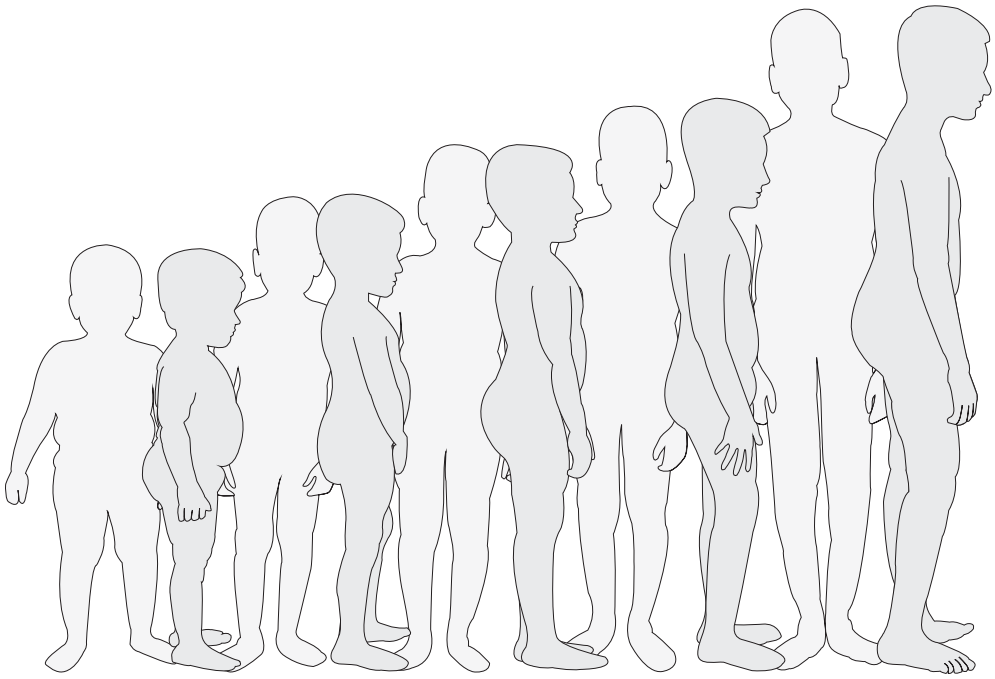
# Growth Hormone Treatment in Children with Prader-Willi Syndrome

## From infancy to adulthood

**Effects on**: Body Composition, Bone Mineral Density, Energy Intake, Quality of Life and IGF Bioactivity

**Nienke E. Bakker**



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**Growth Hormone Treatment in Children with Prader-Willi Syndrome**
**From infancy to adulthood**

Groeihormoonbehandeling in kinderen met het Prader-Willi syndroom
Van baby tot volwassene

**Proefschrift**

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*Voor alle kinderen met het Prader-Willi syndroom en hun ouders*

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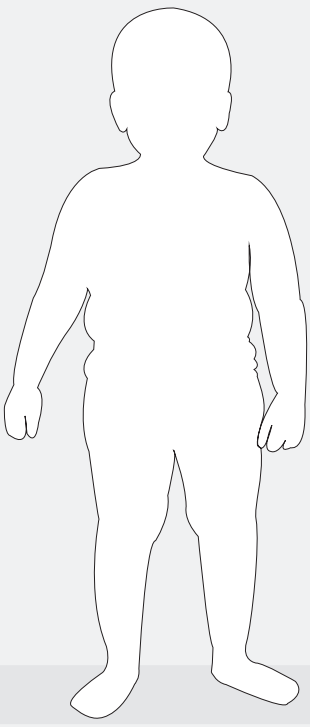

# CHAPTER 1

## General introduction and aims of the study

## INTRODUCTION

This is the fifth thesis of our research group in the field of Prader-Willi syndrome (PWS) and encompasses 6 new studies embedded in the Dutch PWS Cohort study in children and adolescents with PWS.

In 1887, sir Langdon Down described an adolescent girl with short stature, obesity, hypogonadism and cognitive impairment (Figure 1)[1]. Almost a century later, in 1956, 3 endocrinologists Prader, Labhart and Willi described the most characteristic features as "Ein Syndrom von Adipositas, Kleinwuchs, Kryptorchidismus und Oligophrenie nach myotoniertigem Zustand im Neugeborenenalter"[2]. A detailed description of the syndrome was given several years later. The clinical features which were considered characteristic were floppy at birth, obesity, mental retardation, hypogonadism, hypotonia, shortness of stature, prominent forehead, almond-shaped eyes, retroussé nose, small fish-like mouth, short hands and feet and some of them showed a diabetic type of glucose tolerance test[3]. First research topics were predominantly describing the syndrome, at that time also known as syndrome of Hypotonia-Hypomentia-Hypogonadism-Obesity (HHHO)[4], and focused on the relations with diabetes[5, 6] and the cause of hypotonia[7, 8]. In the 70s studies reported on low growth hormone (GH) levels[9-11] and the first clinical trial with GH treatment was published in the 80s[12].

Since 2002, our research group has been investigating the effects of GH treatment in children with PWS in the Dutch PWS Cohort study.

Knowledge about different aspects of PWS has vastly increased over the past 50 years, although new questions and dilemmas are met and need further investigations.

This chapter describes the clinical manifestations in different stages of life, the genetic background, the hypothalamic – pituitary axis in children with PWS. In the scope of this


**Figure 1. A** Girl with PWS described by L. Down, 1887[13]. **B** Three girls with PWS during GH treatment, 2015. Photo is depicted with permission from parents and children.

thesis, characteristics of children with PWS are described in combination with the long-term effects of GH treatment. Finally, the aims of the studies described in the following chapters are presented.

## 1.1 Prader-Willi syndrome

Prader-Willi syndrome is a rare genetic disorder caused by a lack of expression of paternally inherited imprinted genes on the Prader-Willi region on chromosome 15[14]. According to European surveys the incidence of PWS is estimated at around 1 in every 15,000 births, equally affecting boys and girls[15, 16]. Social-economic status and ethnicity are not associated with the incidence of PWS. PWS is considered the most common cause of syndromic obesity. Most symptoms of PWS are thought to be the result of hypothalamic dysfunction[17]. Clinical signs and symptoms of children with PWS vary, but can usually be subdivided in 5 phases[18]:

- In utero, decreased fetal movements and some growth restriction compared to unaffected siblings.
- Neonate and infant, first characterized by severe hypotonia and feeding difficulties with failure to thrive, followed by the phase in which the child grows steadily along a growth percentile and weight is increasing at a normal rate.
- Toddler, associated with weight gain without a significant change in appetite or caloric intake, followed by the phase in which the weight gain is associated with an increased interest in food.
- Childhood and adolescence, characterized by hyperphagia, typically accompanied by food-seeking and lack of satiety and particular behavioral features.
- Adulthood, when the insatiable appetite has disappeared and a person with PWS is able to feel full.

### *1.1.1 In utero*

During pregnancy, some mothers report decreased fetal movements and the incidence of polyhydramnios is increased[19]. Growth parameters are usually normal till 24 weeks but in the third trimester, intra-uterine growth restriction and asymmetrical intrauterine growth (increased head/abdomen circumferences ratio) are described[20, 21]. The fetus is often in breech presentation and delivery by caesarian section is more common[19]. Both have been related to hypotonia, but could also be related to an abnormal fetal hypothalamic system[17]. The combination of decreased fetal movements, breech presentation, intra-uterine growth restriction, polyhydramnios and excluding placental insufficiency is a strong indication to consider a molecular cytogenomic diagnosis prenatally[21]. No associations are found between assisted reproductive technology and PWS[22]. Both premature and post term deliveries are frequently observed, but on average babies with PWS are born at a gestational age of 38 weeks[20].

*1.1.2 Neonate and Infant*

Birth weight and birth length are usually reduced with a median (range) birth weight SD score (SDS) of -1.4 (-2.8 to 0.2), with 20% having a SDS less than -2.0, and median birth length SDS of -0.5 (-2.1 to 1.4)[23, 24]. Typical in the neonatal period are severe hypotonia, diminished tendon reflexes, lethargy with decreased arousal, a weak or absent cry and poor suck (Figure 2)[25, 26]. The prevalence of hip dysplasia is approximately 10-fold increased compared with the general population[27]. Almost all neonates require some type of assisted feeding for 3-9 months. Problems with thermoregulation are evident at birth and throughout life[28, 29]. Some neonates suffer from breathing problems, probably caused by hypotonia of the respiratory muscles, but the associated respiratory depression is typically mild and transient[24, 30]. Genital abnormalities can already be observed at birth. In male neonates with PWS, the penis may be small but most characteristic is the hypoplastic scrotum, poorly rugated and pigmented, with unilateral or bilateral cryptorchidism (86-100%)[31, 32]. In female neonates with PWS, the genital hypoplasia is easily missed, but the labia minora and the clitoris are generally small[32]. The combination of low birth weight, severe hypotonia and genital abnormalities should raise suspicion of PWS[26]. The genetic confirmation of PWS can be established nowadays within the neonatal period[24].

Failure to thrive is frequently seen during infancy. This slowly improves and is followed by a phase in which the child grows steadily along a growth percentile and weight is increasing at a normal rate[18]. Despite the low-normal weight, an abnormal body composition is already present, with a low lean body mass and a high fat percentage[33, 34]. Mainly

**Figure 2.** Twins, below a girl with PWS and above her sister, without PWS. Photo is depicted with permission from parents

due to this abnormal body composition and in particular the low lean body mass (LBM), the energy expenditure is 30% lower than normal[34].

Although also present in the neonatal phase, typical features of PWS become more pronounced in infancy; almond-shaped eyes, a narrow bi-frontal diameter, a thin down-turned upper lip, and a narrow nose[35]. Furthermore, hypopigmentation, fair hair and blue eyes are frequently seen, in particular in children with a deletion[36]. Early mental and motor milestones are delayed and on average reached at double the normal age[37, 38].

### *1.1.3 Toddlers*

Weight gain and obesity without a significant change in appetite or caloric intake, starts usually between the age of 1-4 years and is followed by the phase in which the weight gain is associated with a concomitant increased interest in food (Figure 3)[18]. The exact mechanism of the switch in nutritional phase is still unknown. It is speculated that disbalances between insulin, leptin, glucose, peptide YY, ghrelin and pancreatic polypeptide, contribute to the hyperphagia in PWS[39]. A well-balanced diet with a reduced-energy intake and physical training is necessary to prevent obesity[40]. Physical training remains important throughout life, to improve motor development and exercise intolerance[41].

**Figure 3.** 3 Infants and toddlers with PWS. Photo is depicted with permission from parents

### *1.1.4 Childhood and Adolescence*

Childhood and adolescence are characterized by hyperphagia, typically accompanied by food-seeking and lack of satiety and particular behavioral features. In the presence of a reduced metabolic rate and decreased physical activity, this obsessive behavior towards food consumption often leads to morbid obesity in children with PWS[42]. Children with PWS have poor growth, resulting in short stature and small hands and feet.

However, due to early genetic testing, dietary counselling and growth hormone treatment, obesity and short stature are less common in children with PWS[43]. Nevertheless, hyperphagia remains and, for example, ingestion of toxic substrates such as waste, soil, plants, medicine, cosmetics and cleaning products is more prevalent in PWS subjects than in controls (20% vs. 2% of controls)[44].

Due to their impaired LBM and muscle imbalance, scoliosis, kyphosis, gene valgum and pes planus, knock knees and patellofemoral instability are frequently seen[45, 46]. Furthermore, sleep-related breathing disorders are observed in almost all children with PWS[47, 48].

Cognitive impairment becomes evident in childhood. The average IQ is 70, which corresponds to a mild to moderate learning disability[49]. Some children are able to attend regular school, but almost all have learning difficulties[50-52]. Behavioral problems, often in relation with food, become typically more prominent in childhood. Temper tantrums, stubbornness, difficulties with changing routines, repetitive speech, controlling and manipulative behavior and compulsive-like behavior are common[53, 54]. Typical adolescent rebelliousness and food-related behavioral problems are frequently seen and can be challenging for most parents and caretakers. Psychiatric illnesses, like psychosis, can occur, in particular in adolescents with a maternal uniparental disomy (mUPD)[55].

Puberty in children with PWS often starts spontaneously, but in most cases proceeds delayed or incomplete[56, 57]. Precocious puberty and, more frequently, premature adrenarche are also reported[31, 58]. Failure of complete puberty may be caused by a combination of both primary hypogonadism as well as hypothalamic dysfunction[31, 56, 57]. Substitution of sex hormones is controversial in adolescents with PWS, in boys mainly because of behavioral issues and in girls because of hygiene issues.

### *1.1.5 Adulthood*

The transition from childhood to adulthood is complex. Insistence on sameness, pervasive food seeking behavior, mood disorders and inactivity make this phase even more complicated[59]. Ethical issues with regard to autonomy of persons with PWS versus providing high-quality care are challenging[60]. In general, adults with PWS cannot live independently. Although the concept of group homes may at first be rejected by young adults with PWS, the structure provided by these homes improves quality of life and is ultimately preferred. In some adults with PWS, the insatiable appetite disappears and they are eventually able to feel full[18].

Due to an impaired spontaneous growth velocity and the absence of the pubertal growth spurt, median adult height in women is 150 cm and in men 160 cm[23, 61]. Although secondary sex characteristics are often absent or incomplete, a few case reports on pregnancies were published[62, 63]. Paternity has never been described. The adult population of today was usually diagnosed relatively late, when obesity was already present

and was never treated with GH. So far, no studies are available on the clinical picture of PWS adults who have been diagnosed in the neonatal phase and who were treated from infancy onwards with GH, diet and exercise programs. When severe obesity with its complications can be avoided, adults with PWS may have a reasonable life expectancy[64]. To date, the majority of children are diagnosed in the neonatal phase, allowing earlier introduction of care to reduce morbidity and improve quality of life.

## 1.2 Genetic background

Until 1981, the diagnosis of PWS was solely based on clinical findings and a combinations of symptoms listed in the consensus diagnostic criteria[35]. Associations between PWS and chromosomal rearrangements were already made, but in 1981 it was reported that the cause of PWS was an interstitial deletion of the long arm of chromosome 15 at region q11-q13 in the majority of patients[65]. One year later, it was discovered that this deletion only affected the paternally inherited chromosome 15[66]. In 1989, mUPD was discovered[67]. It turned out that PWS is due to lack of expression of paternally inherited genes located on chromosome 15, locus q11-13. Another term for the 15q11-13 locus on chromosome 15 is the 'Prader Willi/Angelman region'[68]. To date, it is known that the expression of the genes in the Prader-Willi region on the paternally inherited chromosome might be lost due to a deletion, an mUPD, an imprinting center defect[69, 70] or a Robertsonian translocation[71, 72].

### *1.2.1 Genomic imprinting*

Deoxyribonucleic acid (DNA) was first described by Watson and Crick in 1953[73]. The long thread of DNA consists of smaller threads, called chromosomes (Figure 4). These chromosomes contain many different genes that carry the genetic information that is used in the development and functioning of all living organisms and some viruses. Humans have 23 pairs of chromosomes in the nucleus of each cell in their body. Children inherit 23 chromosomes from their father and 23 chromosomes from their mother, which form the 23 pairs in each cell. Chromosome 15 is one of these 23 chromosomes.

Genomic imprinting is a genetic phenomenon that plays a role in the activation of genes. Imprinting is a mechanism by which genes are imprinted or silenced during gametogenesis, which leads to a different expression according to the parent of origin. Only a small percentage of the genes is active.

In healthy subjects, the Prader-Willi region of the maternally inherited chromosome 15 is silenced, whereas this region of the paternally derived chromosome is expressed. Abnormal or absent expression of paternally derived genes on the Prader-Willi region causes PWS. PWS is one of the first discoveries of a genomic imprinting disorder in humans[67].

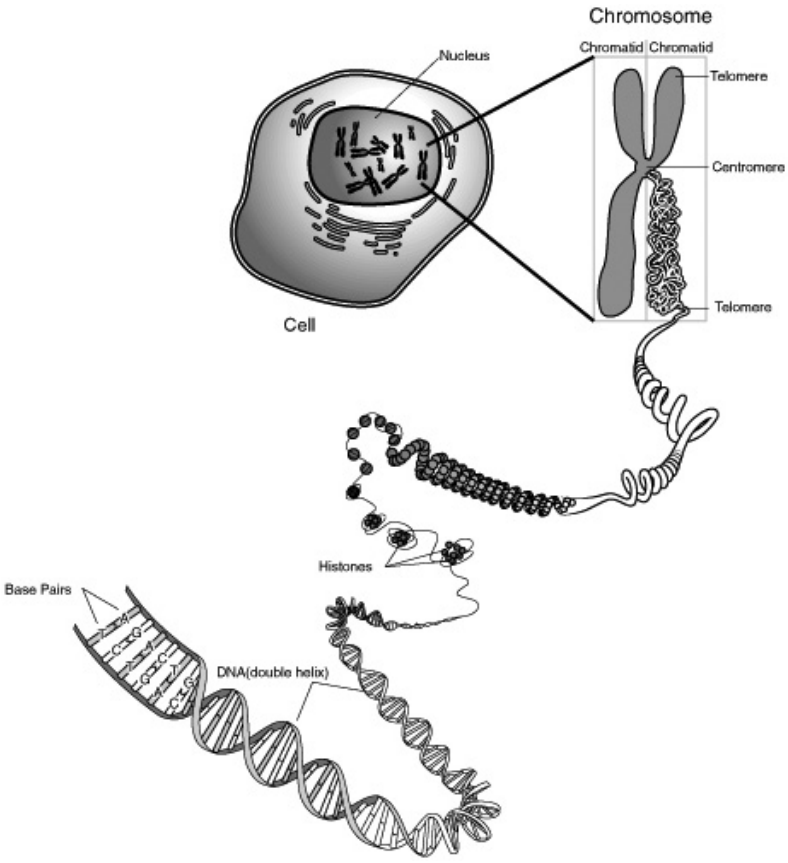


**Figure 4.** Schematic overview of a cell with nucleus, chromosomes and DNA

*1.2.2 Deletion, maternal uniparental disomy, imprinting center defect and translocation*

Prader-Willi syndrome (PWS) is a complex genetic disorder with errors in genomic imprinting (Figure 5), generally due to a paternal deletion of chromosome region 15q11-q13[36]. These de novo deletions occur exclusively on the paternal chromosome[67]. The defect occurs either as a large type I deletion or as a smaller type II deletion. Persons with a deletion have more typical PWS facial features and hypopigmentation, and have a slightly higher performal IQ than those with mUPD[49, 74]. According to the literature, children with a deletion have more behavioral problems like self-injury, food-steeling and compulsive behavior[75].

Maternal disomy 15 (both chromosomes 15 from the mother) is the second most common form of PWS resulting from a gamete complementation by the union of a nullisomic and a disomic gamete or a trisomic conception followed by trisomy rescue in early pregnancy and loss of the paternal chromosome 15[76]. Maternal age at birth is significantly different between mothers of children with a deletion and mothers of

children with an mUPD, with a median age of 29 vs. 35 years, respectively[77]. These findings support the hypothesis that advanced maternal age at childbirth is a predisposing factor for the development of mUPD because of increased meiosis 1 errors. Persons with mUPD have significantly higher verbal IQ scores than those with a deletion[49, 78]. They are at risk for psychiatric problems, like psychosis and autism spectrum disorders[55, 79].

The third, more rare cause of PWS, is an imprinting defect. A mutation in the imprinting control region results in apparently normal chromosomes 15 of biparental inheritance, but the paternal chromosome carries a maternal imprint. This leads to a complete loss of the paternally expressed genes in the Prader-Willi region[69, 70].

Less than 1% of patients have a Robertsonian translocation in which case, part of the paternally inherited chromosome 15 is situated on another chromosome. A balanced translocation results in no excess or deficit of genetic material and causes no health difficulties. In the unbalanced form, Robertsonian translocations cause chromosomal deletions or additions[71, 72].

When one individual in a family has PWS on the basis of the typical deletion or mUPD, recurrence risk is low (probably 1 %). In cases of PWS in which the father carries a translocation, a significant recurrence risk exists. In case of PWS due to an imprinting mutation, recurrence risk is substantial (probably 50%).[80]

A deletion, uniparental disomy, imprinting defect or translocation in the same region on the maternal instead of the paternal chromosome 15, results in a completely different syndrome: Angelman syndrome.

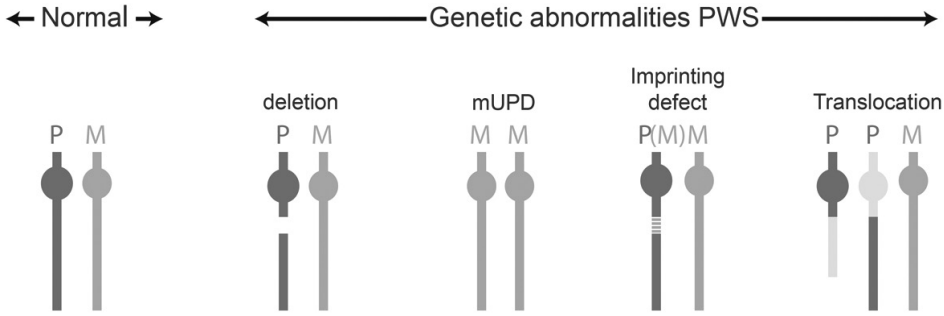


**Figure 5.** Schematic overview of the chromosomal defects described in Prader-Willi syndrome. P=paternally derived chromosome 15, M=maternally derived chromosome 15, mUPD=maternal uniparental disomy, ICD=imprintings center defect

## 1.3 The hypothalamus and pituitary

The hypothalamus is a part of the brain located just above the brain stem, below the thalamus (Figure 6). The hypothalamus contains a number of small nuclei, which control timing of birth, circadian cycle, sleep, hunger, thirst, anger and body temperature. One of the most important functions of the hypothalamus is to link the nervous system to

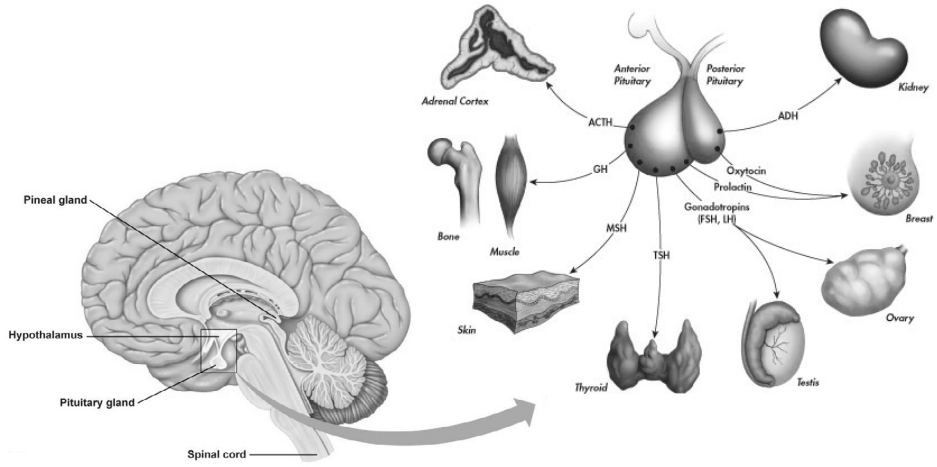


**Figure 6:** The hypothalamus regulates the release of hormones from the anterior pituitary. ACTH= adrenocorticotropin hormone, GH=growth hormone, MSH=melanocyte stimulating hormone, TSH=thyroid stimulating hormone, FSH=follicle stimulating hormone, LH=luteinizing hormone, ADH=anti diuretic hormone

the endocrine system via the infundibulum to the pituitary gland. The pituitary gland, is a protrusion of the bottom of the hypothalamus at the base of the brain and is an important endocrine gland. The hypothalamus synthesizes and secretes neurohormones, which stimulate the anterior pituitary. They are called hypothalamic-releasing hormones, i.e. growth hormone-releasing hormone (GHRH), gonadotropin-releasing hormone (GRH), corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH). These hormones are released into the blood stream and stimulate the anterior pituitary to secrete the following hormones: growth hormone (GH), gonadotrophins (LH/FSH), prolactin (PRL), corticotropin (ACTH) and thyrotropin (TSH).

A number of symptoms in Prader-Willi syndrome might be due to a dysfunction of various hypothalamic systems[17]. Symptoms that have been related to hypothalamic dysregulation including excessive daytime sleepiness, abnormalities of sleep architecture, sleep-related breathing disorders, insatiable hunger, temper tantrums, abnormal temperature control, and hormonal problems such as hypogonadotropic hypogonadism, growth hormone insufficiency and stress-related central adrenal insufficiency[81-84].

## 1.4 Growth hormone treatment in children with PWS

Most children with PWS have short stature and a severely reduced pubertal growth spurt. At birth, children with PWS show a low median birth weight SD score (SDS) of -1.4 and a low-normal median birth length SDS of -0.50[23, 24]. Without GH treatment, mean adult height in women is 150 cm and in men 160 cm[23, 61]. Features of PWS which might

support the presence of lower serum GH levels are short stature, reduced height velocity and relatively low IGF-I levels despite obesity, small hands and feet, increased fat mass and a reduced lean body mass[85]. During testing, however, most children do not fulfil the criteria for GH deficiency[86, 87]. The first report about effects of GH treatment on height velocity and weight gain in children with PWS was published in 1996[87]. Rapidly thereafter randomized controlled trials demonstrated improved growth and body composition during GH treatment in children with PWS[88-91]. In 2000 GH treatment was approved for children with PWS, by the Food and Drug Administration (FDA) for short stature and by the European Medicines Agency (EMA) for short stature and abnormal body composition.

In 2002, the Dutch national growth hormone (GH) trial for children with Prader-Willi syndrome was started to study the effects on growth, body composition, activity level, psychological development and quality of life. At first, children were treated in a randomized controlled trial (RCT), lasting 1 year for infants and 2 years for prepubertal children. After the RCT, children were subsequently followed during continuous GH-treatment in the Dutch PWS Cohort study (for study designs see Appendix 1). To date, most Dutch infants from the age of 6 months onwards are treated in the Dutch PWS Cohort study and followed during GH treatment until they reach adult height.

## 1.5 Unresolved issues

Clinical topics that needed to be investigated were long-term effects of GH treatment on body composition, bone health parameters and quality of life. Because almost no data were available about dietary management, undescended testes in boys with PWS and high IGF-I levels during GH treatment, these topics also needed to be investigated.

### *1.5.1 Cryptorchidism*

Almost all male neonates with PWS are born with unilateral or bilateral cryptorchidism (86-100%) (Figure 7A)[31, 32]. The penis may be small but most characteristic is the hypoplastic scrotum, which is poorly rugated and poorly pigmented[32, 35].

In healthy boys, the descent of the testes is associated with alterations in the circulating concentrations of reproductive hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH) and testosterone. FSH and LH, also called gonadotrophins, are produced by the pituitary gland when stimulated by the hypothalamus, while testosterone is produced by the testes after stimulation by LH. Production of testosteron results in a negative feedback to the pituitary and hypothalamus. This is the male hypothalamic-pituitary-gonadal (HPG) axis (Figure 7B). The HPG-axis plays an important role in the descent of the testes, however, it was also speculated that hypotonia could play a role in cryptorchidism in PWS.

