination for signs of scoliosis and yearly radiographic evaluation of the spine in children with PWS.

Chapter 5

Randomized controlled trial to investigate the effects of growth hormone treatment on scoliosis in children with Prader-Willi syndrome

We have earlier demonstrated a high prevalence of scoliosis in children with PWS, ranging from 30-80%, depending on age. Based on uncontrolled data from the general population, indicating a possible effect of accelerated growth on spinal deformity, scoliosis was often considered a contraindication for GH treatment in children with PWS. However, there were no reports on the effects of GH treatment on scoliosis in children with PWS. We therefore conducted a randomized controlled trial, investigating the effects of GH treatment on onset of scoliosis and curve progression. Thirty-eight infants and 44 prepubertal children were randomized into a GH-treated group (1 mg/m^2·day) or a control group, for one and two years, respectively. Pubertal children were randomized to receive somatropin 1 or 1.5 mg/m^2·day. Annually, x-rays of the spine were taken, and height, weight, truncal lean body mass (with dual energy x-ray absorptiometry), and IGF-I levels were measured. GH-treated children had similar onset of scoliosis and curve progression as randomized controls (p=0.27-0.79 and p=0.18-0.98, respectively). GH treatment, IGF-I SDS, and catch-up growth had no adverse effect on the onset of scoliosis or curve progression, even after adjustment for confounders. HeightSDS, truncal lean body mass, and IGF-I SDS were significantly higher in GH-treated children than in randomized controls. At baseline, a higher IGF-I SDS was associated with a lower severity of scoliosis. Thus, we conclude that scoliosis should no longer be considered a contraindication for GH treatment in children with PWS.

Chapter 6

Randomized controlled GH trial: Effects on anthropometry, body composition, and body proportions in a large group of children with Prader-Willi syndrome

Children with PWS have impaired growth and an abnormal body composition, with high fat% and low lean body mass. Previous one-year controlled studies demonstrated that GH treatment resulted in improved growth and body composition in PWS. These studies, however, described relatively small patient groups of mostly overweight PWS patients. We performed a randomized controlled GH trial in 91 pre-pubertal children with PWS (42

infants, 49 prepubertal children aged 3-14 years), evaluating growth, body composition, and anthropometric outcomes. Infants and prepubertal children were randomly allocated to a GH-treated group (1 mg/m^2·day) or a control group for 1 and 2 years, respectively. Annually, anthropometric assessments were performed and body composition was measured by dual energy x-ray absorptiometry (DXA). Median (iqr) heightSDS increased during GH treatment in infants from -2.3 (-2.8 to -0.7) to -0.4 (-1.1 to 0.0) and in prepubertal children from -2.0 (-3.1 to -1.7) to -0.6 (-1.1 to -0.1). In non-GH-treated children height SDS did not increase. Head circumference improved in response to GH. Body fat percentage and anthropometric outcomes improved in GH-treated children, but did not completely normalize. Lean body mass SDS stabilized in contrast to a persistent decrease in controls.
In conclusion, GH in children with PWS improves height, body composition, head circumference and anthropometric outcomes. Children with PWS are highly sensitive to GH, indicating that monitoring of serum IGF-I levels during GH treatment is indicated.

Chapter 7

Randomized controlled trial investigating bone mineral density and effects of growth hormone treatment in prepubertal children with Prader-Willi syndrome

Bone mineral density is decreased in adults with PWS. Although childhood is the critical period for bone accumulation, reports on BMD in children with PWS are very limited. Patients with PWS have many symptoms resembling GH deficiency. In children with GH deficiency, BMD is decreased and normalizes during GH treatment. We conducted a national multicenter randomized controlled trial evaluating BMD and $BMAD_{LS}$, which corrects for short stature, and investigating the effects GH treatment. Children were randomly assigned to a GH-treated group (1 mg/m^2·day) or a control group for the duration of 2 years. We included 46 prepubertal children with PWS, with a mean ± SD age of 7.1 ± 2.2 years. At start, 6, 12, and 24 months of study, total body and lumbar spine BMD (BMD_{TB} and BMD_{LS}) were measured by dual energy x-ray absorptiometry (DXA) and bone mineral apparent density ($BMAD_{LS}$) was calculated. Baseline BMD_{TB}SDS, BMD_{LS}SDS, and $BMAD_{LS}$SDS were all normal (mean ± SD: -0.2 ± 1.1 SDS; -0.4 ± 1.2 SDS; and 0.40 ± 1.1 SDS, respectively). After start of GH treatment, BMD_{TB} SDS decreased during the first six months, did not change during the second six months, and increased thereafter. The decrease during the initial phase of GH treatment is most likely due to a higher rate of bone expansion compared to mineral acquisition and is also found in other study populations. In contrast to bones in the total body which consist for 80% of cortical bone, the lumbar spine mainly consists of trabecular bone which has a higher rate of bone turnover. Therefore, the equilibrium between bone expansion and mineral acquisition is earlier restored, and this most likely explains why no

significant decrease in BMD_{LS} SDS was found during the initial phase of GH treatment. After 24 months of study, BMD_{TB}SDS, BMD_{LS}SDS, and $BMAD_{LS}$SDS did not significantly differ between GH-treated children and randomized controls (p=0.30, p=0.44, and p=0.47, respectively). Repeated measurements analysis showed a significant positive association between IGF-I SDS and BMD_{TB}SDS and BMD_{LS}SDS, but not with $BMAD_{LS}$.