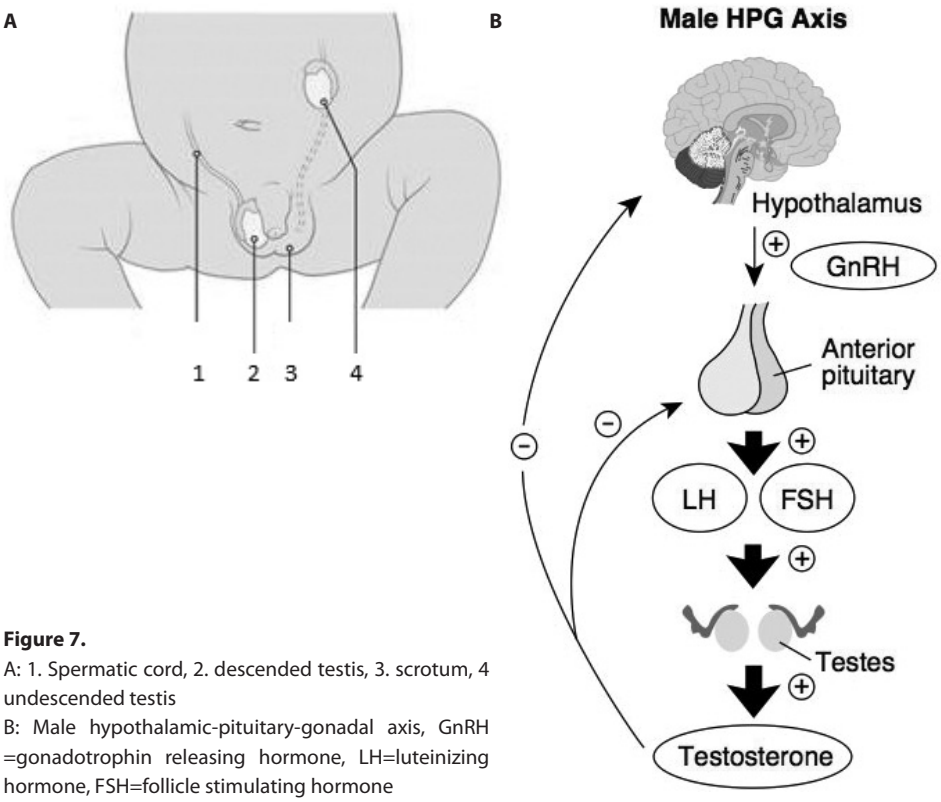


**Figure 7.**
A: 1. Spermatic cord, 2. descended testis, 3. scrotum, 4 undescended testis
B: Male hypothalamic-pituitary-gonadal axis, GnRH =gonadotrophin releasing hormone, LH=luteinizing hormone, FSH=follicle stimulating hormone

Surgery is the most common treatment of cryptorchidism[92]. However, increasingly studies are performed to investigate the effects of hormonal treatment[93, 94]. Even in boys with congenital hypogonadotropic hypogonadism, neonatal gonadotropin treatment have shown beneficial effects on testicular endocrine function and on genital development[95]. The American Academy of Pediatrics recommended a therapeutic trial with human chorionic gonadotropin (hCG) in boys with PWS before surgery is considered, in order to avoid general anesthesia[96]. HCG is a hormone produced by the placenta. The presence of hCG is detected in pregnancy tests. During the first two trimesters of intrauterine life, fetal sex steroid production is driven by maternal hCG[97]. The pituitary analog of hCG is LH. Because of its similarity to LH, hCG can also be used to induce testosterone production in the testes. HCG has been shown to induce testicular descent, presumably by increasing weight and vascularity of testis and also by stimulating testosteron production[98].

*1.5.2 Growth and Body composition*

During the first year of life, infants with PWS grow steadily along their growth percentile, while weight is increasing at a normal rate. Thereafter short stature is present in ap-

proximately 50% of PWS patients[23]. Between 3 and 13 years of age, the 50th percentile for height in PWS is roughly identical with the 3rd percentile in healthy controls. After the age of 2 years, a rapid weight gain occurs and after the age of 10 years the BMI exceeds the normal range in nearly all children[23]. Children with PWS, even underweight infants, have an abnormal body composition, with a low lean body mass and a high fat percentage[33, 34]. This abnormal body composition in combination with short stature resembles a growth hormone deficient state[99]. GH treatment in children with PWS improves body composition, growth, physical strength, and energy expenditure[38, 91, 100-102], but the most important reason for treating these children with GH is to optimize their body composition. Without GH treatment, body composition tends to deteriorate over time[61], resulting in a mean BMI of 35 in boys and girls at adult height (Figure 8)[23]. A high BMI is considered a metabolic and cardiovascular risk, also because this is often related with dyslipidemia and insulin resistance. On the other hand, under pathological conditions of GH excess, the diabetogenic actions of GH can become apparent[103]. The latter was never investigated during long-term GH treatment in PWS. A GH-dose response study showed that children with PWS need a GH dose of at least 1 mg/m$^2$/day to improve and

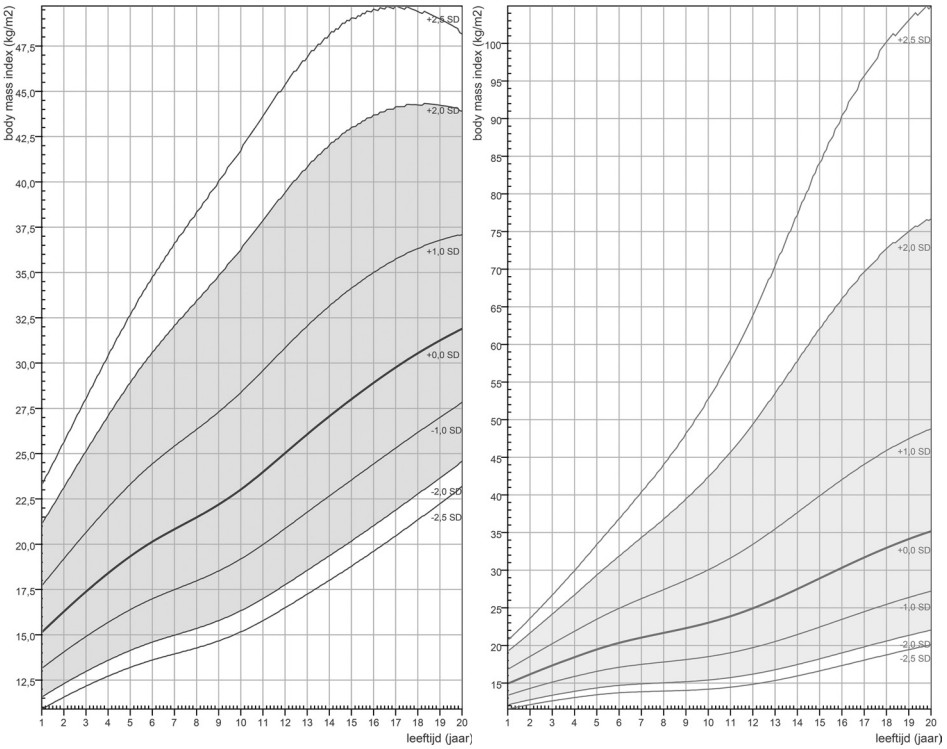


**Figure 8.** PWS specific growth charts for children without GH treatment. Adapted from Hauffa et al.[61] and Growthanalyser (www.growthanalyser.org)

maintain their high-normal body composition. With a lower dose, the body composition deteriorated, while a higher dose improved body composition even more, but also resulted in higher serum IGF-I levels[100]. The question remained whether 1 mg GH /$m^2$/day is safe and able to counteract the clinical course of obesity in PWS, over a long period from childhood to adolescence.

*1.5.3 Bone mineral density and puberty*

Bone mineral density (BMD) is the result of the equilibrium between bone formation and bone resorption and is influenced by several endocrine factors, including parathyroid hormone, thyroxine, GH and sex steroids[104, 105]. BMD increases during childhood and peak bone mass is normally attained between 18-20 in girls and 18-23 years in boys[106]. Bone strength is a major determinant of osteoporosis later in life and largely depends on this attained peak bone mass (Figure 9). Children with PWS are prone to a lower BMD during childhood and adolescence because of low GH levels, hypogonadism and a sedentary lifestyle. On the other hand, extra mechanical stress due to obesity will increase the BMD of the lumbar spine[107], but most GH-treated children with PWS are nowadays not obese anymore[43].

During short-term GH treatment, prepubertal children with PWS have no change in their BMD SDS[101, 108, 109]. Adults with PWS, who did not receive GH treatment during childhood, have a lower BMD than age- and sex-matched controls[110] and, in addition, more osteoporosis and a high fracture risk has been reported[110-112]. These results suggest that there is a decline in BMD in adolescents with PWS, in contrast to age-and sex-matched peers.

In healthy adolescents, testosterone and estradiol levels are the initiators of the prolonged acceleration of bone mineralization[113, 114]. Due to the fact that boys and girls with PWS have a normal onset of puberty, but a delay in pubertal development beyond Tanner stage 2 compared to age- and sex-matched controls[56, 57], it is suspected that BMD will

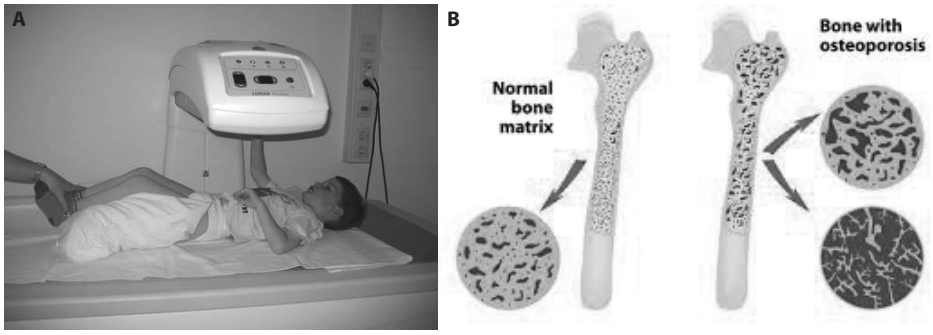


**Figure 9.** A: Dual X-ray scan in a boy with PWS. Photo is depicted with permission from parents and the child

B: Depicts a normal bone and a bone with osteoporosis

decline after Tanner stage 2, when puberty fails to progress. Prior to our study, there were no data on BMD in children and adolescents with PWS during long-term GH treatment.

*1.5.4 Dietary management*

Hyperphagia is the most striking symptom of PWS, marked by an excessive food intake and typically accompanied by food-seeking behavior and a lack of satiety. In the presence of a reduced metabolic rate and a decreased physical activity level, this obsessive behavior towards food consumption will often lead to morbid obesity in children with PWS, when food intake is not restricted[42].

Maintenance of body weight requires an energy balance in which energy intake matches total energy expenditure. The total energy expenditure is the resting energy expenditure (REE) together with the physical activity level[115]. Energy expenditure is closely related to LBM and plays an important role in the development of obesity[116]. One study in infants with PWS reported a reduction in energy expenditure of 30%, mainly due to the abnormal body composition and in particular the low LBM[34]. GH treatment increases fat utilization and REE, the latter partially explained by an increment in LBM[117-119]. But GH treatment is also known to increase energy intake in children born Small for Gestational Age, children with Turner and Silver Russell syndrome[120, 121]. Randomized controlled trials comparing energy intake in children with PWS with and without GH treatment are very scarce[122]. Especially in children with PWS, it is important to obtain insight into the effects of GH treatment on energy intake, REE and physical activity level, to determine which lifestyle interventions might be advisable and beneficial during GH treatment.

*1.5.5 Insulin-like growth factors*

GH is produced by the anterior pituitary, under control of the hypothalamic hormones somatostatin and GH-releasing hormone (GHRH), as well as the stomach hormone ghrelin (Figure 10)[123]. The physiological actions of GH are various and involve multiple organs and physiological systems. Some of the effects of GH are direct, but most effects are mediated via the regulation of insulin-like growth factors (IGFs)[124]. The liver is the main source of circulating IGFs. The IGF family consists of three ligands: insulin, IGF-I and IGF-II and three closely related membrane bound receptors, of which IGF-I is the best known. The growth promoting effects of IGF-I are primarily mediated through the IGF-I receptor[125]. The stability of the IGFs and their interaction with their receptors are mediated by specific IGF binding proteins (IGF-BPs) which are found in the circulation and in extracellular fluids[125]. Humans have six IGFBPs, known as IGFBP-1 to IGFBP-6. In the circulation, almost all IGF-I is sequestered in a stable 150 kD ternary complex with IGFBP-3 (or IGFBP-5) and acid labile subunit (ALS). ALS is essential for the stabilization of circulating IGF-I, and restrains the ability of IGF-I to interact with its receptor[126]. The production of ALS is mostly regulated by GH[127]. Up to 15% of the total plasma IGF-I forms

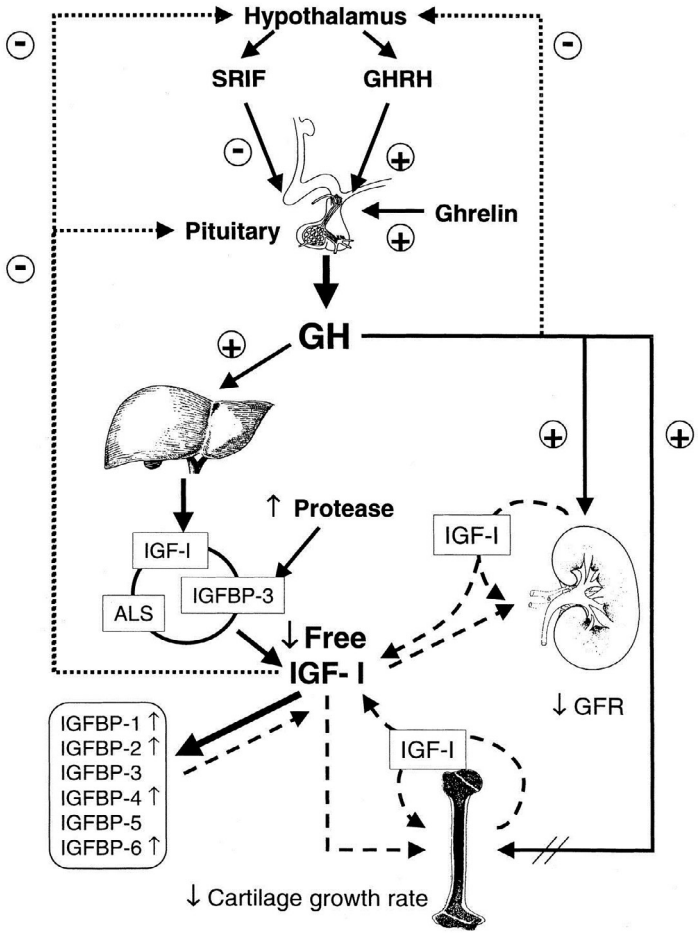


**Figure 10.** Schematic overview of the hypothalamic-pituitary-IGF-I axis. Adapted from Mak et al.[132] SRIF=somatotropin release-inhibiting factor, GHRH=growth hormone releasing hormone, GH=growth hormone, IGF-I=insuline-like growth factor 1, ALS=acid-labile subunit, IGFBP 1-6=insuline like growth factor binding protein 1-6, GFR=glomerular filtration rate

45 kD binary complex with IGFBP-3 (or IGFBP-5). Only 1-5 % of plasma IGF-I is unbound or free[126]. Free IGF-I has a short half-life time of 10-12 minutes, which is much longer in binary complexes (20-30 minutes) and ternary complexes (12-15 hours)[128]. Free IGF-I is able to bind to its receptor, IGF1R, and initiates a complex signaling cascade[129], resulting in longitudinal bone growth and skeletal muscle growth.

Children with PWS often have a reduced GH secretion, low peak GH response to stimulation tests, a decreased spontaneous GH secretion and low serum IGF-I levels[86, 130]. However, during GH treatment IGF-I levels rapidly increase[43, 131]. This has been interpreted as the result of increased GH sensitivity[131]. In children with PWS, elevated serum IGF-I levels during GH treatment are a major concern and often subject of debate[101, 131].

However, to maintain an acceptable body composition with a fat mass percentage at max 2 SDS, children with PWS require relatively high IGF-I levels[100]. There is, however, still a debate whether or not to accept these high immunoreactive IGF-I levels, because of the unknown long-term consequences. In children with PWS, there are no data on the IGF-I complex formation and the ability of IGF-I to phosphorylate its receptor, the IGF-bioactivity.

### *1.5.6 Quality of life*

Health-related quality of life (HRQOL) refers to the impact of health and illness on an individual's quality of life including, emotional, social, home and physical domains[133, 134]. Several generic and disease-specific questionnaires have been developed to measure HRQOL[134, 135]. The advantage of generic HRQOL questionnaires, is that they allow comparisons with normative data and also across disease populations[134]. Disease-specific questionnaires include domains that are only valid for a specified illness, which maximizes content validity and result in a greater sensitivity and specificity in particular patients[134].

To determine a child's HRQOL, the opinion of a child itself is most important[136]. However, due to a different time perspective, dominance of short-term memory and recent incidents and probably the lack of necessary language skills, the use of self-reported questionnaires in children is not always reprehensive. Parents are generally quite able to determine their child's HRQOL, but may over- or underestimate the importance of certain aspects of a child's life. Therefore the method of a combination of child reports and parent reports seems to be most useful[136-138].

GH treatment is an effective and safe treatment for children with PWS[43, 139] and has beneficial effects on cognitive functioning in children with PWS[49]. A few studies showed that GH treatment can improve the physical and psychological aspects of HRQOL in adults with PWS[140, 141], but information about HRQOL or the effect of GH treatment on HRQOL in children with PWS was very scarce.

## 1.6 Aims and outline of the thesis

This thesis presents a detailed description of the studies, which were performed to improve the knowledge about, and the care for patients with PWS. The aims of the studies described in this thesis were to evaluate various characteristic aspects in children with PWS during their development to adolescence, such as testicular decent, body composition, bone health parameters, dietary intake and REE, the high serum IGF-I levels during GH treatment and health-related quality of life and the effects of long-term GH treatment on these characteristic aspects (Figure 11). The study populations consisted of children participating in the Dutch national GH RCT or Dutch PWS Cohort study. Study designs are described in Appendix 1.

**Chapter 1** gives an introduction about PWS and the background of topics described in this thesis.
**Chapter 2** reports the outcome of treatment with human Chorionic Gonadotropin (hCG) on testis position in infant boys with PWS. In addition, in those who underwent orchidopexy, biopsy was taken and testis histology studied. (Cohort study)
**Chapter 3** reports the results of the long-term longitudinal GH study, investigating whether long-term GH treatment could counteract the clinical course of increasing obesity in PWS by maintaining the improved body composition over many years. (Cohort study)
**Chapter 4** reports longitudinal long-term data on bone mineral density (BMD) in children and adolescents with PWS during long-term GH treatment. (Cohort study)
**Chapter 5** presents the outcome of the effect of GH treatment on reported energy-intake in children with PWS, in relation with body composition, resting energy expenditure (REE) and hormone levels. (RCT)
**Chapter 6** presents the results of a study evaluating serum IGF-I, IGFBP-3 and acid labile subunit (ALS) levels, distribution of complex formation and IGF-bioactivity in GH-treated children with PWS compared with untreated healthy controls. (case-control study)
**Chapter 7** describes the effect of GH treatment on health-related quality of life (HRQOL) in children with PWS according to the children and their parents. (RCT and Cohort study)
**Chapter 8** Results and conclusion are discussed in the light of the present literature.
**Chapter 9** Contains an English and a Dutch summary of the results described in this thesis.
**Chapter 10** Contains a list of abbreviations, a list of co-authors affiliations and a list of publications. It further contains the PhD portfolio, acknowledgements and curriculum vitae.

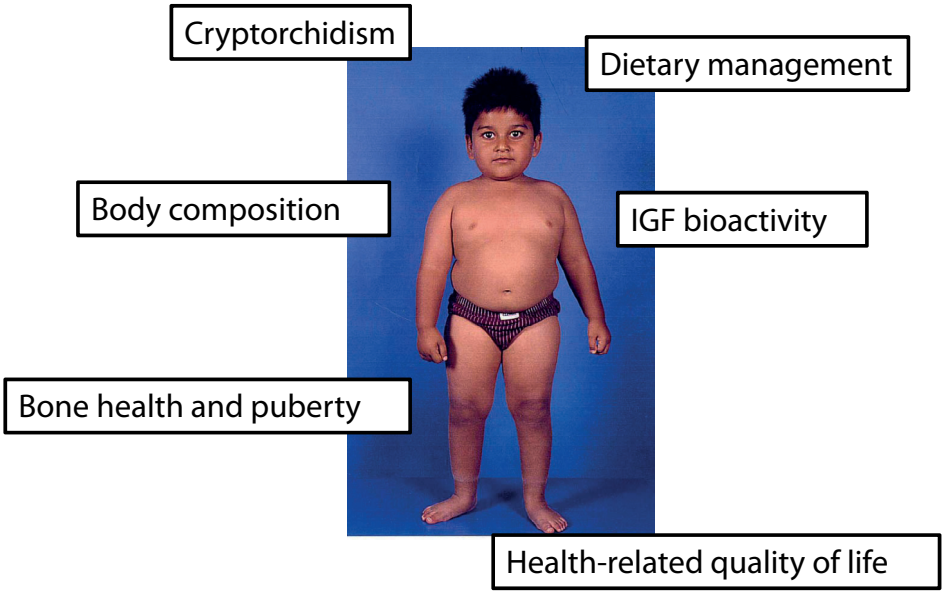


**Figure 11.** Topics of this thesis
3-year old boy with PWS without GH treatment. Photo is depicted with permission from parents and the child

## APPENDIX 1: DUTCH NATIONAL PWS STUDIES

### Project Coordination

Both the Dutch multicenter randomized controlled GH trial (Dutch GH RCT) and the multicenter follow-up study (Dutch PWS Cohort study) are coordinated by the Dutch Growth Research Foundation, Rotterdam, the Netherlands. The PWS research team consists of MD-researchers, a research nurse and a psychologist. Three-monthly, 14 hospitals throughout The Netherlands are visited by the MD-researcher and the 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 once a year.

### Patients

Until 01-01-2015, 163 Dutch children with PWS were included in the Dutch GH RCT and Cohort Study (Figure 2). For both studies, children had to meet the following criteria:

*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 Health 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 PWS Cohort study)

### Design

*Infants*

The RCT infant group consisted of 49 children aged between 6 months and 3.5 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$^2$ per 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$^2$ per day and were prospectively followed in the Dutch PWS Cohort study in collaboration with pediatricians or pediatric endocrinologists throughout The Netherlands.

**Figure 1.** Participating centers

| | |
|---|---|
| Rotterdam: | N.E. Bakker, R.J. Kuppens, S.H. Donze, S.T. Lo, A. Lukoshe, E. Mahabier, Z.C.E. Troeman, E.P.C. Siemensma, R.F.A. Tummers-de Lind van Wijngaarden, D.A.M. Festen, P.M.C.C. van Eekelen, G.C.B. Bindels-de Heus, A.C.S. Hokken-Koelega, Erasmus University Medical Center Rotterdam/ Sophia Children's Hospital (black dot); |
| Nijmegen: | A.A.E.M. van Alfen-van der Velden, B.J. Otten, Radboud University Medical Center |
| Amsterdam: | J. Rotteveel, VU University Medical Center |
| Amsterdam: | N. Zwaveling-Soonawala, Academic Medical Center/ Emma Children's Hospital |
| Apeldoorn: | L. Lunshof, Gelre Hospitals |
| Den Bosch: | P.E. Jira, Jeroen Bosch Medical Center |
| Den Haag: | E.C.A.M. Houdijk, Haga Hospitals/ Juliana Children's Hospital |
| Eindhoven: | R.J.H. Odink, St. Catharina Hospital |
| Enschede: | M. Wegdam-Boer/ R.C.F.M. Vreuls, Medical Center Twente |
| Groningen: | G. Bocca, University Medical Center Groningen/ Beatrix Children's Hospital |
| Harderwijk: | M. van Leeuwen, St. Jansdal Hospital |
| Leeuwarden: | E. van Pinxteren-Nagler, Medical Center Leeuwarden(2002-2014) |
| Leiden: | D.A.J.P. Haring, Alrijne Hospital |
| Leiden: | W. Oostdijk, Leiden University Medical Center/ Willem-Alexander Children's Hospital |
| Lelystad: | R. Lauwerijs, MC Zuiderzee (2002-2013) |
| Nijmegen: | C. Westerlaken, Canisius-Wilhelmina Hospital (2004-2011) |
| Utrecht: | J.J.G. Hoorweg-Nijman, H. van Wieringen, St. Antonius Hospital |
| Zwolle: | E.J. Schroor, Isala Hospital (2002-2014) |

*Prepubertal group*

The RCT prepubertal group consisted of 50 children; girls aged between 3.5 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 2 years (Figure 2). The GH-treated group received somatropin 1 mg/m$^2$ per 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 dif-

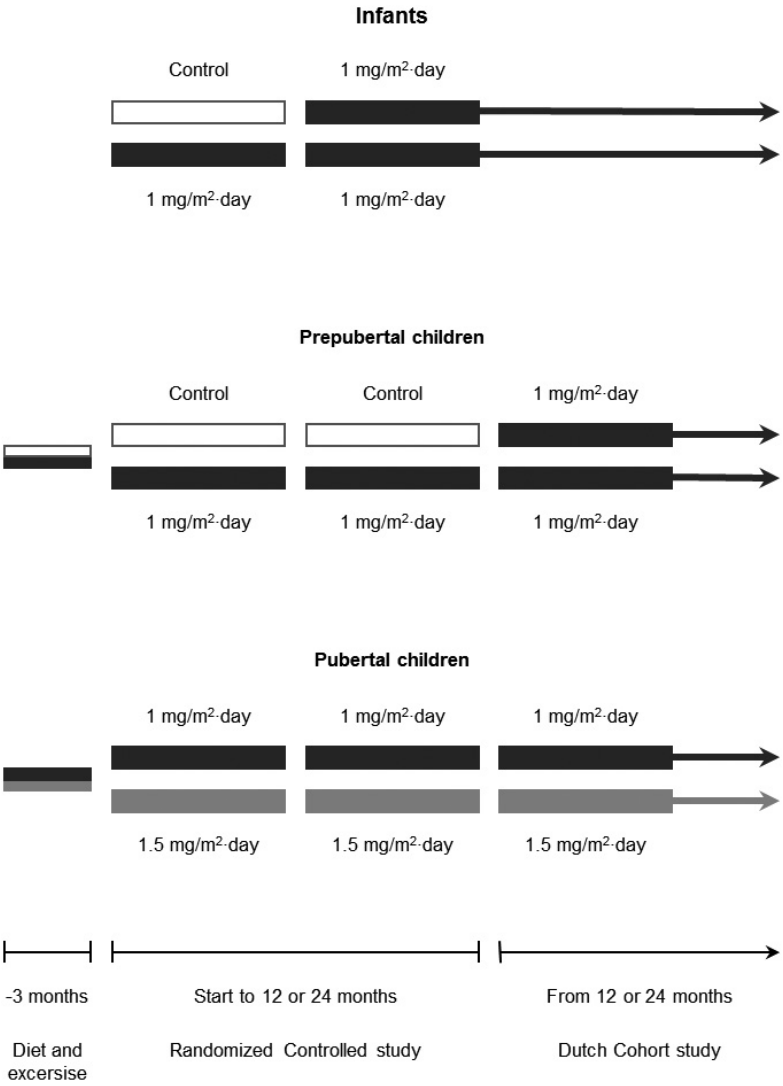


**Figure 2:** Design of the multicenter randomized controlled GH trial and the Dutch PWS Cohort study

ferences. From 24 months of study onwards, all children were treated with somatropin 1 mg/m$^2$ per day and were prospectively followed in the Dutch PWS Cohort study in collaboration with pediatricians or pediatric endocrinologists throughout the Netherlands.