In conclusion, our results show that prepubertal children with PWS have a normal BMD. GH treatment had no effect on BMD, except for a temporary decrease of BMD_{TB}SDS in the first 6 months.

Chapter 8

Cardiovascular and metabolic risk profile and acylation stimulating protein levels in children with Prader-Willi syndrome and effects of growth hormone treatment

Children with PWS have an unfavourable body composition with a high fat%. However, reports on the cardiovascular and metabolic risk profile in children with PWS and the effects of GH treatment were scarce. Acylation stimulating protein (ASP) stimulates glucose uptake and triglyceride storage in adipose tissue. Acylation stimulating protein (ASP) stimulates glucose uptake and triglyceride storage in adipose tissue and is an important marker for fat accumulation and dyslipidemia. However, ASP levels of children with PWS have never been reported.

We conducted a randomized controlled trial in 85 young children with PWS to assess the baseline cardiovascular and metabolic risk profile and ASP levels and to investigate the effects of GH treatment. Thirty-five infants and 50 prepubertal children were randomly allocated to either a GH-treated group (1 $mg/m^2 \cdot day$) or a control group, for one and two years, respectively. The mean ± SD age was 4.9 ± 3.0 years. The mean ± SD fat% was 28.4 ± 6.2 in infants and 36.9 ± 8.5 in prepubertal children. Fat%SDS was elevated in 95% of prepubertal children. In addition to high fat%, 63% of infants and 73% of prepubertal children had dyslipidemia, indicating an unfavourable cardiovascular profile. Systolic and diastolic blood pressure were normal in nearly all children. Forty percent of infants and prepubertal children demonstrated at least one component of the metabolic syndrome, whereas 5% demonstrated at least three and could therefore be diagnosed with the metabolic syndrome. Glucose and insulin levels were normal in nearly all children. The mean ± SD baseline ASP level was 107 ± 45 nmol/l (normal<58 nmol/l) and correlated positively with fat mass and inversely with serum triglyceride levels. GH improved fat%SDS and the HDLc to LDLc ratio (p<0.0001 and p=0.04). GH had no significant effect on ASP levels.

In conclusion, the majority of young children with PWS have a high fat% and dyslipidemia, indicating an unfavorable cardiovascular and metabolic profile. Systolic and diastolic blood

pressure were normal in most children, as well as insulin and glucose levels. As ASP stimulates glucose uptake and triglyceride synthesis and storage into adipose tissue, the high ASP levels in children with PWS may contribute to the high fat% and may also keep glucose and insulin levels within normal range. GH improved fat% and the HDLc to LDLc ratio, but had no effect on blood pressure, glucose homeostasis, and serum lipids. The high ASP levels did not change in response to GH and may therefore contribute to the lack of normalization of fat% during GH treatment in patients with PWS.

Chapter 9

Efficacy and safety of long-term continuous growth hormone treatment in children with Prader-Willi syndrome

Patients with PWS have an abnormal body composition with increased fat mass and decreased lean body mass, and short stature. Some studies showed normalization of adult height when GH treatment was started before onset of puberty. Although improvement of body composition is considered the most important effect of GH in children with PWS, there are no reports regarding effects of long-term GH treatment on body composition in a large group of patients. We investigated long-term efficacy and safety of GH treatment on body composition, growth, bone maturation, and safety parameters, in 55 children with PWS included in a 4-year national multicenter prospective follow-up study. The mean ± SD age at start of study was 5.9 ± 3.2 years. All children received somatropin 1 mg/m^2·day. The following data were annually obtained in one center: fat% and lean body mass (LBM) by dual-energy x-ray absorptiometry, height, weight, head circumference, bone age, blood pressure, and fasting IGF-I, IGFBP-3, glucose, insulin, HbA1c, total cholesterol, HDL, and LDL. SD-scores were calculated according to Dutch and PWS reference values (SDS and SDS_{PWS}).

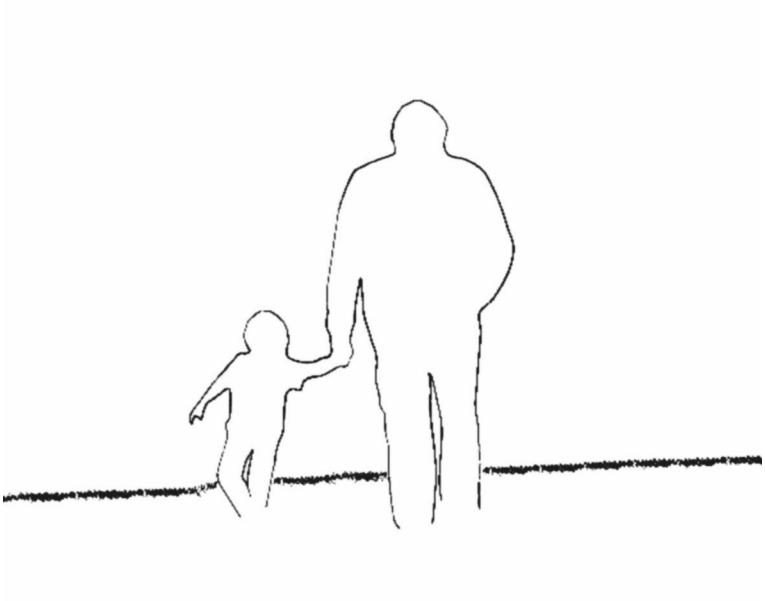
Fat%SDS was significantly lower after 4 years of GH treatment ($p<0.0001$). LBMSDS significantly increased during the first year ($p=0.02$), but returned to baseline values the second year and remained unchanged thereafter. Thus, LBM stabilized during long-term continuous GH treatment, which is in contrast to the persistent decrease of LBMSDS commonly observed in untreated children with PWS. Mean ± SD height normalized from –2.27 ± 1.2 SDS to –0.24 ± 1.2 SDS ($p<0.0001$). Head circumference SDS increased from –0.79 ± 1.0 at start to 0.07 ± 1.1 SDS after 4 years. $BMISDS_{PWS}$ significantly decreased. Mean ± SD IGF-I and the IGF-I/IGFBP-3 ratio significantly increased to 2.08 ± 1.1 and 2.32 ± 0.9 SDS, respectively. GH treatment had no adverse effects on bone maturation, blood pressure, glucose homeostasis, and serum lipids.

Our study shows that 4 years of continuous GH treatment with a standard dose (1 mg/m^2·day) had a significant favorable effect on body composition, heightSDS, $BMISDS_{PWS}$, and

head circumference SDS, without adverse effects on blood pressure, glucose homeostasis, and serum lipids. Importantly, the favorable effect on body composition persisted during the 4 years of study. Based on our findings, it is recommended to keep IGF-I levels between 2 and 3 SDS for optimal effects in children with PWS, without adverse effects. In conclusion, long-term continuous GH treatment is an effective and safe therapy for children with PWS.

Chapter 12

Samenvatting



Dit proefschrift beschrijft verschillende studies die zijn verricht om de kennis van PWS te vergroten en de zorg voor patiënten met PWS te verbeteren. Studies zijn uitgevoerd binnen het kader van de landelijke groeihormoonstudie voor kinderen met PWS. Algemene aspecten van PWS werden onderzocht, zoals de prevalentie van bijnierinsufficiëntie, alsmede slaapgerelateerde ademhalingsstoornissen, scoliose, botdichtheid en cardiovasculaire risicofactoren. Ook werd onderzoek verricht naar de effectiviteit en veiligheid van groeihormoonbehandeling op korte en lange termijn. In het bijzonder werden bestudeerd of de volgende factoren beïnvloed werden door GH: lichaamssamenstelling, lengte, hoofdomtrek en overige antropometrische uitkomsten, botrijping, scoliose, botdichtheid, bloeddruk, glucosehuishouding en lipiden. Dit hoofdstuk geeft een samenvatting van deze studies en de belangrijkste uitkomsten.

Hoofdstuk 2

Hoge prevalentie van bijnierinsufficiëntie bij patiënten met Prader-Willi syndroom