*Pubertal group*

The RCT pubertal group consisted 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$^2$ per day or 1.5 mg/m$^2$ per 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.

*Inclusion directly in the PWS Cohort study*

Since 2008, 55 infants between 6 months and 3.5 years were directly included in the PWS Cohort study for follow-up during long term GH treatment until final height. All children were treated with somatropin 1 mg/m$^2$ per day and were prospectively followed in the Dutch PWS Cohort study in collaboration with pediatricians or pediatric endocrinologists throughout the Netherlands.

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# CHAPTER 2

## Testes in infants with Prader-Willi Syndrome: hCG treatment, surgery and histology

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## ABSTRACT

***Purpose*** Boys with Prader-Willi syndrome (PWS) often have undescended testes. Prospective studies on treatment of cryptorchidism in boys with PWS are lacking. Our aim was to evaluate the effects of human Chorionic Gonadotropin (hCG) treatment on testis position. In those who underwent orchidopexy, biopsy was taken and testis histology studied.

***Materials and Methods*** Sixteen male PWS infants with cryptorchidism, median (interquartile range) age 1.6 (1.2-1.8) years, underwent a hCG stimulation test. After a positive test, hCG treatment was initiated (250-500 IU depending on age, intra-muscular, 6 weeks, twice a week).

***Results*** We found one testis in a stable scrotal position, one vanished and one atrophic abdominal testis. Of 29 testes that could respond to hCG, 23% reached a stable scrotal position, 62% a lower position and 14% didn't change position. Thus 22 testes required additional orchidopexy.

Of 17 obtained biopsies in 12 infants, two had germ cells in > 60% of seminiferous tubules, three in 30-60%, seven in < 30%, four had Sertoli cell-only syndrome and one was a vanished testis. In infants who underwent orchidopexy, a younger age, higher Inhibin B levels and higher testosterone increase after hCG stimulation were associated with higher number of germ-cell containing tubules.

***Conclusions*** HCG treatment resulted in an anatomical lower testis position in most infants with PWS and 23% of the testes reached a stable scrotal position. Seventy-six percent required an additional orchidopexy to ensure a stable scrotal position.

## INTRODUCTION

Prader-Willi syndrome (PWS) is a complex neurodevelopmental disorder characterized by muscular hypotonia, developmental delay, short stature, abnormal body composition, hypogonadism and cryptorchidism[1, 2]. Genital abnormalities can already be observed at birth. In male neonates with PWS, the penis may be small but most characteristic is the hypoplastic scrotum, poorly rugated and pigmented, with unilateral or bilateral cryptorchidism (86-100%)[1, 3]. These are major diagnostic criteria for PWS[4] and suggest hypogonadism prenatally[5]. However, normal increase of gonadotropins and testosterone levels postpartum has been reported, indicating normal minipuberty[5, 6].

Several studies investigated the complex and heterogeneous nature of the hypothalamic-pituitary-gonadal axis (HPG-axis) in PWS[7-9]. All forms of hypogonadism, of central origin, peripheral or combined, are found in males with PWS[8], but in general a sufficient HPG-axis in childhood and primary testicular failure in adulthood is reported[7-9].

The HPG-axis plays an important role in the descent of the testes. Data on hCG treatment in non-PWS cryptorchid boys are contradictory[10]. Treatment with hCG in non-PWS boys was more effective in those with bilateral cryptorchidism than in boys with unilateral cryptorchidism and complete bilateral testicular descent was achieved in 23% of the patients[11, 12]. The age might be crucial, one study reported better results in younger boys[12], but others found better results between 3-4 years of age[13]. One paper questioned the use of hCG treatment in infants, because it might suppress the number of germ cells[14].

Recently the American Academy of Pediatrics recommended a therapeutic trial with human chorionic gonadotropin (hCG) in boys with PWS before surgery is considered, because avoidance of general anesthesia is desirable[15]. One study in 4 patients with PWS showed that gonadotropin treatment facilitated testicular descent in 3 patients[16].

We hypothesized that hCG treatment in infants with PWS results in a scrotal position of the testes. In addition, a positive relation between scrotal position before hCG treatment, Inhibin B levels and testosterone increase after hCG stimulation and histology was expected. We therefore evaluated the effects of hCG treatment on testis position in our Dutch PWS Cohort study and performed testis histology in case of orchidopexy.

## METHODS

### Patients

Genetically confirmed PWS male infants, below the age of 3.5 years, participating in the Dutch PWS Cohort study since June 2007 with unilateral or bilateral undescended testes were included. All were treated with growth hormone, somatropin 1 mg/m$^2$/day. At time

of enrollment, no orchidopexy or surgery was planned or performed and all infants were naïve for hCG treatment or other hormonal treatment, which could interfere with the development of male reproductive tissues.

### Design

Infants were seen by the PWS Team of the Dutch Growth Research Foundation together with the pediatric urologist, they were not blinded to clinical characteristics. During examination, the lowest position of the testis was recorded. In case of impalpable testis during examination (KW), an ultrasonography was performed.

Birth weight standard deviation scores (SDS) were calculated with Growth Analyser 4.0 (available at www.growthanalyser.org), according to gestational age and gender[17]. Neonatal testicular position was obtained from medical records.

Prior to treatment, all infants underwent an hCG stimulation test. A single dose of 1500IU i.m. hCG, Pregnyl® (Organon, The Netherlands) was administered[18]. Baseline and 72-hours after hCG, serum levels of LH, FSH, testosterone, Inhibin B and anti-Mullarian hormone (AMH) were measured. The hCG stimulation test was positive when the maximum testosterone level after 72-hours was 2-20 times higher than baseline in infants aged 3-12 months and 5-10 times higher, between 2.5 and 9.0 nmol/l, in infants aged 1-4 years[19]. After a positive test, hCG treatment was initiated with intra-muscular injections twice a week for 6 weeks. The hCG dose was age-dependent; 250IU for infants aged 3-12 months of age and 500IU for infants aged 1-4 years[20, 21]. Location of testes were determined by the PWS team and the pediatric urologist, at baseline and during the 3-monthly visits. We defined a stable scrotal position when the testis was palpable in the scrotum during at least two follow-up visits.

The study protocol was approved by the Medical Ethics Committee. Written informed consent was obtained from the parents.

### Hormone assays

Blood samples were collected for assessment of serum FSH, LH T, Inhibin B and AMH, of which intra- and interassay coefficients of variation (CV) are described elsewhere[7].

Serum AMH levels were assessed using the AMH Gen II ELISA (Beckman Coulter Inc, USA). The intra-assay CVs were 3.0% and 3.4% at concentrations of 0.49 and 4.24 ug/L respectively.

### Surgery

In case of a bilateral stable scrotal position, hCG treatment was defined successful. If the testis was located in the scrotum, and a patent processus vaginalis (PV) was suspected, the infant was followed for another six months. Patent PV, symptomatic inguinal hernia, or a non-stable scrotal position of the testis were indications for orchidopexy in

combination with hernia repair or closure of the patent PV. In case of an impalpable and ultrasonographically invisible testis, surgery started with laparoscopy, followed by standard inguinal orchidopexy. In case of testicular atrophy or a vanished testis, orchidectomy was performed. During surgery, the position of the testis (abdominal, inguinal canal, external ring, scrotal), size of the testis measured with a ruler and patency of the PV were recorded. During surgery, a testicular biopsy of up to 2.0x0.5 mm was taken, immediately fixed in formalin during 24 hours and embedded in paraffin. All biopsies showed at least 30 seminiferous tubules and were therefore considered to be representative for the remaining testis.

### Histology

Sections of 4 µm were prepared and stained with hematoxylin and eosin (H&E). To identify germ cells, additional specific immunohistochemical markers were used: DEAD (Asp-Glu-Ala-Asp) box polypeptide 4, abbreviated to DDX4 (i.e. VASA) and Testis Specific protein on Y chromosome (TSPY).

The number of germ-cell positive tubules was scored as percentage of the total number of tubules according to the Tubular Fertilization Index (TFI). The TFI varied from: I: nearly normal number of germ cells (> 60% germ-cell positive tubules); II: fairly number of germs cells (30%-60%); III: severely reduced number of germ cells (< 30%) and IV: Sertoli cell only syndrome (SCO)[22]. All histological slides were reviewed by an experienced pathologist and one of the authors.

### Statistics

Statistical analyses were performed with SPSS 21.0. Our data were not normally distributed and therefore expressed as median (interquartile range, IQR). Correlations were calculated with non-parametric bivariate correlations and expressed by Spearman's rho, because of the size of the group. Mann-Whitney test was used to calculate differences between groups.

## RESULTS

### Baseline

Sixteen male infants with PWS, median (IQR) age 1.3 (0.9-2.7) years, underwent an hCG stimulation test (Table 1).

**Table 1.** Baseline characteristics in 16 male infants with PWS

| | Median or n | IQR |
|---|---|---|
| Gestational age (weeks) | 39.3 | 38.0 to 41.1 |
| Birth weight SDS | -1.2 | -1.4 to -0.7 |
| Genetics, n | | |
| Deletion | 11 | |
| UPD | 5 | |
| Position testes at birth, n | | |
| Palpable in scrotum | 1 | |
| Unilateral chryptorchidism | 2 | |
| Bilateral chryptorchidism | 13 | |
| Age at hCG stimulation test (years) | 1.3 | 0.9 to 2.7 |

Data are expressed as median (IQR) or number n, SDS according to gestational age and sex matched values[17].

## hCG treatment

Only 1 testis had a stable scrotal position before the hCG stimulation test. Table 2 shows the hormone levels of the hCG test. Medium (IQR) increase of testosterone was 5.9 (3.5-9.2) nmol/L, indicating a good response.

Table 3 shows the results of the hCG treatment. Twenty-five of the 31 testes (81%) descended during treatment to a lower position. After exclusion of the vanished testis and the abdominal testis, 23% reached a stable scrotal position, 62% a lower position and 14% didn't change position. All testes on the left site were palpable after hCG treatment, on the right side three testes remained impalpable (Figure 1).

During hCG treatment, 6 infants developed some transient pubic hair growth and 3 infants had minor fluid retention. Therefore, treatment was terminated after 5 instead of 6 weeks. All symptoms were without clinical consequences and disappeared within a few weeks after discontinuation of hCG treatment.

**Table 2.** HCG stimulation test results

| | **Baseline** | | **72 hours after hCG** | | |
|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | Reference levels |
| LH (U/L) | <0.1 | <0.1 - 0.2 | 0.1 | <0.1 - 0.3 | < 1.5 |
| FSH (U/L) | 1.0 | 0.5 - 1.8 | 0.4 | 0.2 - 0.7 | < 2 |
| Testosterone (nmol/L) | <0.1 | <0.1 - <0.1 | 6.3 | 3.7 - 10.0 | 0.3 - 0.5 |
| Δ Testosterone (nmol/L) | | | 5.9 | 3.5 - 9.2 | |
| Inhibine B (ng/L) | 98.0 | 41.0 - 148.3 | 126.5 | 78.8 - 146.8 | 0 - 1 year -> 68 - 630 |
| | | | | | 1 - 2 years -> 87 - 419 |
| | | | | | 3 - 5 years -> 42 - 268 |
| AMH (ug/L) | 98.3 | 68.5 - 113.3 | 116.9 | 46.9 - 71.5 | 35 - 95 |

**Table 3.** HCG treatment results

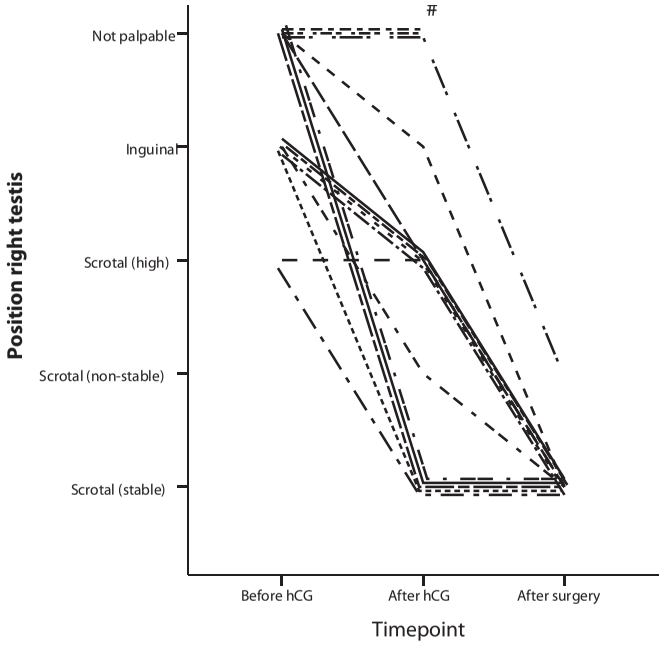
| | Median | IQR | | | | | |
|---|---|---|---|---|---|---|---|
| Age at hCG treatment (years) | 1.6 | 1.2 - 2.8 | | | | | |
| HCG dose (IU) | 500 | 500 - 500 | | | | | |
| Duration (weeks) | 5 | 5 - 6 | | | | | |
| GH treatment (years) | 0.6 | 0.2 - 1.1 | | | | | |
| | **Right testis** | | | | **Left testis** | | | |
| | Before hCG | | After hCG | | Before hCG | | After hCG | |
| Testis position | n | % | n | % | n | % | n | % |
| Scrotal (stable) | 0 | 0 | 5 | 31.3 | 1 | 6.3 | 1 + 4 | 31.3 |
| Scrotal (not stable) | 0 | 0 | 1 | 6.3 | 2 | 12.5 | 1 | 6.3 |
| Scrotal (high) | 2 | 12.5 | 6 | 37.5 | 1 | 6.3 | 5 | 31.3 |
| Inguinal | 6 | 37.5 | 1 | 6.3 | 7 | 43.8 | 5 | 31.3 |
| Not palpable | 8 | 50.0 | 3 | 18.8 | 5 | 31.3 | 0 | 0 |
| Adverse Events | n | % | | | | | | |
| Pubic hair growth | 6 | 37.5 | | | | | | |
| Edema | 3 | 18.8 | | | | | | |

## Surgery

Three infants, infant 1 and 2 with initially bilateral cryptorchidism and infant 8 with initially unilateral cryptorchidism, did not require orchidopexy, because of stable scrotal testes after hCG treatment. Two infants (7 and 10) had a unilateral stable scrotal testis after hCG treatment and required surgery. Twenty-two testes of 12 infants underwent successful orchidopexy (Table 4). The PV was patent in all infants.

In two infants with an unilateral impalpable testis, laparoscopy was performed. Laparoscopy in infant 7 showed a small-caliber vas entering the closed internal ring, and blind-ending atretic vessel, suggestive of a vanished testis. Subsequent inguinal exploration revealed a testicular nubbin which was removed. His left testis, located in the inguinal region, descended to a stable scrotum position after hCG treatment and remained untouched. In infant 14, an atrophic intra-abdominal right testis (not to be positioned scrotal), was removed. His left testis had become palpable in the inguinal region after hCG treatment, and could subsequently been brought into the scrotum by standard orchidopexy.

Infant 8 was treated with hCG for an inguinal palpable testis on the right side, which subsequently descended into the scrotum. Three months after hCG treatment a strangulated right inguinal hernia was repaired. Scrotal testis position was additionally secured by orchidopexy.



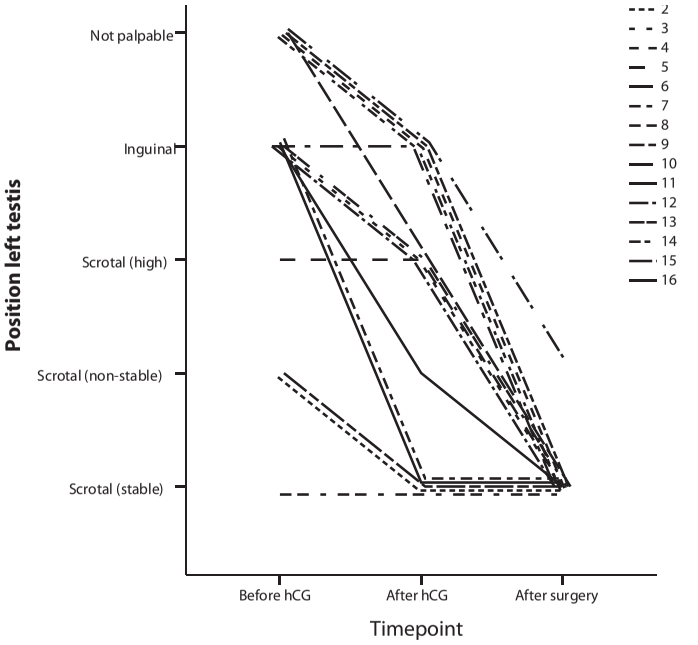




**Figure 1.** Position of the testes before and after hCG treatment and after surgery
*One vanished testis and one orchidectomy.

### Testis histology

Seventeen testicular biopsy specimen, 5 bilateral and 7 unilateral, of 12 infants were available for investigation (Table 4). Representative examples are illustrated in Figure 2. Infant 8 underwent an emergency herniorrhaphy on the right side, without a biopsy. One infant was operated in another center, without a testis biopsy.

Two specimen were obtained of abdominal testes; one vanished testis and one without germ cells (SCO). Of the 15 biopsies obtained during orchidopexy, two showed TFI I; three TFI II; seven TFI III; and three biopsies showed SCO-syndrome, TFI IV.

### Relation between hormones, testis position and histology

A younger age correlated with lower TFI (indicating more germ cells: ρ=0.769, *P*=0.006; Figure 3.a), higher testosterone level after hCG stimulation (ρ=-0.53, *P*=0.05). In line with these results, higher testosterone increase after hCG stimulation associated with lower TFI (ρ=-0.740, *P*=0.009; Figure 3.b). Higher Inhibin B levels during hCG stimulation correlated with higher number of germ-cell containing tubules (ρ=-0.651, *P*=0.041; Figure 3.c). Position of the testes before or after hCG treatment was not associated with TFI, and genetic background and AMH levels not with histology.

### Follow-up

At a median (IQR) age of 4.9 (2.6-6.8) years and a follow-up time of 3.3 (1.4-4.9) years, 27 of the 29 testes with a scrotal position after treatment were still in the same position. One moved from an initially scrotal position to an inguinal position, 5.5 years postoperatively, and one initially scrotal positioned testis was clinically impalpable, 11 months postoperatively (Table 4). As testicular biopsies in both cases showed SCO, a non-intervention policy was chosen. The median (IQR) follow-up time in the boys with 1 or 2 testes who did not undergo surgery was 1.4 (0.5-4.4) years.

## DISCUSSION

We investigated the effect of hCG treatment on testis position in a group of 16 infants with PWS. In total, 81% of the testes descended to a lower position after hCG treatment. After exclusion of one vanished testis and one atrophic testis without an inguinal canal, 23% of the testes reached a stable scrotal position and, after 1.4 years of follow-up, did not require further surgery and 62% reached a lower position. Twenty-two testes of 12 patients required further orchidopexy but could be easily secured in the scrotum. In infants who underwent orchidopexy, a younger age at hCG treatment, higher Inhibin B levels and a higher testosterone increase after hCG stimulation were significantly associated with a higher number of germ-cell containing tubules at time of surgery.

**Table 4.** Surgery outcome and histology

| | | Right testis | | | | Left testis | | | | Follow-up | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Infant | Age | Position after surgery | Length (mm) | % Germ Cell Pos Tubules | Remark | Position after surgery | Length (mm) | % Germ Cell Pos Tubules | Remark | Age | Position Right | Position Left |
| 1 | # | No surgery | 10 | | Stable scrotal position after hCG treatment | No surgery | 13 | | Stable scrotal position after hCG treatment | 2.03 | Scrotal | Scrotal |
| 2 | # | No surgery | | | Stable scrotal position after hCG treatment | No surgery | | | Stable scrotal position after hCG treatment | 8.84 | Scrotal (high) | Scrotal |
| 3 | 1.29 | Scrotal | 12 | > 60% | | Scrotal | 15 | | | 3.84 | Scrotal | Scrotal |
| 4 | 1.36 | Scrotal | 13 | 30-60% | | Scrotal (high) | 12 | < 30% | | 2.47 | Scrotal | Scrotal |
| 5 | 1.77 | Scrotal | | < 30% | | Scrotal | | < 30% | | 2.26 | Scrotal | Scrotal |
| 6 | 1.92 | Scrotal | | | | Scrotal | | | | 5.85 | Scrotal | Scrotal |
| 7 | 2.01 | Vanished testis | | | | No surgery | 13 | | Stable scrotal position after hCG treatment | 3.07 | No testis | Scrotal |
| 8 | 2.03 | Herniorrhaphy | | | Stable scrotal position after hCG treatment | No surgery | | | Stable scrotal position since birth | 2.54 | Scrotal | Scrotal |
| 9 | 2.15 | Scrotal | 21 | | | Scrotal | 14 | 30-60% | | 4.78 | Scrotal | Scrotal |
| 10 | 2.80 | No surgery | | | Stable scrotal position after hCG treatment | Scrotal | | > 60% | | 5.20 | Scrotal | Scrotal |
| 11 | 3.01 | Scrotal | 13 | < 30% | | Scrotal | 13 | < 30% | | 5.15 | Scrotal | Scrotal |
| 12 | 3.22 | Scrotal | 12 | < 30% | | Scrotal | 13 | | | 5.88 | Scrotal | Scrotal |
| 13 | 3.23 | Scrotal | | < 30% | | Scrotal | 15 | < 30% | | 8.84 | Scrotal | Scrotal |
| 14 | 3.96 | Orchidectomy | <10 | SCO | No inguinal canal | Scrotal | 10 | | | 9.41 | No testis | Inguinal |
| 15 | 4.05 | Scrotal (high) | 20 | SCO | | Scrotal | 15 | SCO | Glandular hypospadias | 4.97 | Scrotal (high) | Impalpable |
| 16 | 4.15 | Scrotal | | < 30% | Hydrocele | Scrotal | | | Hydrocele | 7.08 | Scrotal | Scrotal |

# No age at surgery, because testes on both sides attained a stable scrotal position after hCG treatment

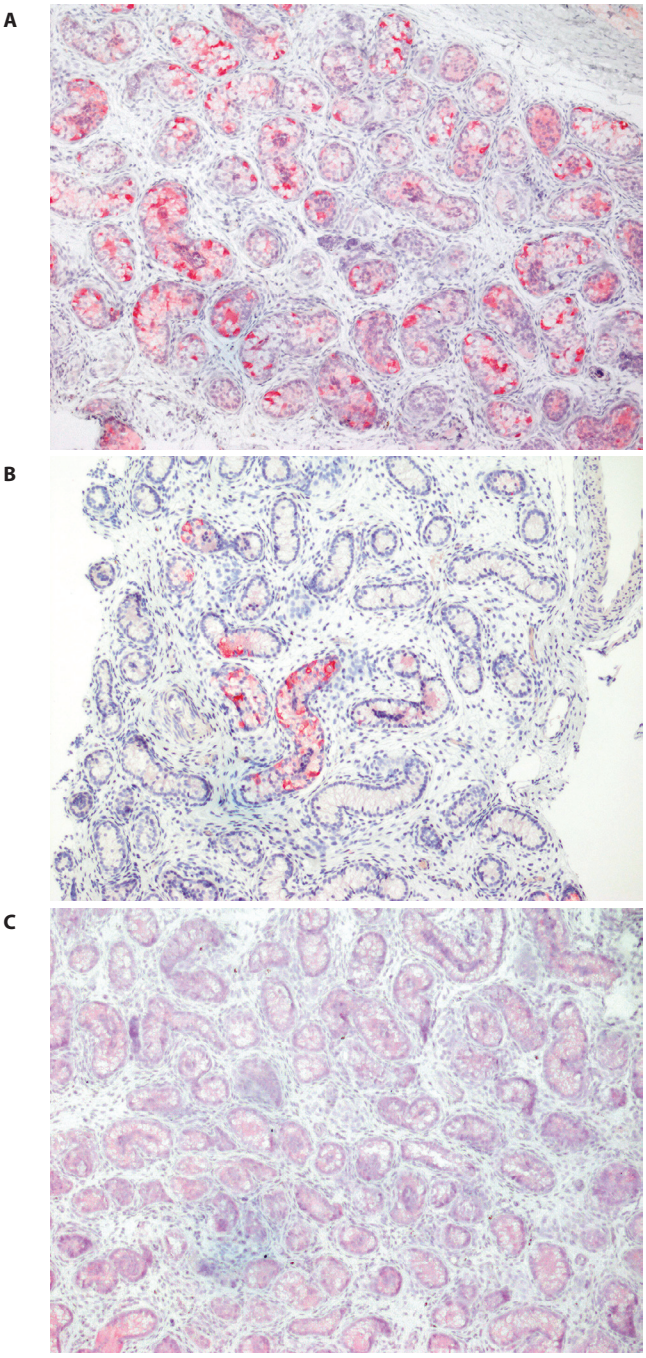
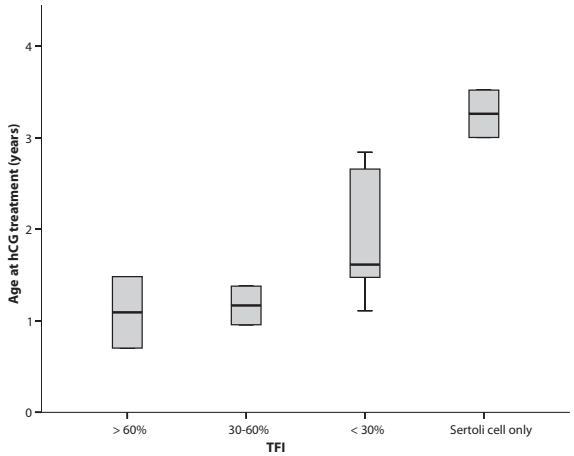


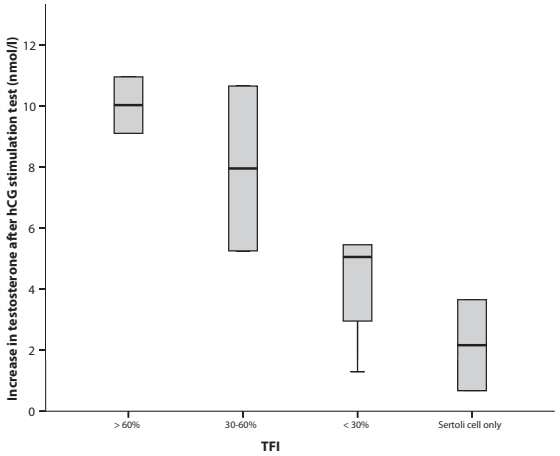

**Figure 2.** Histology cuts, TSPY, 10x.
A. TFI I, TSPY staining in a testis biopsy from a 15 months old boy with PWS. Germ cells stain red.
B. TFI III, TSPY staining in a testis biopsy from a 4 years old boy with PWS.
C. TFI IV, TSPY staining in a testis biopsy from a 4 years old boy with PWS. There are no germ cells (SCO).

A




*P*=0.006

B

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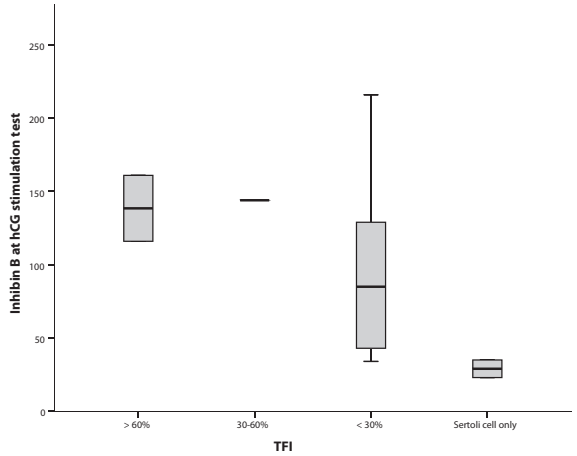
C




*P*=0.041

**Figure 3.** Associations between the age at hCG treatment (A), the increase in serum testosterone levels after hCG stimulation test (B) and serum Inhibin B levels at hCG stimulation test (C) with the TFI. Correlations were calculated with non-parametric bivariate correlations.

This is the first study in which infants with PWS were systematically treated with hCG and had biopsies during surgery. Literature on the optimal treatment of undescended testes in boys with PWS is scarce. Two papers evaluated hypogonadism and pubertal development in PWS: *Uehling et al.* described that gonadotropin treatment facilitated testicular descent in 3 out of 4 patients[16], and *Crinò et al.* described that hormonal treatment in 28 boys, mean age of 4.6 years, gave no clinical results[3]. The high percentage of testicular descent in our study could be explained by the early intervention age and the presence of predominantly bilateral cryptorchidism. Our results are in line with a recent paper, reporting that hCG treatment induced testicular descent, at least one level down, in 81.8% of the testes in a cohort of healthy boys[23]. In healthy boys with bilateral cryptorchidism, hCG treatment was more effective at a younger age[12, 24], although still debated[25, 26].

Based on our results it is, in our opinion, worth giving hCG treatment to PWS infants with cryptorchidism. Most boys with PWS had testes in a lower position after the hCG treatment. In these boys with a higher anesthetic risk, a lower position will ease the orchidopexy procedure and reduce the operation time. However, we acknowledge that our study was not designed to investigate the latter.

We observed a wide range in testicular histology, from a normal number of germ cells to SCO. Our findings are in line with Belgian data, showing a wide variation in germ cell number in eight older boys, without hCG treatment[22]. Remarkably, in the infants who underwent orchidopexy after hCG treatment, we found a relation with age: the younger the better. This was also observed in non-PWS boys who underwent an orchidopexy with biopsy[27]. Data in small groups of adult males with PWS showed total absence of spermatogonia[16, 28]. This might suggest that the number of germ cells in PWS declines over the years. Testicular failure, particularly after the onset of puberty in boys with PWS has been described by our group[7]. According to our present histology results, most boys with PWS already show insufficient germ cell numbers during infancy. We suspect that genes located in the PWS critical region15q11-13 might play a role, directly or indirectly, in this process of early gonadal failure. For example, MAGEL2, supported by data on Magel2-null mice, showing reduced fertility with early reproductive decline and termination[29]. In addition, C15orf2, of which biallelic transcription is observed exclusively in testis, with an important role in spermatogenesis[30]. Although data are lacking, it seems likely that C15orf2 is not expressed in PWS testes, resulting an inadequate testicular development and function.

During follow-up, only two testes changed position, one became inguinal and one impalpable. Postoperative atrophy in the last case is less likely, considering the time relapse of 5.5 years after postoperative confirmation of a viable, scrotal positioned testis. Testicular biopsies showed SCO in both cases, justifying a non-intervention policy. In general the orchidopexy was more easy to perform than in the past, because all patients, except 1, had adequate length of the spermatic cord for successful scrotal fixation.

Because PWS is a rare disorder, the number of infants was limited. Unfortunately, there was no scoring list to evaluate the effect of hCG treatment on the orchidopexy procedure and we had no control group to compare our results. A future prospective controlled study is desirable.

In conclusion, our study demonstrates that most infants with PWS had testes in a lower position after hCG treatment, with 23% of the testes in a stable scrotal position and 76% requiring additional orchidopexy to ensure stable scrotal position. A younger age at orchidopexy was associated with a better histological outcome but the effect of hCG treatment at an early age on testicular function in and after puberty has to be awaited.

## ACKNOWLEDGMENTS

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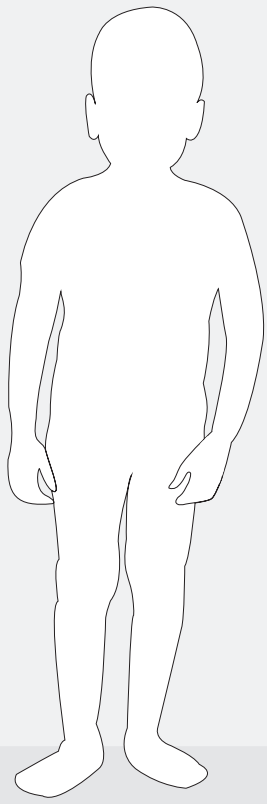

# CHAPTER 3

## Eight years of growth hormone treatment in children with Prader-Willi Syndrome: maintaining the positive effects

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## ABSTRACT

***Background*** The most important reason for treating children with Prader-Willi syndrome (PWS) with GH is to optimize their body composition.

***Objectives*** The aim of this ongoing study was to determine whether long-term GH treatment can counteract the clinical course of increasing obesity in PWS by maintaining the improved body composition brought during early treatment.

***Setting*** Multicenter prospective cohort study.

***Methods*** We have been following sixty prepubertal children for 8 years of continuous GH treatment (1 mg/m$^2$/day ≅ 0.035 mg/kg/day), and used the same dual-energy x-ray absorptiometry (DXA) machine for annual measurements of lean body mass (LBM) and fat percentage (%).

***Results*** After a significant increase during the first year of GH treatment ($P < 0.0001$), LBM remained stable for 7 years at a level above baseline ($P < 0.0001$). After a significant decrease in the first year, fat% SDS and body mass index (BMI) SDS remained stable at a level not significantly higher than at baseline ($P = 0.06$, $P = 0.14$, resp.). However, BMI $SDS_{Prader\text{-}Willi\ Syndrome}$ was significantly lower after 8 years of GH treatment than at baseline ($P < 0.0001$). After 8 years of treatment, height SDS and head circumference SDS had completely normalized. IGF-I SDS increased to + 2.36 SDS during the first year of treatment ($P < 0.0001$) and remained stable since then. GH treatment did not adversely affect glucose homeostasis, serum lipids, blood pressure and bone maturation.

***Conclusion*** This 8-year study demonstrates that GH treatment is a potent force for counteracting the clinical course of obesity in children with PWS.

## INTRODUCTION

Prader-Willi syndrome (PWS) is a rare disorder characterized by short stature, hyperphagia, obesity, hypotonia, hypogonadism, developmental delay and behavioral problems[1-3]. Its incidence is estimated between 1:15,000-1:30,000 live births[4, 5]. PWS is caused by the absence of paternally expressed genes on chromosome 15q11-q13, due mainly to a paternal deletion or a maternal disomy[6].

Growth hormone (GH) treatment in children with PWS improves body composition, growth, physical strength, and energy expenditure[7-11], and has a positive effect on cognition[12]. The most important reason for treating these children with GH is to optimize their body composition[7, 10, 13, 14]; without GH treatment, body composition tends to deteriorate over time[15]. *Carrel et al.* showed that even a low GH dose (0.3 mg/m$^2$/day ≅ 0.01 mg/kg/day) was not sufficient to maintain the positive effects on body composition that had previously been obtained with a dose of 1 mg/m$^2$/day[7]. One year of treatment with a GH dose of 1 to 1.5 mg/m$^2$/day had a sustained positive effect on body composition, but the dose of 1.5 mg/m$^2$/day was complicated by very high IGF-I levels[7]. The recommended GH dose in children with PWS is 1 mg/m$^2$/day[2].

There are only very limited data about the effect of more than 4 years of GH treatment on body composition. The studies were limited to very small groups, focused mainly on adult height and were sometimes based on only three visits[11, 16-18]. Furthermore, they didn't have annually obtained data by the same dual-energy x-ray absorptiometry (DXA) machine.

The question remained whether GH treatment can counteract the clinical course of obesity in PWS, over a long period from childhood to adolescence. We hypothesized that, despite GH treatment, the increasing obesity with a higher fat percentage (%) and a lower lean body mass (LBM) will emerge in PWS over the years. To evaluate the course of PWS during 8 years of continuous GH treatment 1 mg/m$^2$/day, we investigated the effects on body composition, height, BMI and safety parameters in our Dutch PWS Cohort Study.

## METHODS

### Patients

Seventy-three prepubertal children who started GH treatment between April 2002 and December 2005 were selected from our Dutch PWS Cohort Study[10, 12, 19]. Sixty children had completed at least 8 years of continuous GH and were eligible for present evaluation of 8 years of GH treatment.

Thirteen children were treated with GH for less than 8 years; nine children reached adult height in 4 to 6 years of treatment, one boy had to stop GH treatment after 4 years because of extremely high IGF-I levels, despite a very low GH dose, one moved to another country and one stopped participation, but not GH treatment[20]. After one year of GH treatment, one boy died at the age of 3 years during an upper airway infection and suspected central adrenal insufficiency, as previously reported[21]. They were only excluded from present evaluation because they were treated with GH for less than 8 years. All participants had a genetically confirmed diagnosis of PWS by a positive methylation test and were naïve for GH treatment at time of enrollment. At start, children were prepubertal, defined as Tanner breast stage < 2 for girls and testicular volume < 4 ml for boys[22]. Only 9 boys and 1 girl remained prepubertal during 8 years of study. Mean ± SD age onset of spontaneous puberty was 10.09 ± 1.30 years in girls and 11.22 ± 1.97 years in boys.

### Design

The primary objective 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, body mass index (BMI), head circumference, anthropometric data, and bone age (BA); and to assess the safety of GH treatment by studying the effect on blood pressure, fasting serum IGF-I, IGF binding protein 3 (IGFBP-3), glucose homeostasis, and serum lipids. Before initiation of GH treatment all children underwent a polysomnography, as well as at 6 months after start of GH treatment. Children were treated with somatropin 1 mg/m$^2$/day once daily at bedtime (Genotropin; Pfizer Inc., New York, NY) for 8 consecutive years. During the first 4 weeks of GH treatment, children received 0.5 mg/m$^2$/day to prevent fluid retention. The somatropin dose was lowered when IGF-I levels increased above 3 SDS. X-rays to monitor the onset and progression of the scoliosis were performed once a year. 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. Annually, the children visited Children's Hospital Erasmus MC- Sophia, Rotterdam, the Netherlands.

The study protocol was approved by the Medical Ethics Committees at Children's Hospital Erasmus MC- Sophia in Rotterdam, the Netherlands, and of the collaborating centers. Written informed consent was obtained from parents and from children older than 12 years; assent was obtained in children younger than 12 years of age.

### Body composition

In all children, fat% and LBM were measured annually by dual-energy x-ray absorptiometry (DXA) (Lunar Prodigy type; GE Healthcare, Chalfont St. Giles, UK). All scans were

made on the same machine, and quality assurance was daily performed. The intra-assay coefficient of variation (CV) for fat tissue was 0.41% to 0.88%; for LBM 1.57% to 4.49%[23]. LBM was calculated as fat-free mass (FFM) minus bone-mineral content. Fat mass was expressed as percentage of total body weight (fat%). Fat% SDS was calculated, according to age- and sex-matched Dutch reference values[24].

### Anthropometric measurements

Standing height was measured with a Harpenden Stadiometer, and supine length with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was assessed on a calibrated scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, the Netherlands). Height, weight and body mass index (BMI) standard deviation scores (SDS) were calculated with Growth Analyser 3.0 (available at www.growthanalyser.org), and were adjusted for gender and age according to Dutch reference values[25, 26] and also according to PWS reference values[15].



### Assays

After overnight fasting, blood samples were collected for assessment of IGF-I, IGFBP-3, insulin, glucose, total cholesterol, HDL and LDL; after centrifugation, they were immediately frozen at -20° until assayed. The different assays with their intra- and interassay CVs used in this study are described elsewhere[20].

SDS values were calculated for IGF-I, IGFBP-3, and IGF-I/IGFBP-3 ratio, according to age- and sex-matched reference values from the Dutch population[27].

Homeostatic model assessment of insulin resistance (HOMA-IR) was performed using the model HOMA-IR = (fasting insulin x fasting glucose)/22.5[28].

### Blood pressure

Systolic and diastolic blood pressure were measured using an appropriately sized cuff while patients were 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[29]. None of the children were receiving antihypertensive therapy.

### Assessment of bone maturation

Radiographs of the left hand and wrist were taken annually. Bone age (BA) was assessed in duplo by two independent observers (N.E.B. and R.J.K.) using the method of Greulich and Pyle[30]. The mean difference (95% confidence interval) between the observers was -0.015 yr (-0.04 to 0.01). The mean BA was used for further analysis. We used mixed models and corrected for age to calculate the intraclass correlation coefficient, which was 0.97. The bone-age-to-calendar-age ratio (BA/CA) was calculated.

### Statistics

Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL). Most of our data did not have a Gaussian distribution, and are therefore expressed as median (interquartile range, IQR). SDS were compared with 0 or +2 SDS using Mann-Whitney U test. Changes over time were analyzed using repeated measurements analysis with years of GH-use as categorical independent variable and an unstructured covariance matrix or when more applicable a first-order autoregressive (AR(1)) heterogeneous covariance matrix for the measurements within each child. Repeated measurements analysis was also performed to investigate the effects of different parameters on various outcomes. Effects are presented as estimated marginal mean (standard error of the mean, SEM). A regression analysis was performed to determine associations of IGF-I SDS with LBM SDS and fat% SDS. To evaluate the effect of a lower GH dose in a subgroup of 10 children, we used the Wilcoxon signed-rank test. *P* values less than 0.05 were considered statistically significant.

## RESULTS

### Baseline

Table 1 shows the baseline characteristics of 60 children with PWS who have been receiving GH treatment for 8 years. Median (IQR) age at start of GH treatment was 5.49 (3.13 to 7.16) years. All children were prepubertal at start of GH treatment and 10 of them remained prepubertal during the study period. Thirty patients had a deletion, 22 a maternal uniparental disomy and 6 an imprinting center defect (ICD) or a translocation on chromosome 15. Two boys had a positive methylation test, but the underlying genetic defect was not identified. Birth weight SDS and birth length SDS were significantly lower than Dutch references ($P < 0.0001$ and $P < 0.05$, resp.). Relative to 0 SDS, median (IQR) fat% was high (2.29 (1.80 to 2.56) SDS, $P < 0.0001$) and LBM was low (-2.53 (-3.13 to -2.01) SDS, $P < 0.0001$), indicating an unfavorable body composition at start of GH treatment. BMI SDS was significantly higher than Dutch references and significantly lower than PWS reference values (both $P < 0.0001$). Height SDS and head circumference SDS were significantly lower than in healthy children (both $P < 0.0001$), but height SDS was normal according to age-and sex-matched children with PWS. IGF-I and IGFBP-3 were lower than normal (both $P < 0.0001$).

The thirteen children who were excluded from analysis because they had received only 1-6 years of GH treatment were significantly older at start (12.02 (4.42 to 13.71) years, $P = 0.004$), had a higher fat% (2.84 (2.12 to 3.01) SDS, $P = 0.039$), and had lower IGF-I levels (-2.93 (-3.52 to -1.69) SDS, $P = 0.025$) than the group included.

**Table 1.** Baseline characteristics

| | N | % | |
|---|---|---|---|
| N (male/female) | 60 (33/27) | 55/45 | |
| Genetics | | | |
| deletion | 30 | 50.0 | |
| UPD | 22 | 36.7 | |
| ICD/translocation | 6 | 10.0 | |
| Unknown | 2 | 3.3 | |
| | Median | IQR | |
| Age at start (years) | 5.49 | 3.13 to 7.16 | |
| Birth weight SDS | -1.22 | -2.21 to -0.24 | [a] |
| Birth length SDS | -0.47 | -1.38 to 0.38 | [d] |
| Fat % SDS | 2.29 | 1.80 to 2.56 | [a] |
| LBM SDS | -2.53 | -3.13 to -2.01 | [a] |
| Height SDS | -2.31 | -3.05 to -1.53 | [a] |
| Height $SDS_{PWS}$ | -0.11 | -0.60 to 0.36 | |
| Weight to height SDS | 1.28 | -0.36 to 2.03 | [a] |
| Head circumference SDS | -0.81 | -1.49 to -0.27 | [a] |
| BMI SDS | 1.12 | -0.21 to 1.69 | [a] |
| BMI $SDS_{PWS}$ | -0.52 | -1.03 to -0.04 | [a] |
| IGF-I SDS | -2.03 | -2.61 to -1.06 | [a] |
| IGFBP-3 SDS | -2.22 | -3.11 to -1.59 | [a] |

Baseline characteristics of 60 children with PWS. Data are expressed as median (IQR), SDS according to age-and sex matched Dutch reference values[24-27]; $SDS_{PWS}$ according to age and sex matched PWS references values[15].
[a] $P<0.0001$; [b] $P< 0.001$; [c] $P< 0.01$; [d] $P< 0.05$, compared to 0 SDS.

## Efficacy

*Body composition*

Figure 1A shows the course of LBM during 8 years of GH treatment. Mean (SEM) LBM was low at baseline (-2.54 (0.18) SDS), but increased significantly ($P < 0.0001$) during the first year of GH treatment. In the subsequent 7 years of GH treatment, LBM remained very stable and without significant changes over time. After eight years of treatment, LBM SDS was still in the low to normal range, and higher than at baseline (-1.5 (0.21) SDS, $P < 0.0001$).

Figure 1B shows the development of fat% during 8 years of GH treatment. During the first year of GH treatment, mean (SEM) fat% decreased significantly ($P < 0.0001$). After the first year of GH treatment, fat% gradually increased over the subsequent 7 years; after 8 years, it was, however, not significantly different than at baseline (2.30 (0.10) SDS, $P = 0.06$). Unfortunately, we could not compare the fat% over the years with those of untreated PWS references, as these do not exist.

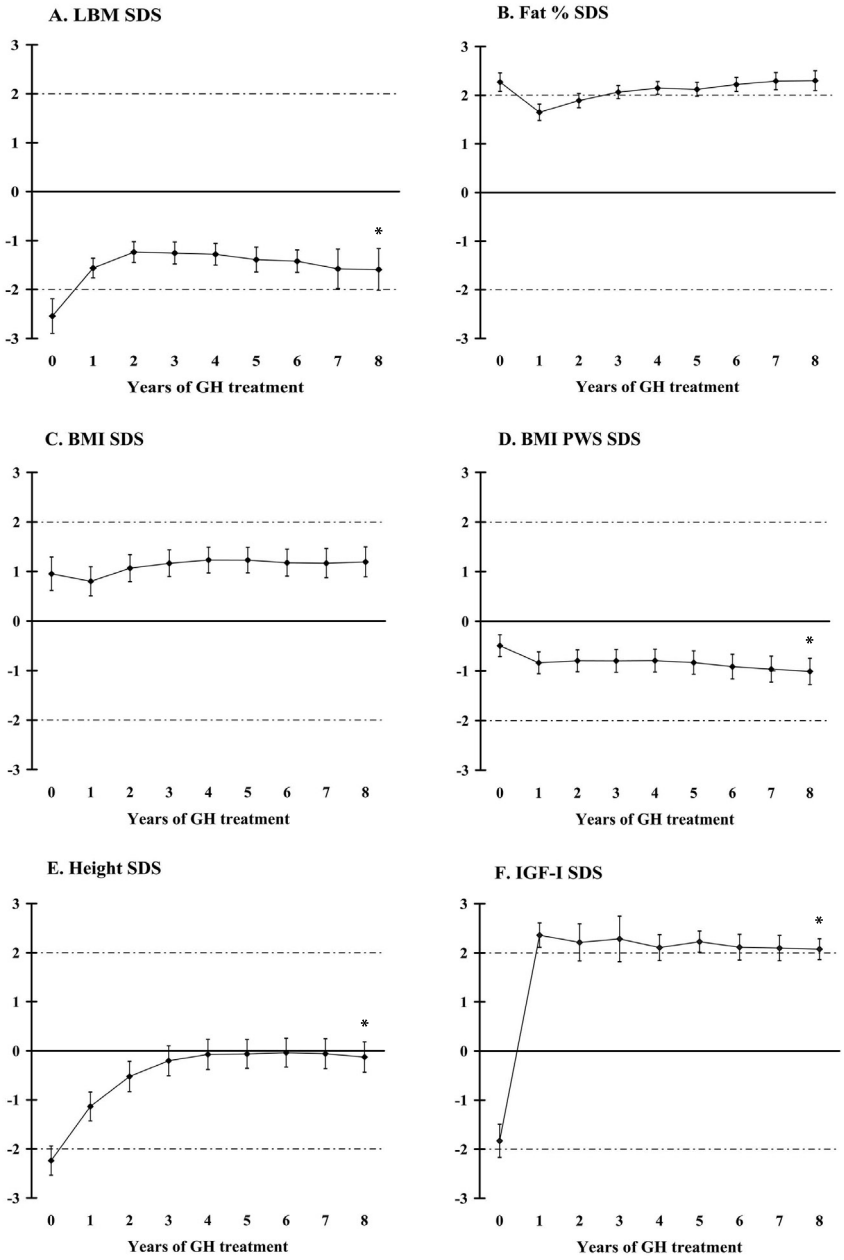


**Figure 1.** Longitudinal changes in Estimated Marginal Means with 95% CI in body composition, growth and IGF-I levels in 60 children with PWS during 8 years of GH treatment. LBM SDS and fat% SDS were calculated according to age- and sex-matched Dutch reference values[24]. BMI SDS and height SDS were according to age- and sex-matched Dutch reference children[25, 26]. BMI $SDS_{PWS}$ according to untreated age- and sex-matched children with PWS[15]. IGF-I SDS was calculated according to age- and sex-matched Dutch reference values[27].

* $P < 0.0001$ compared to baseline

Additionally we investigated the association of IGF-I SDS with LBM SDS and of fat% SDS with IGF-I. After 4 years of GH treatment, the association of IGF-I with LBM SDS was significant, β = 0.34; $p = 0.02$, but no association was found with fat% SDS. After 8 years of treatment, there was no association of IGF-I SDS with LBM SDS ($p = 0.19$) and fat% SDS.

*Body Mass Index (BMI)*

Figure 1C and 1D show BMI SDS and BMI $SDS_{PWS}$. Although mean BMI SDS decreased slightly during the first year of GH treatment, BMI SDS remained stable in the subsequent 7 years and after 8 years it was not significantly different from baseline BMI SDS ($P = 0.14$). Compared to PWS references, however, the mean (SEM) $BMI_{PWS}$ decreased significantly from -0.49 (0.11) SDS to -0.84 (0.11) SDS ($P < 0.0001$) during the first year of treatment. This positive effect persisted during the entire study period. As a result, the $BMI_{PWS}$ after 8 years of GH treatment was -1.01 (0.13) SDS, which was significantly lower than at baseline ($P < 0.0001$) (see also Figure 2).

*Anthropometry*

Figure 1E shows the development of height SDS during 8 years of GH treatment. During the first 4 years, height SDS normalized. Baseline mean (SEM) height improved significantly from -2.24 (0.15) SDS to -0.08 (0.15) SDS ($P < 0.0001$). In the subsequent 4 years of treatment, it remained stable. After 8 years, height SDS was not significantly different than in Dutch reference children ($P = 0.38$) (see also Figure 2).

Mean head circumference SDS increased significantly during the first year of GH treatment ($P < 0.0001$). The size after 8 years of treatment was not significantly different from that in Dutch reference children ($P = 0.74$).

## Safety

Table 2 shows all the safety parameters at baseline and after 2, 4, 6, and 8 years of GH treatment.

*Serum IGF-I and IGFBP-3 levels*

Figure 1F shows the course of IGF-I SDS over 8 years of GH treatment. Mean (SEM) IGF-I increased during the first year of treatment, from -1.83 (0.17) SDS to 2.36 (0.12) SDS ($P < 0.0001$). After the first year, IGF-I SDS levels decreased to 2.11 (0.13) SDS at 4 years of treatment. On average, the IGF-I levels were just above 2 SDS; after 8 years of treatment they were not significantly higher than 2 SDS ($P = 0.42$). Mean (SEM) IGFBP-3 increased significantly during the first year of GH treatment from -2.28 (0.18) to 0.49 (0.13) ($P < 0.0001$) and remained so in the subsequent 7 years. After 8 years of treatment, IGFBP-3 was still significantly higher than at baseline ($P < 0.0001$). The IGF-I/IGFBP-3 molar ratio

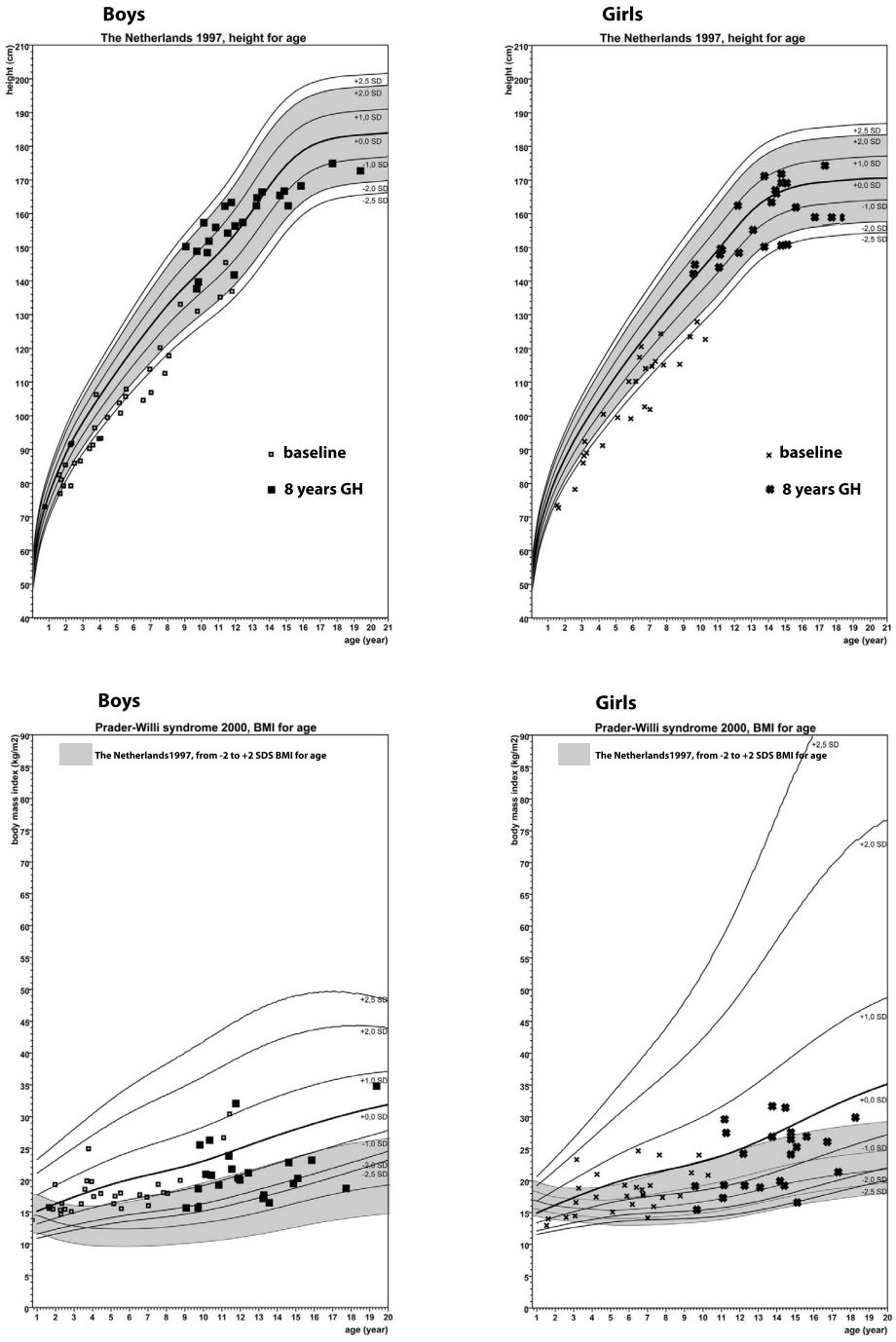


**Figure 2.** Height SDS and BMI SDS and BMI $SDS_{PWS}$ at baseline and after 8 years of GH treatment for boys and girls. SDS according to age- and sex-matched Dutch reference values and PWS reference values[15, 27]. Boys: □ baseline, ■ 8 years of GH treatment; Girls: ✕ baseline, ✖ 8 years of GH treatment

**Table 2.** Safety parameters during 8 years of GH treatment

| | Baseline | 2 yr | 4 yr | 6 yr | 8 yr |
|---|---|---|---|---|---|
| IGF-I SDS | -1.83 (0.17) | 2.21 (0.19)[a] | 2.11 (0.13)[a] | 2.11 (0.13)[a] | 2.07 (0.11)[a] |
| IGFBP-3 SDS | -2.28 (0.18) | 1.75 (0.17)[a] | 2.83 (0.10)[a] | 2.88 (0.09)[a] | 2.86 (0.11)[a] |
| IGF-I/IGFBP-3 ratio SDS | 0.22 (0.01) | 0.47 (0.03)[a] | 0.33 (0.01)[a] | 0.37 (0.01)[a] | 0.40 (0.01)[a] |
| Glucose (mmol/liter) | 4.50 (0.07) | 4.59 (0.07) | 4.69 (0.05)[d] | 4.79 (0.06)[b] | 4.81 (0.05)[b] |
| Insulin (mU/liter) | 8.00 (0.81) | 10.71 (1.36) | 9.82 (0.85) | 11.42 (1.01)[c] | 11.34 (1.07)[c] |
| HOMA-IR | 1.66 (0.18) | 2.18 (0.29) | 2.03 (0.19) | 2.49 (0.23)[c] | 2.41 (0.24)[c] |
| Total cholesterol (mmol/liter) | 4.75 (0.13) | 4.50 (0.10) | 4.40 (0.09)[c] | 4.40 (0.11)[c] | 4.35 (0.09)[c] |
| HDL cholesterol (mmol/liter) | 1.33 (0.10) | 1.24 (0.06) | 1.42 (0.04) | 1.44 (0.04) | 1.49 (0.05) |
| LDL cholesterol (mmol/liter) | 3.07 (0.12) | 2.91 (0.09) | 2.62 (0.08)[b] | 2.54 (0.09)[a] | 2.56 (0.08)[a] |
| Systolic blood pressure SDS | 0.55 (0.13) | 0.47 (0.09) | 0.44 (0.13) | 0.47 (0.11) | 0.10 (0.14)[d] |
| Diastolic blood pressure SDS | 0.53 (0.13) | 0.47 (0.09) | 0.45 (0.08) | 0.53 (0.08) | 0.44 (0.10) |
| BA/CA ratio | 0.79 (0.03) | 0.94 (0.02)[a] | 1.03 (0.02)[a] | 1.04 (0.02)[a] | 1.03 (0.02)[a] |

Safety parameters during 8 years of GH treatment at baseline, 2 years, 4 years, 6 years and 8 years of GH treatment.