Patiënten met PWS hebben een hoog overlijdensrisico. Het sterftecijfer wordt geschat op 3% onder de 30 jaar. Bij kinderen is met name sprake van plotselinge sterfte. Deze overlijdens geschieden grotendeels volgens een bepaald patroon, namelijk een plotselinge verergering tijdens een milde of matige infectie (meestal een bovenste luchtweginfectie). Daarbij hebben enkele studies aangetoond dat overleden kinderen met PWS verkleinde bijnieren hadden bij obductie. In ogenschouw nemend dat patiënten met PWS een disregulatie van de hypothalamus hebben, wijzen deze factoren op het mogelijk bestaan van bijnierinsufficiëntie binnen deze patiëntengroep. Daarom hebben wij middels metyrapontesten de functie van de bijnier onderzocht in 25 at random geselecteerde patiënten met een mediane (25^{e}-75^{e} percentiel) leeftijd van 9,7 jaar (6,8-13,6). Deze testen werden verricht op de Intensive Care Pediatrie van het Sophia Kinderziekenhuis te Rotterdam. Om 23.30 uur werd metyrapon toegediend. Op 23.30, 4.00, 6.00 en 7.30 uur werd bloed afgenomen voor de bepaling van ACTH, 11-desoxycortisol, cortisol en glucose. De afkapgrens voor een correcte oploop van ACTH is >33 pmol/l om 7.30 uur. Dagritmes speekselcortisol werden afgenomen op een andere dag op de volgende tijdstippen: ontwaken, een half uur na het ontwaken, 14.00 uur en 20.00 uur. Alle patiënten waren anderszins gezond tijdens beide testen. Allen werden behandeld met GH en werden vóór de test niet behandeld met glucocorticoïden.

Vijftien patiënten (60%) hadden centrale bijnierinsufficiëntie (CAI), gedefinieerd als onvoldoende ACTH oploop tijdens een metyrapontest. Om 04.00 uur en 06.00 uur was al een duidelijk significant verschil aanwezig in ACTH oploop tussen kinderen die later gediagnosticeerd werden met CAI en de groep zonder CAI. Er was geen significant verschil in leeftijd, geslacht, genotype en BMISDS tussen kinderen met en zonder bijnierinsufficiëntie.

Geen van de kinderen had een afwijkende cortisolspiegel in de ochtend of een afwijkend cortisoldagritme. Dit duidt erop dat CAI alleen aanwezig is tijdens stress (bijvoorbeeld ziekte). De hoge prevalentie van CAI bij patiënten met PWS zou kunnen bijdragen aan het hoge sterftecijfer, met name tijdens infectiegerelateerde stress. Op basis van ons onderzoek wordt geadviseerd om patiënten met PWS een stressdosering hydrocortison toe te dienen in geval van stress, tenzij CAI recent werd uitgesloten door middel van een metyrapontest.

Hoofdstuk 3
De relatie tussen centrale bijnierinsufficiëntie en slaapgerelateerde ademhalingsstoornissen bij patiënten met Prader-Willi syndroom

In het voorgaande hoofdstuk werd een hoge prevalentie van CAI bij kinderen met PWS aangetoond. Echter, in het verleden werd ook gesuggereerd dat slaap-apneus een belangrijke rol zouden hebben in het plotseling overlijden van patiënten met PWS. Daarom werd de relatie tussen CAI en slaapgerelateerde ademhalingsstoornissen onderzocht. Van 20 kinderen die in de studie beschreven in hoofdstuk 2 werden onderzocht, waren polysomnografische gegevens beschikbaar, die verkregen waren in het kader van de landelijke groeihormoonstudie voor kinderen met PWS. Daarnaast werd van 10 kinderen de slaap gemeten tijdens de metyrapontest.

De mediane (25^{e}-75^{e} percentiel) leeftijd bij de metyrapontest was 8,4 jaar (6,5-10,2). Na toediening van metyrapon werd een significante stijging van de centrale apneu index (aantal/uur) waargenomen, van 2,2 (0,4-4,7) naar 5,2 (1,5-7,9). De toename in centrale apneu index leek hoger bij de kinderen met CAI t.o.v. kinderen zonder CAI [2,8 (2,0 tot 3,9) t.o.v. 1,0 (-0,2 tot 2,6), p=0,09]. Uit polysomnografische data, die enige maanden voor de metyrapontest verkregen werden, bleek dat de slaapgerelateerde ademhaling significant ernstiger verstoord was bij kinderen die later met CAI gediagnosticeerd werden (p=0,03). Ook leek deze groep kinderen een lagere minimale zuurstofsaturatie te hebben (p=0,07).

Samenvattend laat onze studie zien dat de centrale apneu index significant stijgt na toediening van metyrapon, met name bij kinderen die gediagnosticeerd werden met CAI tijdens stress. Daarnaast hadden kinderen met CAI al enige maanden voor de metyrapontest een significant hogere centrale apneu index dan kinderen zonder CAI. Op basis van ons onderzoek werd een nieuwe hypothese opgesteld met betrekking tot de meest voorkomende oorzaak van plotselinge sterfte in patiënten met PWS (zie Hoofdstuk 10 van dit proefschrift).