Overview of IGF-I, IGFBP-3, bioavailability of IGF-I, glucose homeostasis, serum lipids, and blood pressure in the total study population during 8 years of GH treatment.

Data are expressed as estimated means (SEM), SDS according to age- and sex-matched Dutch reference values[27] or according to height-matched reference values blood pressure[29].

[a] $P < 0.0001$; [b] $P < 0.001$; [c] $P < 0.01$; [d] $P < 0.05$, compared to baseline.

increased significantly during the first year of treatment ($P < 0.0001$). Thereafter the ratio stabilized, resulting in a mean (SEM) of 0.40 (0.01) after 8 years of treatment.

*Fasting glucose, insulin and HOMA-IR*

During the first year of GH treatment, insulin increased significantly ($P = 0.031$). After 8 years of GH treatment, fasting insulin was not significantly higher than after 1 year of treatment ($P = 0.40$), but was still significantly higher than at baseline ($P = 0.006$). Like fasting insulin, HOMA-IR increased significantly in the first year of treatment ($P = 0.031$), but remained stable, and after 8 years was not significantly different than at 1 year of treatment ($P = 0.41$).

During GH treatment, mean (SEM) fasting glucose levels gradually increased from 4.50 (0.07) mmol/L at baseline to 4.81 (0.05) mmol/L after 8 years of treatment ($P < 0.001$). None of the children developed diabetes mellitus (DM) type II. One Caucasian girl developed DM type I after 36 months of GH treatment. She had no positive family history for DM type I or auto immune diseases. At onset of the DM type I, her BMI SDS was 0.77, fat% SDS 1.47 and height SDS 0.90. After 8 years of GH treatment, her BMI, fat% and height were still within the normal range, (0.09 SDS, 1.36 SDS and 0.18 SDS, resp.).

Fat% SDS had a significant association with fasting insulin levels ($\beta$ = 2.25, 95% CI 1.05 to 3.45, $P < 0.0001$) and with HOMA-IR ($\beta$ = 0.42, 95% CI 0.14 to 0.70, $P = 0.003$), but had no significant association with fasting glucose levels ($P = 0.08$).

*Lipids*

During 8 years of GH treatment, total cholesterol and LDL levels decreased significantly compared to baseline ($P = 0.005$ and $P < 0.0001$, resp.). HDL levels did not change significantly during GH treatment ($P = 0.13$).

Fat% SDS was significantly associated with HDL levels ($\beta$ = -0.09, 95% CI -0.17 to -0.02, $P = 0.017$), but had no significant associations with total cholesterol levels and LDL levels.

*Blood pressure*

After 8 years of GH treatment, systolic blood pressure SDS decreased significantly compared to baseline ($P < 0.05$). Diastolic blood pressure SDS did not change during 8 years of treatment ($P = 0.64$).

*Bone maturation*

To evaluate the progression of bone maturation and the influence of GH treatment on this process, we calculated BA/CA ratios. Before GH treatment, bone age was delayed, with a mean (SEM) BA/CA ratio of 0.79 (0.034) ($P < 0.0001$, compared to 1). During the subsequent 7 years of GH treatment, the BA/CA ratio was not significantly different compared to 1 ($P = 0.129$).

*GH dose*

Although all children started GH treatment with 1 mg/m$^2$/day, GH dose was reduced in 10 children (16.67%; 8 boys) to a median (IQR) dose of 0.5 (0.5 to 0.66) mg/m$^2$/day, because of high IGF-I levels above 3 SDS. This occurred after a median (IQR) duration of 4 (1.75 to 6) years of GH treatment. One year after treatment on the lower GH dose, IGF-I had significantly decreased to 2.45 (2.17 to 3.24) SDS ($P = 0.017$) but fat% had increased significantly to 2.25 (1.21 to 2.44) SDS ($P = 0.043$). In these 10 children, the IGF-I/ IGFBP-3 molar ratio, LBM SDS and height SDS did not significantly change after one year of GH treatment on the lower dose.

## DISCUSSION

In this first 8-year longitudinal study in a large group of 60 children with Prader-Willi syndrome treated with GH 1 mg/m$^2$/day, we demonstrate that GH treatment has positive long-term effects on maintaining body composition, and thus on inhibiting the clinical

course of PWS. LBM SDS, BMI $SDS_{PWS}$, height SDS and head circumference SDS improved significantly during the first year of GH treatment, remaining at the same level over the subsequent 7 years of treatment. Safety parameters such as total cholesterol, LDL levels and the systolic blood pressure also significantly improve.

Our study shows that long-term GH treatment in combination with a strict diet and exercise programs has a continued beneficial effect on LBM. After 8 years of treatment, LBM SDS was still higher than at baseline. This improvement in LBM is very important for children with PWS with regard to psychomotor development. The positive effects of long-term GH treatment on fat% SDS are more complex. We hypothesized that 8 years of GH treatment would maintain the improved body composition, but that, over the years, the clinical course of PWS, reflected in a higher fat% and a lower lean body mass (LBM), would interfere with it. While it did increase gradually over the subsequent 7 years, fat% SDS in children with PWS improved significantly during the first year of GH treatment. After 8 years of GH treatment, it had returned to baseline SDS. An explanation for the gradual increase in fat% SDS over the years is the clinical course of PWS[31, 32], which is not fully restrained by GH treatment. As fat% SD scores for untreated PWS children do not exist, we could make no comparisons with reference data on body composition in PWS. Such a comparison was possible for BMI SDS. BMI SDS after 8 years of GH treatment was not significantly lower than at baseline, but had remained within the normal range. Having a BMI SDS in the upper normal range of Dutch healthy reference children is very acceptable for children with PWS. BMI $SDS_{PWS}$ also improved over the years and after 8 years was still significantly lower than at baseline. These data indicate GH treatment has a positive effect on body composition. We therefore conclude that long-term GH treatment, in combination with a strict diet and exercise programs, is able to inhibit the natural development of increasing obesity in children with PWS, it may improve the future health for these children.

Height SDS completely normalized during 8 years of GH treatment, which is in line with previous studies with a shorter follow-up period[9, 11, 16]. The same was true for head circumference SDS. The clinical relevance of achieving a normal head circumference is uncertain, but parallels the positive effects of GH treatment on cognition[12].

Our findings on safety parameters indicate that GH treatment had no adverse effects on glucose parameters, lipid profile and blood pressure. Fasting glucose and insulin levels increased slightly during 8 years of GH treatment, but remained within the normal ranges. No cases of non-insulin dependent diabetes mellitus occurred. It is known that adults with PWS have a high incidence of DM II[33]. Our results are in accordance with earlier studies[9, 11, 34] and indicate that GH had a beneficial effect on body composition and prevents the development of DM II in children and adolescents with PWS during treatment. We previously published that GH treatment did not adversely affect apnea index and sleep, measured by polysomnography, and onset and progression of scoliosis[19, 35]. Comparable results were found during the 8-years follow-up study.

Total cholesterol and LDL serum levels slightly improved during 8 years of GH treatment. HDL levels did not significantly change during GH treatment, in line with earlier studies[9, 36]. GH treatment significantly improved the systolic blood pressure in line with *Van Dijk et al.*[37], but had no effect on the mean diastolic blood pressure.

The BA/CA ratio showed that bone age at baseline was slightly delayed. Eight years of GH treatment resulted in a complete normalization of bone maturation, indicating that GH treatment represents no risk of rapid bone maturation.

The mean IGF-I level increased in the first year of treatment to just above +2.0 SDS, remaining there for the subsequent 7 years. *Colmenares et al.* published the same levels of IGF-I SDS during a 3-year study[9]. The IGF-I/IGFBP-3 molar ratio increased after the first year of treatment and then stabilized around 0.4, a normal value for healthy children[38]. This is in line with data by *Feigerlova et al.* in a 2-year study that evaluated GH treatment in children with PWS and children with GH deficiency[39]. Despite the higher IGF-I levels in the PWS study group, they found the same IGF-I/IGFBP-3 ratio in both groups after 2 years of GH treatment and concluded that the unbound IGF-I levels were the same in both treatment groups, thus moderating the risks of high IGF-I levels in children with PWS[39]. Also other studies found high IGF-I levels in parallel with high IGFBP-3 levels in response to GH treatment[7, 9, 39]. Therefore, GH dose titration based on the IGF-I/IFGBP-3 ratio might be more adequate in children with PWS. To maintain a good body composition, children with PWS seem to require relatively high IGF-I levels. In our 8-year study, 10 children were changed to a lower GH dose due to IGF-I levels > 3 SDS. The GH dose was reduced to 0.50-0.67 mg/m$^2$/day, dependent on the IGF-I levels. Unfortunately, this resulted in a less favorable body composition measured by DXA. We also found a positive association between IGF-I SDS and LBM SDS after 4 years of treatment, indicating that a 1 SDS higher IGF-I level associates with a 0.34 SDS higher LBM. After 8 years of treatment, however, no association was found, probably due to a wide variation in LBM SDS, likely due to a wide variation in pubertal stages.

In a study by *Carrel et al*, a low dose of 0.3 mg GH/m$^2$/day did not improve body composition, while 1 mg/m$^2$/day and 1.5 mg/m$^2$/day did[7], demonstrating that fat% and LBM are GH-dose dependent in children with PWS. In children with PWS, it appears difficult to find the right balance between the positive effects of GH on body composition and possible risks of higher serum IGF-I levels.

To analyze body composition, we included only children who had received 8 years of consecutive GH treatment. We did not analyze children who had dropped-out or had reached adult height and did not receive 8 years of GH. As this occurred randomly, no biases were introduced into our results.

In conclusion, our study demonstrates positive long-term effects of GH treatment in children with PWS on improving and then maintaining their body composition, height SDS and head circumference SDS. These results show that GH treatment in combination

with a healthy lifestyle can counteract the clinical course of PWS, which is very important, because development towards morbid obesity is a major threat to these patients. There were no adverse effects on glucose parameters, lipid profile and blood pressure. As GH treatment with 1 mg/m$^2$/day results in relatively high IGF-I levels, it is recommended that IGF-I levels should be monitored closely and that GH dose should be adjusted when indicated.



## ACKNOWLEDGEMENTS

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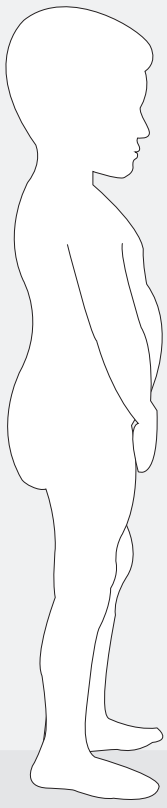

# CHAPTER 4

## Bone mineral density in children with Prader-Willi Syndrome: a longitudinal study during puberty and 9 years of growth hormone treatment

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## ABSTRACT

***Context*** Longitudinal data on bone mineral density (BMD) in children and adolescents with PWS during long-term GH treatment are not available.

***Objective*** To determine effects of long-term GH treatment and puberty on BMD of total body ($BMD_{TB}$), lumbar spine ($BMD_{LS}$) and bone mineral apparent density of the lumbar spine ($BMAD_{LS}$) in children with PWS.

***Design*** Prospective longitudinal study.

***Setting*** Dutch PWS Cohort.

***Participants*** Seventy-seven children with PWS who remained prepubertal during GH treatment for 4 years and 64 children with PWS who received GH treatment for 9 years.

*Intervention* GH treatment 1 mg/$m^2$/day (≅0.035 mg/kg/day).

***Main outcome measures*** $BMD_{TB}$, $BMD_{LS}$ and $BMAD_{LS}$ by using the same dual-energy x-ray absorptiometry (DXA) machine for all annual measurements.

***Results*** In the prepubertal group, $BMD_{TB}$SDS and $BMD_{LS}$SDS significantly increased during 4 years of GH treatment while $BMAD_{LS}$SDS remained stable. During adolescence, $BMD_{TB}$SDS and $BMAD_{LS}$SDS decreased significantly, in girls from the age of 11 years and in boys from the age of 14 and 16 years, resp., but all BMDs remained within the normal range. Higher Tanner stage tended to be associated with lower $BMD_{TB}$SDS ($P = 0.083$) and a significantly lower $BMAD_{LS}$SDS ($P = 0.016$). After 9 years of GH treatment, lean body mass SDS was the most powerful predictor of $BMD_{TB}$SDS and $BMD_{LS}$SDS in adolescents with PWS.

***Conclusions*** This long-term GH study demonstrates that $BMD_{TB}$, $BMD_{LS}$ and $BMAD_{LS}$ remains stable in prepubertal children with PWS but decreases during adolescence, parallel to incomplete pubertal development. Based on our findings, clinicians should start sex hormone therapy from the age of 11 in girls and 14 in boys, unless there is a normal progression of puberty.

## INTRODUCTION

Prader-Willi syndrome (PWS) is a neurogenetic developmental disorder caused by the absence of paternal expression of genes on chromosome 15 at the locus q11-q13, due to a paternal deletion, maternal uniparental disomy, imprinting defects or paternal chromosomal translocation. The syndrome is characterized by short stature, hypogonadism, hypotonia, mild mental retardation and early onset obesity with behavioral problems[1]. Many of the symptoms in children with PWS may be explained by hypothalamic dysfunction, with endocrinopathies like growth hormone deficiency, hypogonadism, central adrenal insufficiency during stress and sometimes hypothyroidism. Growth hormone (GH) treatment improves body composition, adult height, psychomotor development and cognition[2-5]. Other hormone replacement therapies are hydrocortisone in case of stressful events and sex hormones[6-8].



Bone mineral density (BMD) is influenced by activity and endocrine factors, including parathyroid hormone, thyroxine, GH and sex steroids[9, 10]. BMD increases during childhood and peak bone mass is normally attained between the age of 18-20 years in girls and 18-23 years in boys[11]. Bone strength later in life largely depends on this attained peak bone mass. Children with PWS might be prone to a lower BMD during childhood and adolescence because of low GH levels, hypothyroidism, hypogonadism and a sedentary lifestyle. Extra mechanical stress due to obesity will increase the BMD of the lumbar spine[12], but most GH-treated children with PWS are not obese anymore[2].

Studies in prepubertal children with PWS showed that patients not treated with GH had a normal axial and appendicular BMD[13] while total body BMD ($BMD_{TB}$) SDS and lumbar spine BMD ($BMD_{LS}$) SDS remained unchanged during 2 or 3 years of GH treatment[14-16]. However, in 17-37 year old adults with PWS, who did not receive GH treatment during childhood and only one men received testosterone, a lower BMD than age- and sex-matched controls was reported, albeit still in the normal range[17]. In addition, more osteoporosis and a high fracture risk has been observed in adults with PWS[17-20]. In 42 adults with PWS, mean age 28.5 ± 6.7 years, the baseline $BMD_{TB}$ Z-score of -1.0 and $BMD_{LS}$ of -1.4 did not improve during two years of GH treatment[21], 15 of the 42 patients received sex hormone replacement therapy. This suggests that there is a decline in all bone mineral density parameters (BMDs) in adolescents with PWS, in contrast to age- and sex-matched peers. Due to the fact that children with PWS have a normal onset of puberty, but a delay in pubertal development beyond Tanner stage 2 compared to age- and sex-matched controls[22, 23], it might well be that BMDs decline after Tanner stage 2, when puberty fails to progress in patients with PWS.

Currently, there are no data on BMDs in children and adolescents with PWS during long-term GH treatment. We hypothesized that BMDs SDS would remain stable during 4 years of GH treatment in children who remained prepubertal during that period, but

would decline during adolescence, due to incomplete pubertal development. For that reason, we investigated $BMD_{TB}$SDS, $BMD_{LS}$SDS and lumbar spine bone mineral apparent density ($BMAD_{LS}$) SDS during 9 years of GH treatment in children with PWS. In addition, we studied the relation between age, pubertal stage and $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS.

## METHODS

### Patients

For the first part of our study, we included 77 prepubertal children who remained prepubertal during 4 years of GH treatment in our Dutch PWS Cohort Study, and for the second part, 64 children who continued GH treatment for 8 to 11 years. The Dutch PWS Cohort was established in 2002 with the start of a randomized controlled GH trial[24], which was continued by a longitudinal GH study including the majority of Dutch children with PWS[2, 25]. Thirty-three children participated in the prepubertal and the long-term study. At time of GH start, in the prepubertal group, girls were 2 to 10 years old and boys were 2 to 11 years old, and in the long-term group, girls were younger than 11 years with Tanner breast stage 1 and boys were younger than 12 years with Tanner genital stage 1 and a testicular volume less than 4 ml[26]. All children were naive to GH treatment at the start of study and had continuous GH treatment for 4 resp. 9 years.

During the study, six girls (6/31) and five boys (5/33) received sex hormone replacement treatment because of a combination of low bone mineral density (< -1SDS), low serum estradiol or testosterone levels and a personal preference of the child and the parents. We included their data in our analyses until they started sex hormone replacement treatment. The other children did not receive sex hormone replacement treatment, mainly because of parental fear for behavioral issues in boys and hygiene concerns in girls.

All children had a genetically confirmed diagnosis of PWS. The study protocol was approved by the Medical Ethics Committees at Children's Hospital Erasmus MC- Sophia in Rotterdam, the Netherlands, and of collaborating centers. Written informed consent was obtained from parents and children over 12 years. Assent was obtained from children under 12 years.

### Design

The primary objective of our study was to evaluate the effects of GH treatment on $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS in prepubertal children with PWS. The secondary objective was to evaluate the effects of GH treatment on $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS in children and adolescents with PWS during 9 years of GH treatment. Our

final objective was to investigate the relation between age, pubertal stage and $BMD_{TB}$-SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS.

Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY) 1 mg/$m^2$/day was administered subcutaneous once daily at bedtime. The first four weeks of GH treatment, children received 0.5 mg/$m^2$/day to prevent fluid retention[27]. 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. The GH dose was lowered when immunoreactive IGF-I levels increased above +3 SDS. Pubertal stage according to Tanner was determined by the PWS research team of the Dutch Growth Research Foundation at the time of blood sampling and during the 3-monthly visits. All measurements described in this study were yearly performed in the Erasmus University Medical Center Rotterdam-Sophia Children's Hospital.



### Dual-energy x-ray absorptiometry (DXA)

In all children, bone mineral content (BMC; in grams) and BMD (in grams per square centimeter) of the total body and lumbar spine, fat mass, and lean body mass (LBM) were measured by 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 $BMD_{LS}$ is underestimated by the areal presentation and should be corrected for bone size by calculating the $BMAD_{LS}$[28]. $BMAD_{LS}$ was calculated using the following

model: $BMAD_{LS} = BMD_{LS}*[4/(\pi*width)]$, 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[28]. $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS were calculated to age- and sex-matched reference values from the Dutch population[29, 30]. Fat mass was expressed as percentage of total body mass (fat%). Fat% SDS and LBM SDS were calculated according to sex- and height-matched reference values of the Dutch population[29, 30].

### 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; Servo Berkel Prior, Katwijk, The Netherlands). Height SDS and BMI SDS were calculated with Growth Analyser Research Calculation Tools 4.0 (available at www.growthanalyser.org), according to Dutch age- and sex-matched reference values[31].

### Hormone assays

Blood samples were collected for assessment of serum IGF-I, testosterone and estradiol. The first 2 years of study, serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intra- and interassay CVs were 4 and 6%, respectively. After 2 years, IGF-I was measured with the Immulite 2000 (Siemens Healthcare Diagnostics, Deerfield, IL), with interassay CV of 6.5%.

Total serum testosterone levels were determined by coated tube RIA (Siemens DPC). The intra- and interassay CVs were below 6 and 9%, respectively. Lowest detectable level was 0.1 nmol/l. These levels were all higher than those calculated from blank values (+ 3 SD of the blank). Reference values prepubertal (nmol/l) boys 0.3-0.5, pubertal boys 3.0-6.5 and man 10-30. Serum estradiol levels were measured using coated tube radioimmuno-assays (Immulite, Diagnostic Products Corp., Los Angeles, CA, USA). Sensitivities of the assay was 10 pmol/l and the intra- and interassay CVs were less than 5% and 7%. Reference values woman (pmol/l) early follicular 50-250, late follicular 250-1000, LH peak 400-1500, mid luteal 250-1000, late luteal 150-250 and post-menopausal < 50.

### Statistics

Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL). Most of our data did not have a Gaussian distribution, and were therefore expressed as median [interquartile range, IQR]. SDS were compared with 0 using Mann-Whitney U test. Changes over time were analyzed using repeated measurements analysis with years of GH use as categorical independent variable and a first-order autoregressive (AR(1)) or the AR(1) heterogeneous covariance matrix for the measurements within each child. The effects of genetic background, gender, age and Tanner stage were determined by using these variables as factors (in case of nominal or ordinal data) and as covariates (in case of scale variables) in the model. Effects are presented as estimated marginal mean (standard error of the mean, SEM). Multiple linear regression analysis was performed to determine the association of $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS with age at start GH, fat% SDS and LBM SDS. In all multiple linear regression models, adjustments were made for gender, height SDS and Tanner stage. All regression coefficients are presented as a standardized Beta (β) for better interpretation of the results. Median age at reaching each pubertal Tanner mammae (M2-5) or genital (G2-5) stage was estimated separately for girls and boys with PWS, by Kaplan Meier survival estimates. *P* values less than 0.05 were considered statistically significant.

## RESULTS

### Baseline characteristics

Table 1 shows the baseline characteristics of 77 children with PWS who remained prepubertal during GH treatment for 4 years (prepubertal group) and 64 children with PWS who have been receiving GH treatment for 9 years (long-term group). Median [IQR] age at start of GH treatment was 5.5 [3.2 to 7.5] years in the prepubertal group and 5.6 [3.4 to 7.5] years in the long-term group.

In the prepubertal group, median [IQR] baseline $BMD_{TB}$SDS was normal according to age- and sex-matched healthy children (0.0 [-1.0 to 0.8]) and $BMD_{LS}$SDS was significantly lower than 0 SDS, but in the normal range (-0.3 [-1.0 to 0.0]). In the long-term group, $BMD_{TB}$SDS and $BMD_{LS}$SDS were normal according to age- and sex-matched healthy children (0.2 [-0.6 to 0.7] and -0.3 [-1.1 to 0.7], resp.). Height SDS was significantly lower than Dutch references (-2.3 [-3.1 to -1.5], $P < 0.0001$ and -2.4 [-3.1 to -1.8], $P < 0.0001$, resp.). Because of the short stature in children with PWS, we also calculated $BMAD_{LS}$SDS.



**Table 1.** Baseline characteristics at start of GH treatment

| | Prepubertal group | | | Long-term group | | |
|---|---|---|---|---|---|---|
| | N | % | | N | % | |
| N (male/female) | 77 (37/40) | 48/52 | | 64 (33/31) | 52/48 | |
| Genetics | | | | | | |
| Deletion | 34 | 44.2 | | 30 | 46.9 | |
| UPD | 34 | 44.2 | | 25 | 39.1 | |
| ICD/translocation | 4 | 5.2 | | 5 | 7.8 | |
| Unknown | 5 | 6.5 | | 4 | 6.3 | |
| | Median | IQR | | Median | IQR | |
| Age at start GH (years) | 5.5 | 3.2 to 7.5 | | 5.6 | 3.4 to 7.5 | |
| Age at start puberty females (years) | | | | 9.9 | 9.1 to 11.2 | |
| Age at start puberty males (years) | | | | 11.5 | 10.6 to 12.6 | |
| $BMD_{TB}$SDS | 0.0 | -1.0 to 0.8 | | 0.2 | -0.6 to 0.7 | |
| $BMD_{LS}$SDS | -0.3 | -1.0 to 0.0 | [d] | -0.3 | -1.1 to 0.7 | |
| $BMAD_{LS}$SDS | 0.4 | -0.2 to 1.3 | [c] | 1.0 | -0.2 to 1.8 | [b] |
| BMC SDS | -2.1 | -2.7 to -1.6 | [a] | -2.0 | -3.0 to -1.3 | [a] |
| Fat% SDS | 2.3 | 2.0 to 2.8 | [a] | 2.5 | 2.2 to 2.7 | [a] |
| LBM SDS | -2.2 | -2.7 to -1.8 | [a] | -2.3 | -2.9 to -1.9 | [a] |
| Height SDS | -2.3 | -3.1 to -1.5 | [a] | -2.4 | -3.1 to -1.8 | [a] |
| BMI SDS | 1.1 | 0.0 to 1.8 | [a] | 1.2 | -0.1 to 1.8 | [a] |

Data are expressed as median (IQR), SDS according to age-and sex-matched Dutch reference values[29-31].
[a] $P<0.0001$; [b] $P< 0.001$; [c] $P< 0.01$; [d] $P< 0.05$, compared to 0 SDS

Baseline $BMAD_{LS}SDS$ was well within the normal range, being 0.4 [-0.2 to 1.3] in the prepubertal group and 1.0 [-0.2 to 1.7] in the long-term group, respectively.