Hoofdstuk 4

Scoliose in Prader-Willi syndroom: Effecten van leeftijd, geslacht, body mass index, spiermassa en genotype

Aangezien er slechts weinig kennis was van het vóórkomen van scoliose bij kinderen met PWS, werd een cross-sectioneel onderzoek verricht naar de prevalentie van scoliose bij een groep van 96 kinderen met PWS. Jaarlijks werden röntgenfoto's genomen van de wervelkolom. Ook werden de lengte, het gewicht, de totale spiermassa en de spiermassa van de romp (middels een DXA-scan) gemeten. De mediane (25^{e}-75^{e} percentiel) leeftijd was 4,8 (2,1-7,5) jaar. De prevalentie van scoliose was 30% bij kinderen onder de 10 jaar tot 80% bij kinderen van 10 jaar en ouder. Vijftig procent van de kinderen had een Cobbse hoek die groter was dan 20°, waarvoor verwijzing naar een orthopedisch chirurg noodzakelijk is. Daarnaast had 13% van de kinderen een behandeling voor scoliose ondergaan, bestaande uit een korset of een operatie. Bij kinderen met PWS komen twee typen scoliose voor: 1) de "long C-curve type" scoliose, die met name vóórkomt in jonge kinderen met een lage ratio van de spiermassa van de romp t.o.v. het lichaamsoppervlak (trunkLBM:BSA); en 2) een vorm die lijkt op idiopathische scoliose (IS), die met name voorkomt in oudere kinderen met minder hypotonie van de rompmusculatuur.

Op basis van ons onderzoek wordt aangeraden om kinderen met PWS regelmatig te controleren op tekenen van scoliose middels lichamelijk onderzoek en om jaarlijks röntgenfoto's te laten maken van de wervelkolom.

Hoofdstuk 5

Gerandomiseerde gecontroleerde studie: effect van groeihormoon-behandeling op scoliose bij kinderen met Prader-Willi syndroom

Eerder toonden wij een hoge prevalentie van scoliose aan bij kinderen met PWS. De prevalentie was 30% bij kinderen onder de 10 jaar tot 80% bij kinderen van 10 jaar of ouder. In de algemene populatie is een snelle groei geassocieerd met het ontstaan en de progressie van scoliose. Daarom werd scoliose vaak gezien als contra-indicatie voor groeihormoonbehandeling, ook voor kinderen met PWS. Echter, de effecten van groeihormoonbehandeling op scoliose bij kinderen met PWS was helemaal niet bekend. Daarom hebben wij binnen de landelijke groeihormoonstudie gekeken naar de effecten van groeihormoon op het ontstaan en de progressie van scoliose bij 91 kinderen met PWS. Achtendertig kinderen <3 jaar en 44 prepubertaire kinderen >3 jaar werden verdeeld over een groeihormoonbehandelde groep (1 mg/m^2·dag) en een controlegroep, respectievelijk voor de duur van 1 en 2 jaar. Pubers werden verdeeld over twee groepen met een verschillende dosis groeihormoon, namelijk 1 mg/m^2·dag en 1,5 mg/m^2·dag. Jaarlijks werden röntgenfoto's

gemaakt van de wervelkolom en werd de lengte, het gewicht, de spiermassa van de romp (middels een DXA-scan) en IGF-I spiegels gemeten. Er was geen verschil in het ontstaan en de progressie van scoliose tussen groeihormoonbehandelde kinderen en kinderen in de controlegroep (respectievelijk p=0,27-0,79 en p=0,18-0,98). Groeihormoonbehandeling, IGF-ISDS en inhaalgroei hadden geen invloed op het ontstaan en de progressie van scoliose, ook niet na correctie voor mogelijke confounders. De lengte en spiermassa van de romp werden gunstig beïnvloed door groeihormoonbehandeling. Bij start van de studie werd een associatie gevonden tussen hogere IGF-I spiegels en minder ernstige scoliose. Samenvattend tonen onze resultaten aan dat scoliose niet meer als contra-indicatie gezien moet worden voor groeihormoonbehandeling bij kinderen met PWS.

Hoofdstuk 6

Effecten van groeihormoon op antropometrie, lichaamssamenstelling en -proporties bij een grote groep kinderen met Prader-Willi syndroom tijdens de gerandomiseerde gecontroleerde groeihormoonstudie