Body composition in the prepubertal and long-term group showed a high median [IQR] baseline fat% SDS (2.3 [2.0 to 2.8] and 2.5 [2.2 to 2.7], resp.) and a low LBM SDS (-2.2 [-2.7 to -1.8] and -2.3 [-2.9 to -1.9], resp.). BMI SDS was significantly higher than Dutch references (1.1 [0.0 to 1.8], $P < 0.0001$ and 1.2 [-0.1 to 1.8], $P < 0.0001$, resp.).

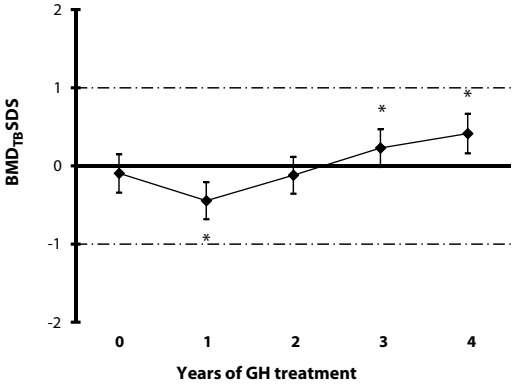
### Bone mineral density during 4 years of GH in prepubertal children

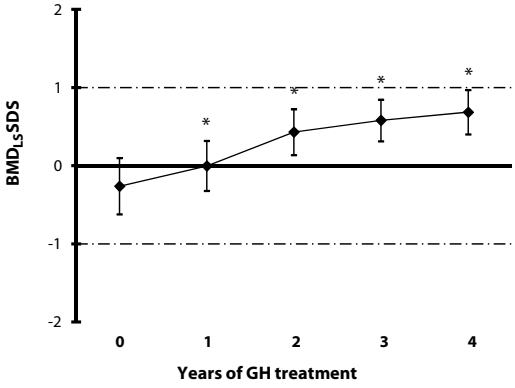
Figure 1 shows the course of $BMD_{TB}SDS$, $BMD_{LS}SDS$ and $BMAD_{LS}SDS$ during 4 years of GH treatment in 77 prepubertal children. During the first year of GH treatment, $BMD_{TB}SDS$ decreased significantly ($P < 0.001$). After the first year of GH treatment, mean (SEM) $BMD_{TB}SDS$ gradually increased to 0.42 (0.13) after 4 years of GH treatment ($P < 0.001$ compared to baseline). $BMD_{LS}SDS$ increased significantly ($P = 0.001$) during the first year of GH treatment. After the first year of GH treatment, $BMD_{LS}SDS$ remained significantly higher than at baseline, 0.69 (0.14); $P < 0.001$. $BMAD_{LS}SDS$ remained very stable over time, and was after 4 years of treatment not significantly different from baseline (0.90 (0.15); $P = 0.229$). Thus, during 4 years of GH treatment, $BMD_{TB}SDS$ and $BMD_{LS}SDS$ increased and $BMAD_{LS}SDS$ remained stable, between 0 and 1 SDS.

### Bone mineral density during 9 years of GH treatment

Figure 2 shows the course of $BMD_{TB}SDS$, $BMD_{LS}SDS$ and $BMAD_{LS}SDS$ during 9 years of GH treatment in 64 children, who became pubertal during this long-term study. After 9 years of GH treatment, the $BMD_{TB}SDS$ was not significantly different compared to baseline (-0.03 (0.16); $P = 0.716$). However, the $BMD_{TB}SDS$ was significantly lower after 9 years of GH treatment compared to the $BMD_{TB}SDS$ after 4 years of GH treatment (0.39 (0.14); $P = 0.001$). Also the $BMD_{LS}SDS$ was not significantly different compared to baseline (-0.01 (0.19); $P = 0.251$), after 9 years of GH treatment. However, also the $BMD_{LS}SDS$ was significantly lower after 9 years of GH treatment compared to the $BMD_{LS}SDS$ after 4 years of GH treatment (0.63 (0.17); $P < 0.001$). The $BMAD_{LS}SDS$ remained stable during the first 4 years (0.89 (0.16); P = 0.740) and then decreased to 0.10 (0.15), after 9 years of GH treatment ($P < 0.0001$, compared to 4 years).

Thus, despite the fact that BMDs improved or remained stable during the first 4 years of GH treatment, we observed a decline after 4 years of GH treatment in $BMD_{TB}SDS$, $BMD_{LS}SDS$ and $BMAD_{LS}SDS$. Because almost all patients became pubertal during the 9 year study, we investigated the course of $BMD_{TB}SDS$ and $BMAD_{LS}SDS$ according to age and pubertal development in the long-term group.



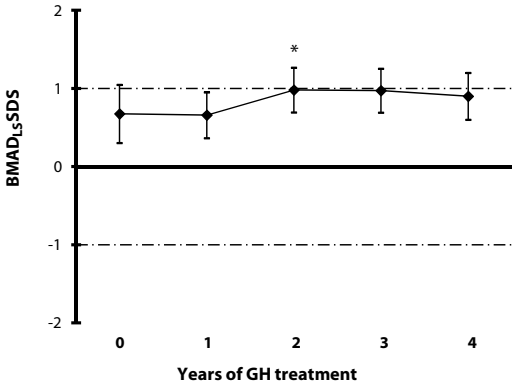
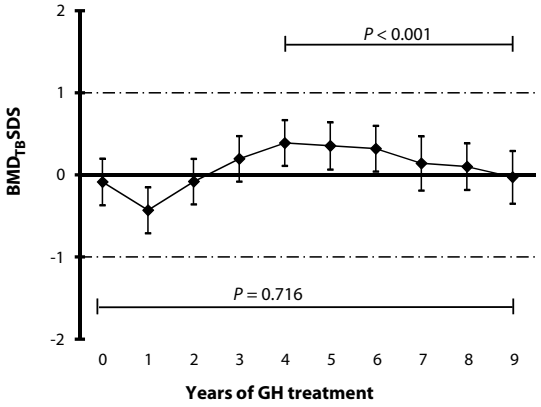
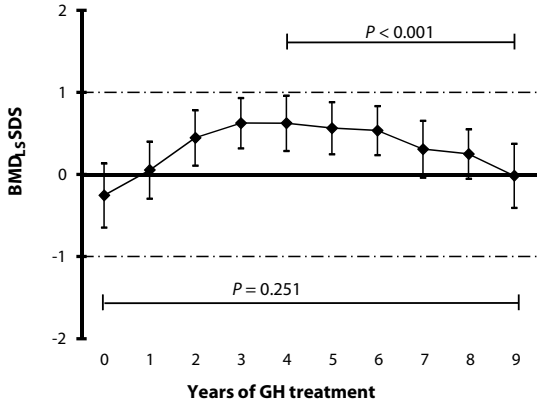




**Figure 1.** $BMD_{TB}$, $BMD_{LS}$ and $BMAD_{LS}$ SDS in prepubertal children with PWS during 4 years of GH treatment
*$P<0.05$, compared to baseline



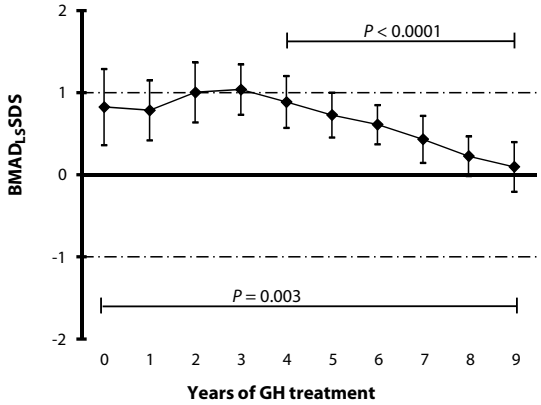




**Figure 2.** $BMD_{TB}$, $BMD_{LS}$ and $BMAD_{LS}$ SDS during 9 years of GH treatment

### Influence of variables on $BMD_{TB}SDS$ and $BMAD_{LS}SDS$

In the following analyses we used $BMAD_{LS}SDS$ instead of $BMD_{LS}SDS$, because the $BMAD_{LS}SDS$ is corrected for bone size.

*Genotype*

The course of $BMD_{TB}SDS$ and $BMAD_{LS}SDS$ over time was not significantly different between children with a deletion or an mUPD ($P = 0.300$). Other genotypes were not statistically tested since the numbers were too low.

*Age*

In girls, without estrogen substitution, mean (SEM) $BMD_{TB}SDS$ declined significantly from 0.46 (0.19) at the age of 11 years to 0.28 (0.19) at the age of 12 years ($P = 0.004$), with a further decline to -0.59 (0.27) at the age of 17 years, ($P < 0.0001$). $BMAD_{LS}SDS$ also declined significantly from 0.56 (0.19) at the age of 11 years to 0.30 (0.16) at the age of 12 years ($P = 0.020$). Subsequently, $BMAD_{LS}SDS$ decreased to -0.68 (0.21) at the age of 17 years ($P < 0.001$) (Figure 3A).

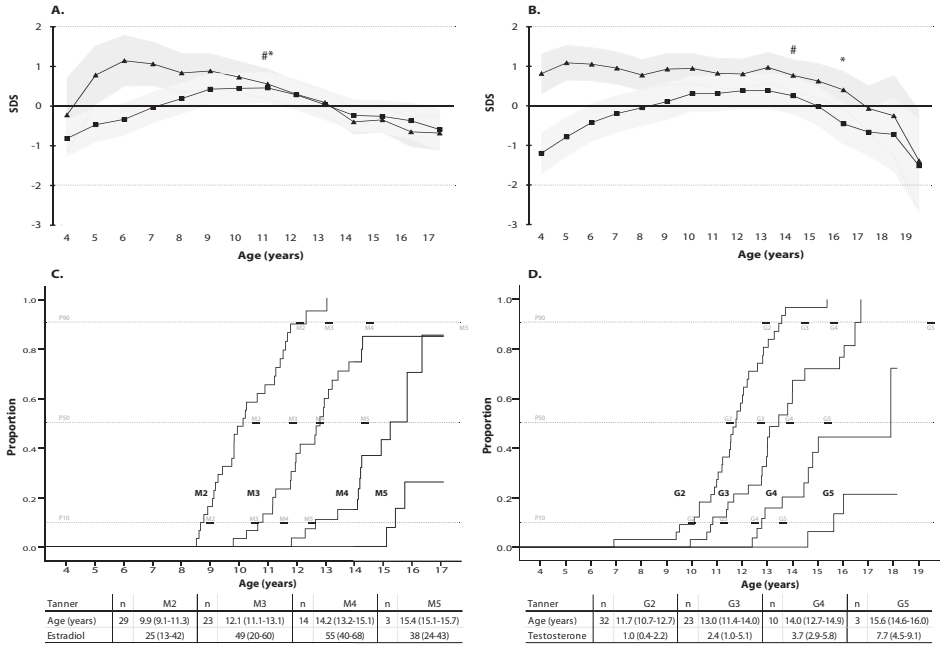
In boys, without testosterone substitution, mean (SEM) $BMD_{TB}SDS$ declined significantly from 0.25 (0.23) at the age of 14 years to -0.01 (0.25) at the age of 15 years, ($P = 0.048$), with a further decline to -1.52 (0.59) at the age of 19 years ($P = 0.002$). $BMAD_{LS}SDS$ declined significantly from 0.40 (0.24) at the age of 16 years to -0.07 (0.29) at the age of 17 years, ($P = 0.026$). Subsequently, $BMAD_{LS}SDS$ decreased to -1.39 (0.64) at the age of 19 years ($P = 0.005$) (Figure 3B).

*Tanner stages*

By introducing only Tanner stages in the mixed model analysis, we investigated the effect of Tanner stages on BMDs. Mean (SEM) $BMD_{TB}SDS$ declined from -0.02 (0.14) at T2 to -0.20 (0.16) at T4, ($P = 0.106$) and $BMAD_{LS}SDS$ declined significantly from 0.45 (0.12) at T2 to 0.12 (0.016) at T4, ($P = 0.015$).

The median (P10-P90) ages at attaining each of the pubertal M stages (M2-M5) in girls and G stages (G2-G5) in boys with PWS are presented in Figure 3C and D. Compared with healthy references[32], the median ages at attaining M2 and 3 in girls and G2 and 3 in boys with PWS were not significantly different, whereas the progression to pubertal stage 4 and 5 was delayed (in girls $P = 0.05$ and $P = 0.025$, resp., and in boys $P = 0.009$ and $P = 0.018$, resp.).

The panels under Figure 3 C and D show the median (IQR) ages and serum estradiol and testosterone levels corresponding to the median ages at attaining M/G2, M/G3, M/G4 and M/G5. Remarkably, despite an increase in Tanner stages, almost no increase in serum sex hormone levels was observed.




| Tanner | n | M2 | n | M3 | n | M4 | n | M5 |
|---|---|---|---|---|---|---|---|---|
| Age (years) | 29 | 9.9 (9.1-11.3) | 23 | 12.1 (11.1-13.1) | 14 | 14.2 (13.2-15.1) | 3 | 15.4 (15.1-15.7) |
| Estradiol | | 25 (13-42) | | 49 (20-60) | | 55 (40-68) | | 38 (24-43) |

| Tanner | n | G2 | n | G3 | n | G4 | n | G5 |
|---|---|---|---|---|---|---|---|---|
| Age (years) | 32 | 11.7 (10.7-12.7) | 23 | 13.0 (11.4-14.0) | 10 | 14.0 (12.7-14.9) | 3 | 15.6 (14.6-16.0) |
| Testosterone | | 1.0 (0.4-2.2) | | 2.4 (1.0-5.1) | | 3.7 (2.9-5.8) | | 7.7 (4.5-9.1) |

**Figure 3.** $BMD_{TB}$ SDS and $BMAD_{LS}$ SDS in boys and girls
A. ■ $BMD_{TB}$SDS and ▲ $BMAD_{LS}$SDS, Estimates Marginal Means with 95% CI in girls
B. ■ $BMD_{TB}$SDS and ▲ $BMAD_{LS}$SDS, Estimates Marginal Means with 95% CI in boys
# $P<0.05$, first significant decline in $BMD_{TB}$SDS compared to the previous year
* $P<0.05$, first significant decline in $BMAD_{LS}$SDS compared to the previous year
C. Represents pubertal development in 31 girls with PWS
D. Represents pubertal development in 33 boys with PWS
Estradiol and testosterone levels are medians with IQRs by entering Tanner stage 2,3,4 and 5 resp.

Thus, Figure 3 shows that the decline in BMD compared to age- and sex-matched peers is paralleled with low serum sex hormone levels.

*Sex hormone treatment*

Six girls, between 12 and 18 years, started with estrogens during the last 1 to 5 years of GH treatment. Five boys, between the age of 13 and 17 years, started testosterone treatment during the last 1 to 3 years of GH treatment. During 9 years of GH treatment, these 11 children had a lower $BMD_{TB}$SDS ($P = 0.003$) and $BMAD_{LS}$SDS ($P = 0.003$) over time. They started GH treatment significantly later than the rest of the long-term group (age: 8.8 [5.2 to 9.9] vs. 5.3 [3.1 to 6.8] years; $P = 0.002$) and also entered puberty significantly later (11.7 [11.2 to 12.9] vs. 10.6 [9.5 to 11.6] years; $P = 0.01$). Because of the wide variation in age at start, dosage and duration of the treatment and the small sample size, we were not able to study the effects of sex hormone treatment on BMD.

### Multiple regression analysis in the long-term group

Table 2 shows the results of the most recent DXA scan in the long-term group at a median [IQR] age of 14.5 [12.8 to 15.8] years. The median duration of GH treatment had been 9 [8 to 10] years. $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS were not significantly different compared to 0 SDS. We performed a multiple regression analysis to determine associations between age at start of GH treatment, LBM SDS and fat% SDS with $BMD_{LS}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS (Table 3). Age at start of GH treatment was negatively associated with $BMD_{TB}$SDS and $BMD_{LS}$SDS ($\beta = -0.21$, $P = 0.049$ and $\beta = -0.22$, $P = 0.040$, resp.), indicating that one year earlier start of GH treatment will result in a higher $BMD_{TB}$ and $BMD_{LS}$ of +0.21 SDS and +0.22 SDS, resp. $BMD_{TB}$SDS and $BMD_{LS}$SDS were positively associated with LBM SDS ($P < 0.001$ and $P < 0.001$). $BMAD_{LS}$SDS was not associated with LBM SDS. $BMD_{TB}$SDS was also positively associated with fat% SDS ($P = 0.008$), but less than LBM SDS. $BMD_{LS}$SDS and $BMAD_{LS}$SDS were not associated with fat% SDS.



**Table 2.** Last DXA scan in the long-term group

| | N | % | |
|---|---|---|---|
| N (male/female) | 64 (33/31) | 52/48 | |
| Tanner (M/G) | | | |
| 1 | 4 | 6.3 | |
| 2 | 15 | 23.4 | |
| 3 | 22 | 34.4 | |
| 4 | 18 | 28.1 | |
| 5 | 4 | 6.3 | |
| Unknown | 1 | 1.6 | |
| | Median | IQR | |
| Age (years) | 14.5 | 12.8 to 15.8 | |
| Years of GH treatment | 9 | 8 to 10 | |
| $BMD_{TB}$SDS | -0.2 | -0.8 to 0.6 | |
| $BMD_{LS}$SDS | 0.0 | -0.9 to 0.9 | |
| $BMAD_{LS}$SDS | 0.1 | -0.7 to 0.9 | |
| BMC SDS | -0.8 | -1.3 to 0.6 | [d] |
| Fat% SDS | 2.3 | 1.9 to 2.6 | [a] |
| LBM SDS | -2.0 | -2.9 to -1.1 | [a] |
| Height SDS | -0.5 | -1.5 to 0.4 | [b] |
| BMI SDS | 1.2 | 0.2 to 1.9 | [a] |

Data are expressed as median (IQR), SDS according to age- and sex-matched Dutch reference values[29-31].
[a] $P<0.0001$; [b] $P< 0.001$; [c] $P< 0.01$; [d] $P< 0.05$, compared to 0 SDS

**Table 3.** Multiple regression analysis for $BMD_{TB}$SDS and $BMD_{LS}$SDS and $BMAD_{LS}$SDS

| | $BMD_{TB}$SDS | | $BMD_{LS}$SDS | | $BMAD_{LS}$SDS | |
|---|---|---|---|---|---|---|
| | β | *P* | β | *P* | β | *P* |
| Age at start GH | -0.21 | **0.049** | -0.22 | **0.040** | -0.10 | 0.450 |
| LBM SDS | 0.74 | **<0.001** | 0.63 | **<0.001** | 0.19 | 0.177 |
| Fat% SDS | 0.26 | **0.008** | 0.15 | 0.133 | 0.09 | 0.486 |
| Model *P*-value | | **<0.001** | | **<0.001** | | **0.019** |
| R Square | 0.58 | | 0.56 | | 0.22 | |

Adjusted for gender, height SDS and Tanner stages

## DISCUSSION

Our study demonstrates that $BMD_{TB}$SDS and $BMD_{LS}$SDS increased and $BMAD_{LS}$SDS remained stable, around the 0 SDS, during 4 years of GH treatment in prepubertal children with PWS. After 4 years of treatment, the $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS decreased significantly, but remained within the normal range. The $BMD_{TB}$SDS decreased significantly during adolescence, after the age of 11 years in girls and 14 years in boys. $BMAD_{LS}$SDS decreased significantly from the age of 11 years in girls and 16 years in boys. After 9 years of GH treatment, LBM SDS was the most powerful predictor of $BMD_{TB}$SDS and $BMD_{LS}$SDS in adolescents with PWS.

This is the first study investigating the long-term effects of GH treatment on BMDs in PWS. The improvement in $BMD_{TB}$SDS and $BMD_{LS}$SDS and the stable $BMAD_{LS}$SDS in prepubertal children during 4 years of GH treatment indicate that GH treatment has a positive effect on BMD. This finding is in line with a 3-year GH study, which observed a stable $BMD_{LS}$SDS in children with PWS[15]. The improvement in BMD could be due to direct effects of GH treatment, as is also seen in children with GH deficiency[33], or indirectly, by increasing the LBM[34]. At baseline, BMAD was higher than average, probably due to a higher BMI and a relatively early adrenarche[35].

Previously, we observed an increase of LBM SDS in the first 2 years of GH treatment and a stabilization of the LBM around -1.5SDS during continued GH treatment[2]. LBM was a strong predictor of BMD in our patients. Recent studies support our findings that lean body mass has a greater effect on BMD than fat mass[36, 37]. Thus, it seems that physical activity in combination with GH treatment is important to optimize the BMD, especially in PWS patients. Additionally, we observed that a younger age at start of GH was associated with a higher $BMD_{TB}$SDS and $BMD_{LS}$SDS. We found that a 5 year earlier start of GH treatment results in a 1 SDS increase in $BMD_{TB}$ and $BMD_{LS}$ at the age of 14.5 years. Thus our study suggests that early GH treatment has a protective effect on BMDs in children with PWS. However, we acknowledge that our study was not designed to investigate the latter, as all children received long-term GH treatment.

During the long-term treatment, all BMDs remained within the normal range for age- and sex-matched controls. We observed, however, a decline in BMDs SDS after 4 years of follow-up compared to age-and sex-matched reference values. Our data show that adolescents with PWS, girls from the age of 11 and boys from the age of 14, have a decline in $BMD_{TB}$SDS and $BMAD_{LS}$SDS. This is in line with literature describing $BMD_{TB}$ and $BMD_{LS}$ in the low to normal range in young adults with PWS[18]. Abnormal pubertal development in PWS is previously described by our group[22, 23]. After a normal onset of puberty, a lack of pubertal progression is observed during adolescence. We observed low serum levels of estradiol and testosterone and a lack of pubertal progression, with only few adolescents progressing to Tanner stage 5. A higher Tanner stage was associated with a trend to a lower $BMD_{TB}$SDS and in a significantly lower $BMAD_{LS}$SDS. This negative association may be explained by the lack of pubertal progression and low sex hormone levels. We observed that the children who started sex hormone therapy were older at start of GH treatment and entered puberty later. Starting low doses of sex hormones should be considered when girls are 11 and boys are 14 years old, unless there is a normal progression of puberty. The importance of which is underlined by our results, showing a low BMD in children aged 17 to 19 years, which will most likely result in osteopenia and osteoporosis at an early age if not timely intervened. In girls with Turner syndrome, low doses estrogens started in childhood and slowly increased during adolescence, resulted in a better adult height, improved BMD and had neurocognitive and behavioral benefits[38, 39]. Recently, positive effects of testosterone replacement therapy without behavioral problems were described in 16-48 years old males with PWS[7].

Due to the decline in BMD during adolescence, it is very likely that young adults with PWS will not attain the same peak mass as healthy peers. This might increase the fracture risk in later life[11, 20]. Healthy girls attain their peak bone mass at the mean age of 19.9 years and boys at 20.1 years[11]. Because GH treatment is only registered until patients with PWS reach adult height, we were not able to study the effect of GH treatment on BMD in young adults above the age of 19 years. Further research should study BMD during long-term GH treatment in combination with sex hormone replacement therapy in young adults with PWS, in order to find the optimal treatment combination. Besides, continuation of GH treatment during transition from late adolescence to early adulthood in patients with PWS must be studied, because in young adults with GHD, BMDs deteriorate after discontinuation of GH treatment[40-42].

In conclusion, $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS in GH-treated prepubertal children with PWS remain completely within the normal range compared to age- and sex-matched reference values. However, during adolescence a decrease in BMDs is observed to low-normal values. To avoid a higher risk for osteoporosis in later life, we recommend to stimulate physical activity to improve lean body mass and to closely monitor BMD from the age of 11 years in girls and 14 years in boys with PWS. To prevent the decline

in $BMD_{TB}$ or $BMAD_{LS}$, clinicians should be aware of this problem and start sex hormone therapy when serum sex hormone levels remain low in girls from the age of 11 and in boys from the age of 14.

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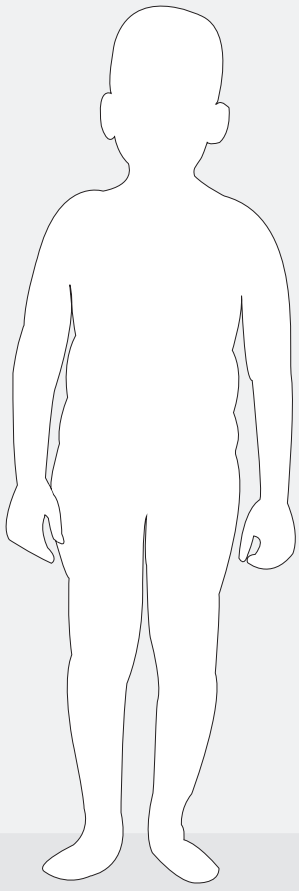

# CHAPTER 5

## Effects of growth hormone treatment on dietary energy intake, body composition and resting energy expenditure in children with Prader-Willi Syndrome: a randomized controlled trial

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## ABSTRACT

***Background/Aims*** Dietary management is a difficult, but key aspect of care in children with Prader-Willi syndrome. We therefore investigated the effect of growth hormone (GH) treatment on reported energy intake in children with PWS, in relation with body composition, resting energy expenditure (REE) and hormone levels.

***Methods*** In a randomized controlled GH-trial including 47 children with PWS, we assessed 5-day dietary records and DXA for body composition. REE was calculated by the equation of Müller, based on fat mass, fat free mass and gender.

***Results*** Baseline energy intake of children with PWS was lower than normal daily energy requirements ($P < 0.001$), and decreased with age to 50% in prepubertal children. Energy intake in infants (m/f: 11/8; median (IQR) age 2.7 (1.5-3.2) years) increased after 1 year GH treatment ($P = 0.008$), this tended to be higher in the GH-group than untreated group ($P = 0.07$). In prepubertal children (m/f: 14/14; median (IQR) age 6.8 (5.1-8.1) years), increase in energy intake was higher in the GH-group, but this was not different compared to the untreated group. REE was not different between GH-group and untreated group. Increase in energy intake during 2 years GH treatment was correlated with lower fat% SDS ($P = 0.037$), and higher adiponectin levels ($P = 0.007$).

***Conclusion*** Our study demonstrates that parents of children with PWS are very well capable to restrict energy intake up to 50% compared to daily energy requirements for age- and sex-matched healthy children. GH-treatment was associated with slight increase in energy intake, but also improved body composition and adiponectin levels, which suggests a protective effect of GH treatment.

## INTRODUCTION

Prader-Willi syndrome (PWS) is a genetic disorder which arises from a lack of expression of paternally inherited imprinted genes on chromosome 15q11-q13, caused by deletion, uniparental disomy (UPD), imprinting centre defect, or balanced translocation[1]. According to European surveys the incidence of PWS is estimated at around 1 in every 15,000-30,000 births, equally affecting boys and girls[2, 3]. Children with PWS have obesity, muscle hypotonia, short stature, hyperphagia, hypogonadism, behavioural problems and developmental delay[4].

Hyperphagia is the most striking symptom of PWS, marked by an excessive food intake after the age of 2 years. In the presence of a reduced metabolic rate and decreased physical activity, this obsessive behaviour towards food consumption often leads to morbid obesity in children with PWS[5]. However, due to early genetic testing, dietary counselling and growth hormone treatment, obesity has become less common in children with PWS[6].

Several studies have shown that GH treatment is beneficial for children with PWS. It improves linear growth, body composition, physical strength and agility[7-9]. Fat utilization and resting energy expenditure (REE) are increased by GH treatment, the latter partially explained by an increment in lean body mass (LBM)[10-12]. In addition, we found that cardiovascular and metabolic risk profile improved during GH treatment[13] and adiponectin levels increased[14]. The hormone adiponectin is secreted by adipocytes and regulates glucose and lipid metabolism and energy homeostasis. High adiponectin levels are thought to be protective with regards to cardiovascular disease and type 2 diabetes mellitus[15, 16].

Maintenance of body weight requires an energy balance in which energy intake matches total energy expenditure, the latter being the product of REE and an index often referred to as physical activity level[17]. Especially in children with PWS, it is important to obtain insight into the effects of GH treatment on energy balance, so as to determine what lifestyle interventions might be possible.

Although GH treatment is known to increase energy intake in children born Small for Gestational Age, children with Turner and Silver Russell syndrome[18, 19], randomized controlled trials comparing energy intake in children with PWS with and without GH treatment are very limited[20].

We hypothesized that children with PWS have a lower energy intake than WHO daily energy requirements for age- and sex-matched healthy children and that GH treatment would not change the energy intake of children with PWS because they are nowadays under strict dietary supervision. We expected to find a positive correlation between energy intake and serum IGF-I levels, height velocity, body composition and REE during GH treatment.

We therefore investigated the food intake in children with PWS by using a 5-day dietary record in combination with measurements of height, fat percentage, LBM, sum of 4 skinfold (SF) measurements, BMI, and serum IGF-I, insulin and adiponectin levels. We compared the baseline intake with the daily energy requirements for age- and sex-matched children. In addition, we investigated the effect of GH treatment on food intake, body composition, REE and hormone levels in our randomized controlled study.

## MATERIALS AND METHODS

### Patients

All children participated in the Dutch randomized controlled GH trial (RCT) for children with PWS[8]. They fulfilled the following inclusion criteria; genetically confirmed diagnosis of PWS, age between 6 months and 12 years (girls) or 14 years (boys), and no signs of puberty[21]. Patient were included by collaborating pediatric endocrinologists.

For the present study, we included infants and prepubertal children who had dietary records at baseline and during the RCT. In accordance with previous studies[8, 13], the study population was divided in an infant group (< 3.5 years of age at start) and a prepubertal group (≥3.5 years at start).

The study protocol was approved by the Medical Ethics Committees at Children's Hospital Erasmus MC- Sophia in Rotterdam, the Netherlands, and of collaborating centers. Written informed consent was obtained from their parents or custodians and the children gave their assent.

### Design

This was a multicentre, randomized controlled, parallel, open-label GH trial. Prior to randomisation with a random number table designed by our statistician, infants were stratified for age and prepubertal children also for BMI. The allocation ratio was 1:1. No sample size calculation was performed, because PWS is a rare syndrome. Infants and prepubertal children were randomly assigned to either the GH-group or untreated group. The GH-group started with GH treatment at a dose of 1.0 mg/m$^2$ body surface/day (≈ 0.035 mg/kg/day) (Genotropin; Pfizer, New York, NY). The control group remained untreated for 1 year in infants and 2 years in prepubertal children and subsequently received the same GH treatment as the GH-group.

Patients and parents of both groups received dietary counselling starting 3 months prior to enrollment in the study. Patients were regularly seen by a physical therapist, who promoted physical activity. Every three monthly, children were examined 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

based on body surface area, to maintain a daily dose of 1 mg/m$^2$. Once a year, children visited the Children's Hospital Erasmus MC- Sophia (Rotterdam, the Netherlands), where the following data were obtained: fat mass, LBM, weight, height, and fasting serum levels of insulin-like growth factor type I (IGF-I), insulin, glucose and adiponectin.

### Energy intake

Dietary energy intake was calculated at baseline and at 1 and 2 years after enrollment in the study, using of a 5-day dietary record. Parents were asked to record the type and amount of food and beverages consumed by the child for 5 consecutive days. Dietary intake was converted into energy, expressed in kcal/day, using computer software based upon a nutrient file compiled from the Dutch Food Composition Table[22]. Macronutrient intake was calculated and expressed as percentage of total energy intake (fat E%, protein E%, carbohydrate E%). The baseline individual energy intake was compared with age-matched daily energy requirements recommended by the WHO for healthy boys and girls[23]. Because of the large age range (3.5-12 years) in the prepubertal group we divided the patients into two age groups: ≥ 3.5-7 and > 7-12 years.

### Anthropometric measurements

Standing height was measured with a Harpenden Stadiometer and supine length with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was assessed on a calibrated scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, the Netherlands). Biceps, triceps, subscapular and suprailiac SFs were measured in all children using one Holtain Skinfold Caliper. For analysis we used the sum of the four measurements. Height, BMI and the sum of the four SF standard deviation scores (SDS) were calculated with Growth Analyser 4.0 (available at www.growthanalyser.org), adjusted for age and sex according to Dutch reference values[24-26].

### Body composition

Fat mass and LBM were annually measured by Dual-energy X-ray Absorptiometry (DXA) (Lunar Prodigy type; GE Healthcare, Chalfont St. Giles, UK). LBM was calculated as fat free mass (FFM) minus bone mineral content. Fat percentage was expressed as percentage of total body mass. In the prepubertal group, fat percentage SDS and LBM SDS were calculated, according to age- and sex-matched Dutch reference values, available for children older than 4 years[27].

### Resting energy expenditure

REE (kcal/day) was calculated, in the prepubertal group only, at baseline and after 1 and 2 years[28] using the Müller's equation since this is validated for estimation of REE in Dutch

children and adolescents[29]. The equation is based on fat mass (FM), fat free mass (FFM) and gender:

REE (kcal/day) = (0.0788 x FFM (kg) + 0.02132 x FM (kg) + 0.327 x gender + 2.694) x 1000 / 4.18.

REE adjusted for fat free mass has been shown to be similar in obese children and children with PWS[11]. We verified the accuracy of the Müller's equation in PWS, in a subset of 6 children (age range: 4-15 years), at baseline and after 1 and 2 years in the study, by comparison of predicted REE to open-circuit indirect calorimetry measurements (Deltatrac Metabolic Monitor, Datex, Helsinki, Finland). Children were measured in non-fasting state. Ten percent was subtracted from the measured REE to compensate for the thermogenic effect of food[30]. The predictive equation was evaluated on the basis of the percentage calculated within 10% of measured REE, the root mean squared error (RMSE) and the mean percentage difference between REE predicted by Müller and REE measured by indirect calorimetry. The mean error between the predictive equation and measured value was 7%, with a RMSE of 127 kcal/d. Consequently, we adjusted Müller's REE for the observed 7% overestimation.

When assuming energy balance, the ratio of energy intake to REE estimates physical activity level.

### Assays

Blood samples were collected after overnight fasting for assessment of insulin, glucose and IGF-I and after centrifugation immediately frozen at -20° until assayed. Adiponectin levels were only assessed in the prepubertal group.

Serum insulin levels were measured by immunoradiometric assay (Medgenix, Biosource Europe, Nivelles, Belgium) with intra- and interassay CVs of 2 to 4.7% and 4.2 to 11.3%, respectively.

Serum glucose levels were assessed on an Abbott Architect Clinical Chemistry Analyzer (Abbott Laboratories, Irving, TX), with intra- and interassay CVs of 0.7 and 0.8%.

Serum IGF-I levels were measured using an immunometric technique on an Advantage Automatic Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA). The intra- and interassay CVs were 4 and 6%, respectively.

In the prepubertal children serum adiponectin levels were assessed in duplicate by an ELISA (Human Total Adiponectin/Acrp30 Quantikine ELISA kit, R&D Systems Inc., Minneapolis, MN). The intraassay CV was less than 7%, and the interassay CV was less than 7%. The MDD was 0.246 ng/ml, and no significant cross-reactivity or interference was observed.

### Statistical analysis

Statistical analyses were performed with SPSS 20.0 (SPSS Inc., Chicago, IL). Data were separately analysed for infants and prepubertal children. Each outcome variable was assessed for normality. Baseline characteristics are expressed as median and interquartile range (IQR) or mean and Standard Deviation (SD). Independent Student's *t*-tests were used to compare energy intake between children with PWS and daily energy requirements for age and sex-matched healthy children. Mann-Whitney U tests were used to compare variables between the GH-treated and the untreated group and Wilcoxon Signed Ranks tests were used to compare different variables with baseline.

Spearman's correlation coefficients were used to assess relationships between energy intake and body composition variables. *P* values less than 0.05 were considered statistically significant.

## RESULTS

### Patient characteristics

Of 73 infants and prepubertal children included in the RCT, we received dietary records from 52 subjects. Five children were excluded because of incomplete dietary records or inadequate notation of portion sizes. We analysed 140 complete dietary records of 47 children. There were no statistical differences in height SDS ($P = 0.086$), BMI SDS ($P = 0.193$) and gender ($P = 0.957$) between the children with or without dietary records at baseline and during the RCT.

The baseline characteristics of the 47 children (m/f: 25/22; median (IQR) age 4.7 (3.7-7.0) years) with PWS are summarized in Table 1. Twenty-two patients had a deletion, 22 a maternal uniparental disomy, and 3 had an imprinting center defect. The median (IQR) BMI SDS was 1.0 (0.0-1.8).

In infants, the GH-group and untreated-group showed similar characteristics at start of study except of a lower number of girls and less children with UPD genotype in the control group. In the prepubertal group, the GH-group and untreated group showed similar characteristics at start of study except of a lower number of girls and less children with UPD genotype in the GH-group.

### Energy intake

Reported mean baseline energy intake of infants and prepubertal children with PWS was significantly lower compared to daily energy requirements for age- and sex-matched healthy children ($P < 0.001$). At baseline, infants with PWS eat 16% less than daily energy requirements and prepubertal children 20-38% less, Table 2. During the study, with increasing age, a significant decrease in energy intake as percentage of daily energy

**Table 1.** Baseline characteristics of 47 infants and prepubertal children with PWS.

| | Infants | | Prepubertal children | |
|---|---|---|---|---|
| | GH | Untreated | GH | Untreated |
| *n* (m) | 11 (5) | 8 (6) | 12 (8) | 16 (6) |
| Age (year) | 2.0 (1.6 - 3.1) | 2.8 (1.4-3.2) | 6.9 (5.2-9.2) | 6.4 (5.0-8.1) |
| Height SDS | -2.6 (-3.0- -1.0) | -2.4 (-3.6- -1.6) | -1.9 (-2.8- -1.7) | -2.6 (-3.2- -2.0) |
| Body weight (kg) | 11.3 (7.4-13.3) | 11.8 (9.3-15.0) | 22.3 (17.7-34.8) | 20.0 (17.7-25.7) |
| BMI SDS | -0.7 (-2.0-2.0) | 0.2 (-1.6-1.9) | 1.1 (0.1-2.4) | 1.2 (0.7-1.7) |
| BMI $SDS_{PWS}$ | -0.5 (-1.0-0.4) | -0.1 (-0.5-0.4) | -0.9 (-1.5-0.4) | -0.7 (-0.3- -0.2) |
| LBM (kg) | 8.1 (5.5-9.2) | 8.5 (6.9-9.5) | 13.3 (12.1-18.4) | 12.6 (11.2-14.4) |
| LBM $SDS_{age}$* | | | -2.2 (-2.7- -1.9) | -2.6 (-2.8- -2.3) |
| Fat mass (kg) | 3.1 (2.2-5.4) | 3.0 (1.7-4.7) | 8.1 (5.1-16.3) | 7.3 (5.7-10.3) |
| Fat percentage | 27.1 (21.0-33.2) | 26.6 (23.0-34.0) | 35.3 (28.8-45.0) | 34.9 (31.8-42.0) |
| Fat percentage $SDS_{age}$* | | | 2.3 (1.9-2.9) | 2.2 (2.4-2.7) |

SDS, SD score; LBM, lean body mass; BMI, body mass index.
Data are presented as number or median (IQR).
*Age- and sex-matched reference values were not available for children < 4 years.

**Table 2.** Baseline energy intake of children with PWS.

| | | Energy intake | | Macronutrient distribution (E%) | | |
|---|---|---|---|---|---|---|
| | *n* | kcal/d | % DER | Fat | Protein | Carbohydrate |
| Infants < 3.5 years | 19 | 867 ± 138 * | 84 | 23.7 ± 5.5 | 16.4 ± 3.2 | 59.3 ± 4.6 |
| Prepubertal children 3.5 - 7 years | 16 | 1102 ± 139 * | 80 | 22.4 ± 4.1 | 16.4 ± 2.1 | 60.7 ± 4.5 |
| Prepubertal children 7 - 12 years | 12 | 1147 ± 123 * | 62 | 24.9 ± 4.2 | 16.5 ± 2.6 | 58.3 ± 5.0 |

E%, percentage of total energy intake.
Data are presented as number or mean ± SD.
* *P*<0.001 PWS group *vs.* daily energy requirements (DER) of age- and sex-matched healthy children[23].

requirements was observed (ρ = -0.6, *P* < 0.001), (Figure 1). Children older than 10 years were allowed to eat 50% less than age- and sex-matched healthy children. After 1 year study, infants had no significant difference in energy intake versus daily energy requirements between the GH-group and the untreated group. In the prepubertal children, there was also no difference between the GH-group and the untreated group after 2 years study.

## Effect of GH treatment on energy intake

Figure 2 shows the results of the energy intake and change in energy intake in infants and prepubertal children during the study.

*Infants.* After 1 year, the median (IQR) increase in energy intake in the GH-group was higher than in the untreated group (264 (135-370) vs. 108 (7-193) kcal/day), but this did

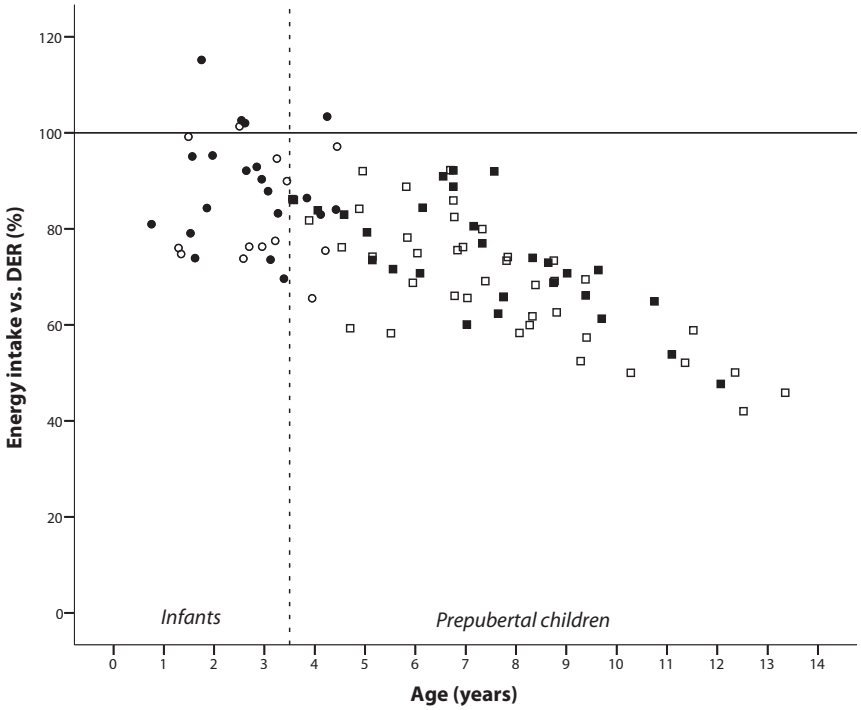


**Figure 1.** Energy intake of children with PWS (n=47) expressed as percentage of daily energy requirements for age- and sex-matched healthy children
Infants: ● GH-group and ○ untreated group
Prepubertal children: ■ GH-group and □ untreated group

not reach significance ($P$ = 0.072). Within groups, energy intake had only significantly increased after one year from baseline in the GH-group ($P$ = 0.008), but there was no significant difference in total energy intake between the GH-group and untreated group (1,077 (1,026-1,114) kcal/d vs. 943 (825-1,226) kcal/day, $P$ = 0.463). GH treatment did not affect the proportion of fat, proteins and carbohydrates to total energy intake (Table 3).

*Prepubertal children*. The GH-group demonstrated a non-significant increase in energy intake of 158 (-77 to 371) kcal/day, whereas children of the untreated group had a non-significant -25 (-98-187) kcal/d decrease in energy intake after 2 years compared to baseline, but no significant difference was observed in the change in energy intake between the GH- and untreated group. After 2 years, the total energy intake was not significantly different between the GH-group and untreated group (1,272 (1,169-1,390) kcal/day vs. 1,156 (1,062-1,279) kcal/day, $P$ = 0.064). There were no differences in the proportion of fat, protein and carbohydrates between the GH- and the untreated group, but when children with PWS became older, the proportion of fat to total energy intake had significantly increased ($P$ = 0.006) and that of carbohydrates decreased ($P$ = 0.001) (Table 3).

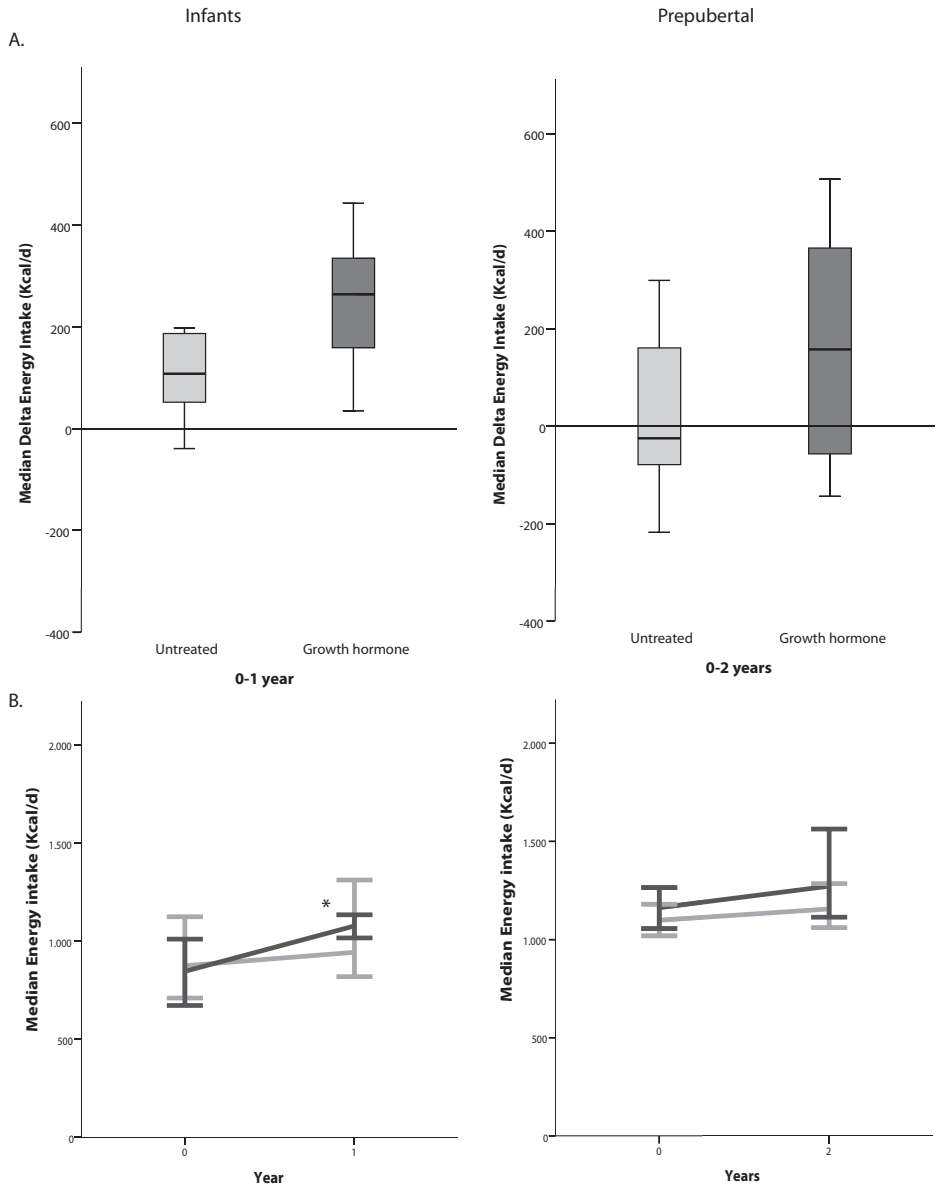


**Figure 2a.** Median Delta Energy intake compared to baseline in infants for 0-1 year and in prepubertal children 0-2 years

**2b.** Median Energy intake compared to baseline in infants for 0-1 and in prepubertal children for 0-2 years.
■ GH-group and ■ untreated group

* $p < 0.05$ compared to baseline

### Body composition

*Infants.* After 1 year, fat percentage had remained stable in the GH-group while it had increased in the untreated group ($P = 0.036$). As a result, the fat percentage was lower in the GH-group than in the untreated group after 1 year ($P = 0.048$). The sum of 4 SFs SDS was also lower in the GH-group than in the untreated group ($P = 0.05$).

The LBM increased in both groups (GH-group $P = 0.003$ and untreated group $P = 0.012$), and was not significantly different between the groups after 1 year (Table 3).

*Prepubertal children.* After 2 years, the fat percentage SDS had decreased during GH treatment ($P = 0.002$) and had increased in the untreated group ($P = 0.003$), compared to baseline. As a result, the fat percentage SDS after 2 years was lower in the GH-group than in the untreated group ($P = 0.016$). The sum of 4 SFs was also lower in the treatment group than in the untreated group ($P < 0.01$).

The LBM SDS had increased during GH treatment ($P = 0.023$) but had deteriorated in the untreated group ($P = 0.001$). After 2 years, the LBM SDS remained higher in the GH-group ($P < 0.001$).

Both in infants and prepubertal children, height SDS had significantly improved during GH treatment compared to the untreated children (infants $P < 0.05$ and prepubertal $P < 0.001$) (Table 3).



### Resting energy expenditure and physical activity level

The REE significantly increased in both the GH-group and the untreated group ($P = 0.003$ vs. $P < 0.001$), but it was not significantly different between the groups.

At baseline, the median (IQR) energy intake to REE ratio was 1.17 (1.04-1.29). After 2 years of GH treatment, the energy intake to REE ratio had not significantly changed (Table 3).

### Relationship between energy intake and body composition

*Infants.* At baseline, there was a significant correlation between the energy intake and the LBM ($\rho = 0.8$, $P < 0.001$), but not with fat percentage ($\rho = 0.2$, $P = 0.386$) and height SDS ($\rho = -0.1$, $P = 0.620$). During the RCT, the increase in energy intake in infants in the GH-group was not correlated with the increase in LBM ($\rho = 0.2$, $P = 0.663$) and also not with the change in fat percentage ($\rho = -0.4$, $P = 0.298$). In the untreated group, there were also no correlations found with LBM ($\rho = 0.6$, $P = 0.257$) and fat percentage ($\rho = -0.1$, $P = 0.847$).

*Prepubertal children.* At baseline, there was a significant correlation between the energy intake and the LBM ($\rho = 0.4$, $P = 0.043$), but not with fat percentage ($\rho = 0.1$, $P = 0.509$) and height SDS ($\rho = 0.3$, $P = 0.157$). During the RCT, the increase in energy intake in prepubertal children in the GH-group was not correlated with the increase in LBM ($\rho = 0.4$, $P = 0.266$), but it was negatively correlated with the change in fat percentage ($\rho =$

**Table 3.** Complete overview of dietary intake and measurements during the RCT in infants and prepubertal children

| | Infants | | | | Prepubertal | | | |
|---|---|---|---|---|---|---|---|---|
| | *Baseline* | *Change during 1 year RCT* | | | *Baseline* | *Change during 2 year RCT* | | |
| | | **GH** | **Untreated** | *P*-value[#] | | **GH** | **Untreated** | *P*-value[#] |
| Energy intake. kcal/day | 858 (769 to 957) | 264 (135 to 370)* | 108 (7 to 193) | ns | 1126 (1044 to 1215) | 158 (-77 to 371) | -25 (-89 to 187) | ns |
| Fat % | 24.2 (20.7 to 26.2) | -0.7 (-3.2 to 0.6) | -3.4 (-6.8 to 1.4) | ns | 22.8 (20.2 to 27.6) | 1.0 (-1.5 to 3.9) | 3.1 (0.5 to 9.2)* | ns |
| Protein % | 17.0 (14.9 to 18.5) | -2.1 (-3.5 to 3.8) | -1.2 (-3.1 to 1.4) | ns | 16.6 (14.5 to 18.3) | -1.0 (-2.5 to -0.1) | 0.7 (-1.4 to 3.2) | ns |
| Carbohydrates % | 59.2 (56.6 to 62.5) | 1.5 (-1.3 to 3.7) | 4.8 (-0.9 to 7.3) | ns | 60.2 (57.3 to 62.5) | -0.9 (-2.7 to 0.8) | -3.4 (-9.1 to -0.8)* | ns |
| | *Baseline* | *After 1 year RCT* | | | *Baseline* | *After 2 year RCT* | | |
| *Anthropometric measurements* | | | | | | | | |
| Height SDS | -2.6 (-3.0 to -1.5) | -1.1 (-2.0 to -0.2) | -2.3 (-3.4 to -1.5) | < 0.05 | -2.2 (-3.0 to -1.9) | -0.2 (-0.4 to 0.4)* | -1.7 (-2.4 to -1.4) | < 0.001 |
| BMI SDS | -0.1 (-1.9 to 2.0) | 1.0 (-1.6 to 1.6) | 1.4 (-0.4 to 2.6)* | ns | 1.1 (0.6 to 1.8) | 0.8 (-0.4 to 1.7)* | 1.3 (1.1 to 1.6) | ns |
| BMI PWS SDS | -0.4 (-0.9 to 0.4) | -0.6 (-1.4 to 0.3) | -0.2 (-0.5 to 0.5) | ns | -0.8 (-1.0 to -0.2) | -1.0 (-2.0 to -0.4)* | -0.6 (-1.0 to -0.5) | ns |
| Sum of 4 skinfolds SDS | 1.9 (0.5 to 2.9) | 0.9 (-1.1 to 3.1) | 4.0 (3.1 to 5.3) | 0.05 | 4.0 (3.2 to 4.8) | 1.6 (1.0 to 3.3) | 4.9 (2.8 to 7.0) | < 0.01 |
| *DEXA* | | | | | | | | |
| Fat mass (kg) | 3.0 (2.0 to 4.7) | 4.7 (1.5 to 5.2) | 6.0 (2.8 to 7.4)* | ns | 7.8 (5.3 to 10.3) | 9.5 (5.4 to 17.1) | 9.6 (8.5 to 14.2)* | ns |
| Fat percentage | 26.9 (22.9 to 33.2) | 26.3 (15.2 to 28.6) | 36.1 (23.9 to 40.9)* | < 0.05 | 35.2 (29.5 to 42.0) | 34.3 (21.5 to 40.5)* | 39.4 (38.0 to 45.1)* | < 0.05 |
| Fat percentage SDS | | | | | 2.3 (2.0 to 2.8) | 2.2 (1.4 to 2.5)* | 2.6 (2.4 to 2.8)* | < 0.05 |
| LBM (kg) | 8.1 (6.8 to 9.3) | 11.1 (9.1 to 12.8)* | 9.7 (9.3 to 11.0)* | ns | 12.8 (11.5 to 16.0) | 20.4 (17.8 to 26.9)* | 14.4 (13.1 to 16.5)* | .001 |
| LBM SDS | | | | | -2.3 (-2.7 to -1.9) | -1.3 (-1.6 to -1.2)* | -3.0 (-3.2 to -2.4)* | < 0.001 |
| *Fasting hormone levels* | | | | | | | | |
| Insulin | 3.0 (2.0 to 6.0) | 7.3 (6.0 to 12.0)* | 9.5 (3.4 to 12) | ns | 6.0 (5.0 to 9.0) | 9.5 (7.0 to 15.8) | 11.0 (5.8 to 21.5)* | ns |
| Glucose | 4.4 (4.4 to 4.7) | 4.5 (4.2 to 4.8) | 4.4 (4.2 to 4.6) | ns | 4.7 (4.4 to 4.8) | 4.7 (4.3 to 4.8) | 4.6 (4.4 to 5.1) | ns |
| Adiponectin (mg/l) | | | | | 14.8 (13.3 to 21.0) | 17.5 (14.1 to 28.3)* | 14.6 (12.8 to 18.7) | ns |
| Delta Adiponectin (mg/l) | | | | | 14.8 (13.3 to 21.0) | 2.8 (1.1 to 3.6)* | -1.2 (-2.6 to 1.0) | < 0.05 |

**Table 3.** (continued)

| | Infants | | | | Prepubertal | | | |
|---|---|---|---|---|---|---|---|---|
| | *Baseline* | *Change during 1 year RCT* | | | *Baseline* | *Change during 2 year RCT* | | |
| | | **GH** | **Untreated** | *P*-value[#] | | **GH** | **Untreated** | *P*-value[#] |
| *Resting energy expenditure* | | | | | | | | |
| REE Muller (kcal/d) | | | | | 899 (865 to 991) | 971 (948 to 1151)* | 1023 (962 to 1095)* | ns |
| Ratio energy intake to REE | | | | | 1.17 (1.04 to 1.29) | 1.16 (0.97 to 1.29) | 1.25 (1.13 to 1.34) | ns |

Data expressed as median (IQR)

* P < 0.05 compared to baseline

[#] *P*-value between GH-group and untreated group

-0.9, $P = 0.003$). In the untreated group, no correlations were found with LBM ($\rho = -0.1$, $P = 0.791$) and fat percentage ($\rho = -0.3$, $P = 0.411$).