Kinderen met PWS hebben een verminderde lengtegroei en een afwijkende lichaamssamenstelling met een hoog vetpercentage en een lage spiermassa. Uit eerdere studies is gebleken dat groeihormoonbehandeling de lengtegroei bevordert en de lichaamssamenstelling verbetert. Echter, de gecontroleerde studies hadden een maximale duur van 1 jaar en onderzochten relatief kleine groepen kinderen met PWS. Bovendien hadden de meeste kinderen in deze studies ernstig overgewicht. Wij hebben daarom een gerandomiseerde gecontroleerde studie uitgevoerd, waarin 91 prepubertaire kinderen met PWS werden geïncludeerd, waarvan 42 kinderen <3 jaar en 49 kinderen tussen 3 en 14 jaar. Wij onderzochten de effecten van groeihormoonbehandeling op lengtegroei, lichaamssamenstelling en –proporties. De kinderen werden onafhankelijk verdeeld in 2 groepen: een groeihormoonbehandelde groep (1 mg/m^2·dag) en een controlegroep. De duur van de studie was 1 jaar voor kinderen <3 jaar en 2 jaar voor kinderen tussen 3 en 14 jaar. Jaarlijks werd antropometrie verricht en werd de lichaamssamenstelling gemeten met behulp van een DXA-scan. De mediane (25^e-75^e percentiel) lengteSDS steeg in kinderen <3 jaar van -2,3 (-2,8 tot 0,7) naar -0,4 (-1,1 tot 0,0) en in prepubertaire kinderen van -2,0 (-3,1 tot -1,7) naar -0,6 (-1,1 tot -0,1). In de controlegroep steeg de lengteSDS niet. Tijdens groeihormoonbehandeling werd ook een verbetering van de hoofdomtrek waargenomen. Het lichaamsvetpercentage en lichaamsproporties verbeterden, maar normaliseerden niet volledig. In tegenstelling tot de controlegroep waarin de spiermassa daalde, werd een stabilisatie van de spiermassa in de behandelgroep waargenomen.

Samenvattend bevordert groeihormoonbehandeling de lengte, lichaamssamenstelling, hoofdomtrek en lichaamsproporties van kinderen met PWS. Omdat de IGF-I spiegels fors stijgen bij een aantal kinden met PWS, wordt regelmatige controle van IGF-I spiegels tijdens groeihormoonbehandeling aangeraden.

Hoofdstuk 7

Gerandomiseerde gecontroleerde studie: botdichtheid en de effecten van groeihormoonbehandeling bij prepubertaire kinderen met Prader-Willi syndroom

Volwassenen met PWS hebben een lage botdichtheid. Ondanks dat de kinderperiode de belangrijkste periode is voor de accumulatie van bot, was zeer weinig bekend over de botdichtheid van patiënten met PWS in de kinderleeftijd. Daarnaast hebben patiënten met PWS veel symptomen die ook voorkomen bij kinderen met groeihormoondeficiëntie. Deze groep kinderen heeft een verlaagde botdichtheid, die normaliseert tijdens groeihormoonbehandeling. Binnen de landelijke groeihormoonstudie voor kinderen met PWS hebben wij de botdichtheid (BMD) en de botdichtheid gecorrigeerd voor de lengte ($BMAD_{LS}$) bestudeerd en hebben wij de effecten van groeihormoon hierop gemeten. Zesenveertig prepubertaire kinderen werden voor de duur van 2 jaar onafhankelijk verdeeld over twee groepen: een groeihormoonbehandelde groep (1 mg/m^2·dag) en een controle groep. De gemiddelde ± SD leeftijd was 7,1 ± 2,2 jaar. Bij start, 6, 12 en 24 maanden, werden middels een DXA-scan de BMD van het gehele lichaam en van de lumbale wervelkolom (BMD_{TB} en BMD_{LS}) gemeten en werd de $BMAD_{LS}$ gecalculeerd. Bij start waren de BMD_{TB}SDS, BMD_{LS}SDS en de $BMAD_{LS}$SDS normaal (gemiddelde ± SD respectievelijk: -0,2 ± 1,1 SDS; -0,4 ± 1,2 SDS; en 0,40 ± 1,1 SDS). Gedurende de eerste 6 maanden van groeihormoonbehandeling daalde de BMD_{TB}SDS. De BMD_{TB}SDS veranderderde vervolgens niet gedurende de tweede 6 maanden, maar vertoonde daarna wel een stijging. Deze tijdelijke daling van de BMD_{TB}SDS werd waarschijnlijk veroorzaakt door een disbalans tussen botgroei en mineraalopstapeling en is ook in andere studies beschreven. Botten in het gehele lichaam (TB) bestaan voor 80% uit corticaal bot. Botten van de lumbale wervelkolom (LS) bestaan met name uit trabeculair bot. Trabeculair bot heeft een sneller botmetabolisme dan corticaal bot. Daarom is de balans tussen botgroei en mineraalopstapeling sneller hersteld in trabeculair bot, waardoor er tijdens de eerste fase van groeihormoonbehandeling geen daling plaatsvindt van de BMD_{LS}. Na 24 maanden was er geen significant verschil in BMD_{TB}SDS, BMD_{LS}SDS en $BMAD_{LS}$SDS tussen de groeihormoonbehandelde kinderen en de kinderen in de controlegroep (respectievelijk p=0,30, p=0,44 en p=0,47). IGF-I SDS toonde een significante positieve associatie met BMD_{TB}SDS en BMD_{LS}SDS, maar niet met $BMAD_{LS}$SDS.

Samenvattend toont onze studie aan dat de botdichtheid van prepubertaire kinderen met PWS normaal is en dat groeihormoonbehandeling geen effect had op de botdichtheid, behoudens een tijdelijke daling van de BMD_{TB} tijdens de eerste zes maanden.

Hoofdstuk 8

Het cardiovasculaire en metabole risicoprofiel en acylation stimulating protein bij kinderen met PWS en de effecten van groeihormoonbehandeling

Kinderen met PWS hebben een ongunstige lichaamssamenstelling met een hoog vetpercentage. Desondanks was er weinig informatie over het cardiovasculaire en metabole risicoprofiel van kinderen met PWS. Ook waren de effecten van groeihormoon op cardiovasculaire en metabole risicofactoren nog niet goed in kaart gebracht. Acylation stimulating protein (ASP) stimuleert de opname van glucose en de opslag van triglyceriden in vetweefsel. Daarmee is een hoog ASP een belangrijke aanwijzing voor dyslipidemie en vetophoping. Er was nog geen onderzoek verricht naar de ASP waarden in kinderen met PWS of de effecten van groeihormoon hierop.

Binnen de landelijke groeihormoonstudie hebben wij gekeken naar het cardiovasculaire en metabole risicoprofiel en de ASP spiegels van kinderen met PWS. Daarnaast hebben wij het effect van groeihormoonbehandeling op deze parameters onderzocht. Vijfendertig kinderen <3 jaar en 50 prepubertaire kinderen >3 jaar werden verdeeld over een groeihormoonbehandelde groep (1 $mg/m^2 \cdot dag$) en een controlegroep, respectievelijk voor de duur van 1 en 2 jaar. De gemiddelde ± SD leeftijd was 4,9 ± 3,0 jaar. Het gemiddelde ± SD vetpercentage was 28,4 ± 6,2 in kinderen < 3 jaar en 36,9 ± 8,5 in de prepubertaire groep. Het vetpercentage uitgedrukt in SDS was verhoogd in 95% van de prepubertaire kinderen. Naast een verhoogd vetpercentage hadden 63% van de kinderen < 3 jaar en 73% van de prepubertaire kinderen dyslipidemie. Deze kinderen hadden dus een ongunstig cardiovaculair risicoprofiel. Daarentegen hadden bijna alle kinderen een normale systolische en diastolische bloeddruk. In 40% van alle kinderen werd tenminste één component van het metabool syndroom gevonden. Vijf procent van alle kinderen had tenminste drie componenten en voldeed daarmee aan de criteria voor het metabool syndroom. Het merendeel van de kinderen had normale nuchtere glucose- en insulinespiegels. Daarentegen waren de ASP spiegels sterk verhoogd. De gemiddelde ± SD ASP spiegel was 107 ± 45 nmol/l (normal < 58 nmol/l). ASP spiegels waren positief gecorreleerd aan de vetmassa en negatief gecorreleerd aan de triglyceridenspiegels in het bloed. Groeihormoonbehandeling had een gunstig effect op het vetpercentage en op de HDL:LDL ratio. GH had geen significant effect op de ASP spiegels.

Onze resultaten tonen aan dat de meerderheid van de jonge kinderen met PWS een hoog vetpercentage en dyslipidemie heeft, wat aanduidt dat de kinderen een ongunstig cardiovasculair en metabool risicoprofiel hebben. Daarentegen hadden bijna alle kinderen een normale systolische en diastolische bloeddruk, evenals normale insuline- en glucosespiegels. Gezien de rol van ASP in de opname van glucose en de synthese en opslag van triglyceriden in vetcellen, zou ASP niet alleen een belangrijke rol kunnen spelen in het verhoogde vetpercentage van kinderen met PWS, maar zou ook kunnen helpen bij het binnen de normale grenzen houden van de glucose- en insulinespiegels. Groeihormoonbehandeling had een gunstig effect op het vetpercentage en op de HDL:LDL ratio, maar had geen effect op bloeddruk, glucosehomeostase en lipiden. Groeihormoon had geen effect op de hoge ASP spiegels, wat een verklaring zou kunnen zijn voor het feit dat het vetpercentage niet normaliseert tijdens groeihormoonbehandeling.

Hoofdstuk 9

Lange termijn effectiviteit en veiligheid van groeihormoonbehandeling bij kinderen met Prader-Willi syndroom

Kinderen met PWS hebben een verminderde lengtegroei en een afwijkende lichaamssamenstelling met een hoog vetpercentage en een lage spiermassa. Enkele studies toonden aan dat groeihormoonbehandeling resulteert in een normale eindlengte, indien gestart voor de puberteit. De verbetering van de lichaamssamenstelling wordt beschouwd als het belangrijkste effect van groeihormoon bij kinderen met PWS. Echter, er waren nog geen studies verricht naar de lange termijneffecten van groeihormoonbehandeling op de lichaamssamenstelling bij een grote groep kinderen met PWS. Wij hebben een studie verricht naar de lange termijneffecten van groeihormoonbehandeling op lichaamssamenstelling, lengtegroei, botleeftijd en veiligheidsparameters. In een landelijke prospectieve follow-up studie werden de 4-jaarsgegevens van 55 groeihormoonbehandelde (1 mg/m^2·dag) kinderen met PWS geanalyseerd. De volgende parameters werden gemeten: vetpercentage en spiermassa (middels een DXA-scan), lengte, gewicht, hoofdomtrek, botleeftijd, bloeddruk, nuchtere spiegels van IGF-I, IGFBP-3, glucose, insuline, HbA1c, totaal cholesterol, HDL-cholesterol, LDL-cholesterol. SD-scores werden berekend op basis van algemene Nederlandse referentiewaarden (SDS) en specifiek voor PWS (SDS_{PWS}). De gemiddelde ± SD leeftijd bij start van de studie was 5,9 ± 3,2 jaar. Het vetpercentage (SDS) was significant lager na 4 jaar groeihormoonbehandeling ($p<0,0001$). De spiermassa (SDS) vertoonde een significante stijging gedurende het eerste jaar ($p=0,02$), maar keerde daarna terug naar het niveau zoals bij start. Daarna veranderde de spiermassa uitgedrukt in SDS niet meer. Groeihormoon resulteerde dus in een stabilisatie van de spiermassa in tegenstelling tot de situatie in onbehandelde kinderen, die gekenmerkt wordt door een

continue daling van de spiermassa (SDS). De gemiddelde ± SD lengte normaliseerde tijdens groeihormoonbehandeling van -2,27 ± 1,2 SDS bij start tot -0,24 ± 1,2 SDS na vier jaar. De $BMISDS_{PWS}$ toonde een significante daling. De gemiddelde ± SD IGF-I en IGF-I/IGFBP-3 ratio stegen beiden significant naar respectievelijk 2,08 ± 1,1 SDS en 2,32 ± 0,9 SDS. Groeihormoon had geen invloed op de botrijping, de bloeddruk, de glucosehuishouding en lipiden. Onze resultaten tonen aan dat 4 jaar groeihormoonbehandeling met een standaard dosering (1 mg/m^2·dag) een gunstig effect heeft op de lichaamssamenstelling, lengteSDS, $BMISDS_{PWS}$, hoofdomtrekSDS en overige lichaamsproporties, zonder ongunstige effecten op bloeddruk, glucosehomeostase en serum lipiden. De gunstige effecten van korte termijn groeihormoonbehandeling blijven gedurende vier jaar gehandhaafd, hetgeen wordt gekenmerkt door een verlaging van het vetpercentage en een stabilisatie van de spiermassa. Op basis van onze studie wordt aangeraden te streven naar IGF-I spiegels tussen 2 en 3 SDS voor optimale effecten in kinderen met PWS zonder bijwerkingen. Samenvattend is groeihormoonbehandeling een effectieve en veilige therapie voor kinderen met PWS.

Chapter 13

Dankwoord
Curriculum vitae
List of publications

Dankwoord

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Curriculum vitae

Roderick de Lind van Wijngaarden was born in The Hague, The Netherlands, on October 2nd, 1982. He graduated cum laude from high school (Christelijk Gymnasium Sorghvliet) in 2000 and started his medical training at the University of Leiden, Leiden University Medical Center.

From September to December 2001 he studied Immunology and Pathology at the Karolinska University, Stockholm, Sweden. In 2002, Roderick participated in the Leiden University Honors Program “Stress: from biology to public issue”. After participating in several committees, Roderick became chairman of the Leiden Medical Students Association (Medische Faculteit der Leidsche Studenten) from September 2003 to September 2004. Thereafter, he continued his study. In January 2005, Roderick started a research project concerning scoliosis and sleep-apneas in children with Prader-Willi syndrome at the Dutch Growth Research Foundation and the Department of Pediatric Endocrinology of the Sophia Children’s Hospital, Rotterdam, under supervision of Prof. A.C.S. Hokken-Koelega. In 2006, he continued his work as a clinical research fellow and study coordinator of the Dutch national growth hormone trial for children with PWS, which has resulted in the present thesis. During his time as a researcher, Roderick gave poster presentations at the annual meetings of the European Society for Pediatric Endocrinology at 2006, 2007, and 2008. Furthermore, he received a grant for a poster presentation at the Fourth International Congress of the Growth Hormone Research Society (GRS) and the International Society for IGF Research in Genoa, Italy, and gave an oral presentation at the Scoliosis Research Society in Salt Lake City, Utah, USA. In April 2009, he continued his medical training in the form of rotations at the Erasmus University Medical Center Rotterdam. Roderick lives in Rotterdam together with his fiancé Celine Tummers.

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