Both in infants and prepubertal children, the increase in energy intake was not correlated with height velocity.

### Fasting adiponectin, IGF-I, glucose and insulin

Median (IQR) adiponectin levels in healthy prepubertal children (aged 5-10.2 years) was 11.8 (9.7–12.5) mg/l. In prepubertal children, the median (IQR) adiponectin levels significantly increased in the GH-group from 14.8 (13.3 to 21.0) mg/l to 17.5 (14.1 to 28.3) mg/l ($P = 0.018$) but not in the untreated group. After 2 years of GH treatment, the increase in energy intake was positively correlated with serum adiponectin levels, ($\rho = 0.7$, $P = 0.007$).

During the study, in both infants and prepubertal children, there were no correlations between the increase in energy intake and IGF-I, fasting glucose or insulin levels.

Energy intake, adiponectin levels and body composition were not different between children with a deletion or a UPD.

## DISCUSSION

In this randomized controlled GH study, we investigated the effect of GH treatment on dietary energy intake, body composition, adiponectin levels and REE, in a large group of 47 children with PWS. Energy intake in infants and prepubertal children with PWS was significantly lower than the daily energy requirements for age- and sex-matched healthy children. In the infant group, aged < 3.5 years, energy intake increased during GH treatment compared to baseline, but the increase was not significantly different from untreated patients. In prepubertal children, aged ≥ 3.5 years, the energy intake did not significantly increase during 2 years of GH treatment, probably because of the large variation in energy intake. During GH treatment, there was an increase in the LBM and decrease in fat percentage in both infants and prepubertal children. Prepubertal children had significantly higher serum adiponectin levels during GH treatment, while no differences on REE and physical activity levels were found in treated and untreated patients.

Most parents and caregivers know the high risk of obesity development in children with PWS and set strict limitations to food intake[31]. We found that the energy intake, expressed as percentage of the daily energy requirements for age- and sex-matched healthy children, decreased with age to 80% in young infants and to 50% in prepubertal children above the age of 10 years. The BMI SDS was actually higher in the prepubertal children, despite a reported decrease in energy intake to 50% of normal. This could

indicate that we deal with the switch in nutritional phases from 1b to 2, described by Miller et al.[32] or suggests that underreporting was a confounding factor. Although we are aware of the possibility of caloric underreporting, our results are in line with a Norwegian study, who assessed energy intake in 6 infants with PWS. They also found a lower energy intake compared to reference values, but didn't evaluate the effect of GH treatment[33]. One would expect that a restricted energy intake is easier to institute when children are younger and under more close supervision, than in older children. However, our data show that the parents of children with PWS are very well capable to restrict the diet of their child, also in the older ones. In addition, we found that when children with PWS became older, the proportion of fat to total energy intake increased and that of carbohydrates decreased. One explanation is that the fat intake by butter and oil remained stable, while the total energy intake, expressed as percentage of the daily energy requirements for age- and sex-matched healthy children, decreased over time.

We hypothesized that there would be no difference in energy intake in the GH-group compared to the untreated group, because of the strict diets. We observed that 1 year of GH treatment in infants resulted in a significant increase of energy intake and an improved body composition compared to baseline, but not differently from untreated children. In prepubertal children, we found an increased energy intake in GH-treated versus untreated children, but because of the large variation, this did not reach significance. The relation between increase in energy intake and decrease in fat percentage SDS during GH treatment is remarkable. We speculate that children, who improved most in body composition, were allowed the largest increase in energy intake by their parents. Our findings indicate that GH treatment is able to improve body composition and does increase energy intake in children with PWS. This is in line with *Galassetti et al.*[20] who observed that prepubertal GH-treated children with PWS consumed 226 kcal/d more than untreated children (1,296 ± 78 vs. 1,070 ± 105 kcal/day), in accordance with other groups of children receiving GH treatment[18, 19].

We expected to find a positive relation between the increase in energy intake, IGF-I levels and height velocity, but we did not find it. This is probably explained by the strict diets of the children, however, caloric underreporting cannot be ruled out.

Maintenance of body weight requires an energy balance. Two important components of the energy balance equation are energy intake and REE[17, 34]. We found no difference in REE between the GH-group and the untreated group, which is in line with a previous study[35]. An increase of REE was observed in a small American GH-study in 12 children with PWS[36]. REE is very difficult to measure, especially in children, which could possibly explain different outcomes in literature.

The ratio of energy intake to REE estimates the physical activity level when assuming energy balance. The baseline physical activity level was lower compared to estimations

of physical activity level in previous studies. Van Mil *et al.* estimated a mean physical activity level of 1.33 in PWS children and 1.55 in healthy, obese children[37], using the ratio of total energy expenditure to REE, measured by doubly labelled water and indirect calorimetry, respectively. As physical agility is known to increase during GH treatment in PWS[35, 38], we expected an increase in physical activity level in GH-treated children. To our surprise, GH treatment did not change the physical activity level. The physical activity level in our study is likely to be explained by a strict diet. Stimulating physical activity remains an important focus to reach energy balance in these children.

Body composition improved during the first year of GH treatment, in line with other GH-studies[8, 35, 39]. It might be that the effect of GH treatment on body composition occurs via a redistribution of body energy stores from fat mass to LBM, without changes in energy balance. The sum of 4 SFs, which represents subcutaneous fat, returned very rapidly to normal SDS values during GH treatment, which underlines the above theory. Long-term GH treatment, in combination with a strict diet and exercise programs, has a continued beneficial effect on LBM[6] and is recommended in the PWS consensus guidelines[40].

Adiponectin is an adipocytokine that is inversely related to insulin resistance and adiposity, and is thought to be protective with regard to cardiovascular disease and type 2 diabetes mellitus[15, 16]. During GH treatment, higher energy intake was not only related with less fat mass and more LBM, but also with higher serum adiponectin levels. A possible explanation for the rise in serum adiponectin levels might be the increase in LBM during GH treatment. Adiponectin plays an important role in the insulin-dependent glucose metabolism in skeletal muscle[41]. Both the increase in LBM and adiponectin levels are in line with the increase in insulin sensitivity during GH treatment in PWS as previously described[42, 43]. It could also be explained by an altered adipocyte function during GH treatment. *Wölfing et al.* showed that GH treatment stimulates secretion of adiponectin in cultured adipocytes[44] and a Japanese study observed a negative relation between the amount of visceral adipose tissue and adiponectin levels in patients with PWS[45]. However, clinical studies in girls with Turner syndrome and in short children born small-for-gestational-age showed no significant relation between GH treatment and adiponectin[46, 47]. This could be the result of the major improvement in LBM and fat mass in the children with PWS, which is probably more clear than in children with other disorders.

We acknowledge that besides the usual underreporting associated with estimation of food intake by use of dietary records[48], children with PWS might obtain access to food without knowledge of their parents. When faced with tempting food, even children who are otherwise well behaving, might steal food. We did not expect such behaviour in infants who were under close supervision of parents. Nevertheless, the 5-day dietary records is the best available method for estimation of energy intake in infants and

prepubertal children with PWS and we used the strongest design, a RCT, for this study. Forgetting to record the food intake was the main reason for not completing the dietary records.

In conclusion, children with PWS have a low to very low energy intake compared to daily energy requirements for age- and sex-matched children. In infants, aged < 3.5 years, the energy intake increased during GH treatment compared to baseline, but it was not significantly different from the untreated ones. In prepubertal children, aged ≥ 3.5 years, the energy intake did not significantly increase. In contrast to the energy intake, the children had a significant decrease in fat percentage and an increase in adiponectin levels, suggesting a protective effect of GH treatment with regard to the development of obesity and diabetes mellitus type II development in infants and children with PWS. The focus of attention for parents to keep energy balance is to stimulate physical activity.

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None of the authors have a conflict of interest to declare.

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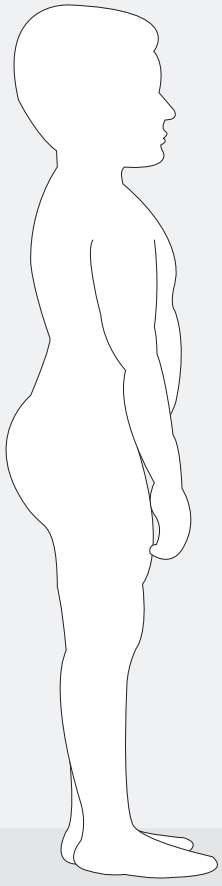

# CHAPTER 6

# IGF-I levels, complex formation and IGF-bioactivity in growth hormone treated children with Prader-Willi Syndrome

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**Submitted**

## ABSTRACT

***Context*** Children with Prader-Willi syndrome (PWS) attain high serum immunoreactive IGF-I levels during standard dose growth hormone (GH) treatment, which leads to concern, but lowering the dose, deteriorates their body composition.

***Objectives*** To evaluate serum IGF-I, IGFBP-3 and acid-labile subunit (ALS) levels, complex formation and IGF-bioactivity in GH-treated PWS children.

***Design and Setting*** This was a cross-sectional study of a Dutch PWS Cohort.

***Participants*** Forty GH-treated PWS children compared with 41 age- and sex-matched healthy controls.

***Interventions*** GH treatment (1.0 mg/m$^2$/day ≅ 0.035 mg/kg/day).

***Main outcome measures*** Serum IGF-I, IGFBP-3 and ALS levels, complex formation and IGF-bioactivity by IGF1 receptor kinase activation assay (KIRA).

***Results*** Serum IGF-I, IGFBP-3, ALS levels and IGF-I/IGFBP-3 ratio were significantly higher in GH-treated PWS children than in healthy controls. The 150 kD ternary complex formation was, however, also significantly higher than in controls, indicating that most of serum IGF-I is sequestered in the ternary 150 kD complex with ALS and IGFBP-3. Young GH-treated PWS children, median (IQR) age 5.2 (4.3 to7.2) years, exhibited higher serum IGF-bioactivity than controls, but no difference was observed in IGF-bioactivity between older GH-treated PWS children, age 14.9 (13.8 to 16.2) years, and controls. The proportion of IGF-bioactivity of total serum IGF-I was, however, lower in GH-treated PWS children than in controls. Serum immunoreactive IGF-I levels did not correlate with IGF-bioactivity in GH-treated children with PWS, in contrast to a strong positive correlation in healthy controls.

***Conclusions*** In GH-treated PWS children, most off serum IGF-I is sequestered in the 150 kD complex. Higher IGF-bioactivity was only found in young GH-treated PWS children and not in the older ones. IGF-bioactivity during GH showed a wide variation and there was a disrupted correlation with immunoreactive IGF-I levels, which makes immunoreactive IGF-I levels an inappropriate indicator of GH-dosing in PWS children.

## INTRODUCTION

Prader-Willi syndrome (PWS) is a complex genetic disorder caused by the lack of expression of the paternally inherited genes on chromosome 15q11-q13[1, 2]. The syndrome is characterized by hypotonia, abnormal body composition, short stature, developmental delay and a distinctive behavioral phenotype[3, 4]. Multiple studies showed that growth hormone (GH) treatment results in a significantly improved growth and an improved body composition[5-7]. Long-term follow-up studies confirmed the beneficial effects of GH treatment in children with PWS[8, 9]. Nowadays, GH treatment in children with PWS is used in many countries.

High serum IGF-I levels during GH treatment in children with PWS are a major concern[9-11]. This has been interpreted as the result of increased GH sensitivity[10]. However, to maintain an acceptable body composition with a fat mass percentage at max 2 SDS, children with PWS require relatively high IGF-I levels[9, 12]. Despite the high serum IGF-I levels, several growth parameters as height, head circumference, hand length, foot length, tibia length, arm span and facial growth, significantly improved during GH treatment, but did not rise above the 0 SDS[13, 14]. Studies showed no signs of acromegaly, indicating no increased IGF-bioactivity. There is, however, still debate whether or not to accept these high immunoreactive IGF-I levels, because of the unknown long-term consequences. Controversial long-term data in GH-treated patients with GH deficiency have been published[15, 16], but there are no long-term data on mortality and causes of death in adults with PWS treated with GH during childhood, as GH treatment has been registered for less than fifteen years in most countries[17].

Humans have six insulin-like growth factor binding proteins (IGFBPs), which can bind IGF-I and form a stable binary complex. Under normal circumstances, the binary complex represents 10-15% of the total serum IGF-I. Most of the remaining serum IGF-I is present in ternary complexes with one molecule of IGFBP-3 or IGFBP-5 and one molecule of acid-labile subunit (ALS). Only 1-5% of IGF-I is unbound or free[18]. Free circulating IGF-I is able to bind to its receptor, IGF1R, and then initiates a complex signaling cascade[19]. In children with PWS, there are no data on IGF-I complex formation and the ability of serum IGF-I to phosphorylate its receptor, a measure of IGF-bioactivity. It is suggested that IGF-I bioactivity is more sensitive for monitoring the effects of GH treatment than immunoreactive IGF-I levels[20].

We hypothesized that GH-treated children with PWS have a normal IGF-bioactivity, despite the high serum immunoreactive IGF-I levels. Hence, we investigated the serum levels of immunoreactive IGF-I, IGFBP-3, ALS and ternary complex formation. In addition, we studied IGF-bioactivity in children with PWS compared to an age- and sex-matched healthy control group and the relation with the serum levels of immunoreactive IGF-I, IGFBP-3 and ALS and anthropometric measurements.

## METHODS

### Patients

We randomly included a group of 40 children participating in the Dutch PWS Cohort study, consisting of 20 children aged 2-8 years (GH-treated young PWS group), and 20 children, aged 12-18 years (GH-treated older PWS group).

### Control group

The reference group consisted of 41 healthy normal statured age- and sex-matched controls, divided in two groups; aged 1-10 years (young controls) and aged 11-18 years (older controls). Fasting blood samples were obtained during minor elective surgery. Children were excluded in case of a birth weight and/or birth length SDS below -2 SDS, systemic disorder, chromosomal disorder or syndrome.

### Design

We investigated serum IGF-I, IGFBP-3 and acid-labile subunit (ALS) levels, complex formation and IGF-bioactivity in GH-treated children with PWS, compared to healthy age- and sex-matched controls.

Children with PWS were treated with somatropin 1 mg/m$^2$/day once daily at bedtime (Genotropin; Pfizer Inc., New York, NY)[17]. During the first 4 weeks of GH treatment, children received 0.5 mg/m$^2$/day to prevent fluid retention. The somatropin dose was lowered when serum IGF-I levels increased above +3 SDS. Children were 3-monthly 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. Annually, the children visited the Erasmus University Medical Center- Sophia Children's Hospital, Rotterdam, the Netherlands. The study protocol of the Dutch PWS Cohort study and the Control study was approved by the Medical Ethics Committees at Children's of the Erasmus MC-Sophia in Rotterdam, the Netherlands, and of the collaborating centers. Written informed consent was obtained from parents and from children older than 12 years; assent was obtained in children younger than 12 years of age.

### Anthropometric measurements

Standing height was measured with a Harpenden Stadiometer, and supine length with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was assessed on a calibrated scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, the Netherlands). Height, weight and body mass index (BMI) standard deviation scores (SDS) were calculated with the Growth Analyser Research Calculation Tools 4.0 (available at www.growthanalyser.org), and were adjusted for gender and age according to Dutch refer-

ence values[21, 22]. Delta height SDS and delta BMI SDS were derived from changes in the respective SDS values during the previous year.

### Assays

After overnight fasting, blood samples were collected for assessment of serum levels of IGF-I, IGFBP-3 and ALS. After centrifugation, serum samples were immediately frozen and stored at -80° until assayed. Measurements of serum IGF-I, IGFBP-3 and ALS were performed in one laboratory. Total IGF-I levels were measured using an immunometric technique on an IMMULITE 1000 Analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, USA). The lower limit of detection was 12 ng/mL (1.6 nmol/l) and inter-assay variation was <7.5% at concentrations ranging from 46-420 ng/ml (6.0 to 55.0 nmol/l) (n = 31). IGFBP-3 levels were measured using a specific RIA, as described previously[23]. The intra- and interassay CVs were 4.0 and 6.0%, respectively. Levels of serum IGF-I and IGFBP-3 were expressed in SDS, adjusting for age and gender[23].

Serum ALS levels were determined using the ELISA kit of Mediagnost (Reutlingen, Germany). Intra-assay variations were 6.6 and 6.8% at mean levels of 911 and 1338 mU/ml (n = 16), respectively. Inter-assay variations were 9, 8 and 8% at mean levels of 931, 1061 and 1926 mU/ml (n = 10), respectively. Smoothed references for ALS levels were constructed by the LMS method[24].

### Column chromatography

The different molecular size classes of circulating IGF-IGFBP complexes were determined by gel filtration through a 1.6x60 cm Hi-Load Superdex 200 column. Prior to size exclusion chromatography, 250 µl of each sample was incubated with 100 µl $^{125}$I-hIGF-I as previously described[25]. The ratio between $^{125}$I-hIGF-I in the ternary 150 kD and in the binary 40-50 kD peak fractions was calculated, and expressed as the percentage of the total radioactivity recovered in the eluate. In addition, we calculated SDS values, adjusted for age and gender[25].

### IGF receptor tyrosine kinase activation assay

In order to assess serum IGF-bioactivity and several pro-IGF2 isoforms, we developed an IGF1 receptor kinase activation assay (KIRA) based on MCF7 cells transfected with the human IGF1R gene[26, 27] (Appendix 1). In brief, the KIRA was performed in 12 well flat-bottom culture plates (Corning, Rochester, NY), to which was added 1 ml per well of cells suspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum. The plates were incubated for 48 h at 37°C in 5% CO2, after which the cells were switched to serum-free Dulbecco's Modified Eagle's medium (DMEM) and kept for another 24 h. After the medium had been discarded, either 1 ml of a series of IGF-I standards or diluted serum was added (for either 4 or 8-fold dilutions similar results

were obtained) and incubated for 30 min at 37°C. IGF-I standards and sera were diluted in serum-free DMEM. After incubation, supernatants were aspirated, the cells rinsed two times with phosphate-buffered saline (PBS), and the cells lysed (0.5 ml lysis buffer/well) for 1 h at 5°C on a plate shaker. The lysis buffer consisted of 1% NP-40 alternative, 20mM Tris (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA, 1 mM activated sodium orthovanadate, and a complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany).

Determination of levels of total IGF1R and phosphorylated IGF1R was performed with the human IGF1R ELISA kit and human phospho-IGF1R ELISA kit (R&D systems Inc. Minneapolis, MN, USA), respectively. All determinations were performed in duplicate. The operational range of the phopho-IGF1R assay was between 1.0-50 ng/ml (i.e. 0.13-6.54 nmol/l) of standard purified hIGF-I added. The within- and between-assay coefficients of variation at a mean level of 0.29 nmol/l IGF bioactivity were <6.5% and < 11% (n = 5), respectively. In the bioassay, the activity of purified hIGF-II and insulin were, on a molar base 20 % and < 1% of that of standard hIGF-I peptide, respectively. The level of phosphorylated IGF1R in the cell lysates after cell stimulation with either hIGF-I or various sera was normalized by that of total IGF1R. Bioactive IGF in the various sera was calculated from the hIGF-I bioactivity standard curves and expressed as nmol/l.

### Statistics

Statistical analyses were performed with SPSS 21.0 (SPSS Inc., Chicago, IL). Data were separately analysed for young and older children (see Patients). Results were expressed as median (interquartile range, IQR). Mann-Whitney U test was used to calculate differences between groups. Correlations were calculated with non-parametric bivariate correlations and expressed by Spearman's rho to assess relationships between IGF-bioactivity and serum levels of total IGF-I, IGFBP-3, ALS and anthropometric measurements. Multiple linear regression analysis was performed to determine the association of IGF-bioactivity with delta height SDS and delta BMI SDS in all children with PWS. In all multiple linear regression models, adjustments were made for age and gender. All regression coefficients are presented as a standardized Beta (β) for better interpretation of the results. *P* values less than 0.05 were considered statistically significant.

## RESULTS

### Baseline

Table 1 shows the baseline characteristics of the children with PWS. GH-treated young children had a median (IQR) age of 5.2 (4.3 to 7.2) years and GH-treated older children 14.9 (13.8 to 16.2) years. The median duration of GH treatment was 4.0 (3.0 to 5.7) years and 9.0 (8.1 to 10.8) years, respectively. GH dose in mg/m$^2$/day was lower in the young

**Table 1.** Clinical characteristics GH-treated children PWS

| | GH-treated young PWS children | GH-treated older PWS children | *P* |
|---|---|---|---|
| Male/Female | 12/8 | 10/10 | 0.530 |
| Genotype | | | 0.890 |
| Deletion | 11 | 11 | |
| mUPD | 8 | 7 | |
| ICD | 1 | 2 | |
| Age at start of GH treatment (years) | 1.35 (0.97 to 1.70) | 6.05 (3.65 to 7.73) | **<0.001** |
| *At blood sampling* | | | |
| Age (years) | 5.2 (4.3 to 7.2) | 14.9 (13.8 to 16.2) | |
| Duration of GH treatment (years) | 4.00 (2.95 to 5.70) | 9.04 (8.14 to 10.77) | **<0.001** |
| Dose GH (mg/m$^2$/day) | 0.67 (0.50 to 1.00) | 1.00 (1.00 to 1.00) | **0.002** |
| Height SDS | -0.07 (-0.61 to 0.63) | -0.32 (-1.48 to 0.16) | 0.194 |
| BMI SDS | 0.89 (0.25 to 1.85) | 1.24 (0.23 to 1.66) | 0.646 |

Data are expressed as median (IQR), or number N
SDS according to age-and sex-matched reference values[21,22].

children than in de older children with PWS (0.7 (0.5 to 1.0) vs. 1.0 (1.0 to 1.0), $P = 0.002$, respectively).

### Serum IGF-I, IGFBP-3 and ALS levels

All PWS children were treated with 1mg GH/m$^2$/day, but the dose was lowered when IGF-I levels reached +3 SDS or higher. In the young children, we had to adjust the GH dose more frequently than in the older children (50% vs. 10%).

All GH-treated PWS children had significantly higher serum SDS values for IGF-I, IGFBP-3, IGF-I/IGFBP-3 ratio and ALS than healthy controls ($P < 0.001$) (Table 2). Serum IGF-I SDS and ALS SDS levels were not significantly different between young and older PWS children, but IGFBP-3 SDS was significantly higher in the young ones (1.3 (1.0 to 1.7) vs 0.9 (0.6 to 1.0), $P = 0.002$). As a result, the IGF-I/IGFBP-3 ratio was significantly lower in young children than in older children with PWS (0.38 (0.27 to 0.44) vs 0.67 (0.59 to 0.73), $P < 0.001$, respectively).

### Column chromatography

In GH-treated young PWS children, the 150 kD ternary complex formation was significantly higher than in the healthy young controls (0.99 (0.59 to 1.43) vs 0.46 (-0.17 to 0.93) SDS, $P = 0.022$) (Figure 1). The 150 kD to 40-50 kD peak ratio was also higher, but this did not reach significance (1.10 (0.55 to 1.88) vs. 0.09 (-0.55 to 1.65) SDS, $P = 0.070$).

In GH-treated older PWS children, 150 kD ternary complex formation was also significantly higher than in the healthy older controls (0.85 (0.31 to 1.51) vs -0.12 (-0.56 to 0.67)

**Table 2.** Overview of serum levels, column chromatography and IGF-bioactivity

| | Young GH-treated PWS children | Healthy young controls | *P* | Older GH-treated PWS children | Healthy older controls | *P* |
|---|---|---|---|---|---|---|
| Male/Female | 12/8 | 11/10 | 0.63 | 10/10 | 11/9 | 0.755 |
| Age (years) | 5.23 (4.31-7.15) | 5.58 (4.02-7.40) | 0.84 | 14.85 (13.82-16.15) | 14.77 (13.10 to 15.59) | 0.499 |
| IGF-I (nmol/l) | 37.8 (25.2 to 41.6) | 10.6 (8.3 to 19.6) | **<0.001** | 85.2 (70.6 to 92.4)* | 47.1 (40.1 to 53.8) | **<0.001** |
| IGF-I SDS | 2.2 (1.6 to 2.7) | -0.2 (-1.2 to 0.1) | **<0.001** | 2.5 (1.9 to 3.1) | 0.6 (0.1 to 0.8) | **<0.001** |
| IGF BP3 (nmol/l) | 93.8 (85.0 to 103.7) | 59.7 (51.6 to 75.2) | **<0.001** | 126.8 (114.5 to 133.2)* | 82.0 (70.5 to 91.3) | **<0.001** |
| IGF BP3 SDS | 1.3 (1.0 to 1.7) | -0.3 (-0.9 to 0.2) | **<0.001** | 0.9 (0.6 to 1.0)* | -0.6 (-1.1 to -0.2) | **<0.001** |
| Ratio IGF-I/BP3 | 0.38 (0.27 to 0.44) | 0.20 (0.15 to 0.25) | **<0.001** | 0.67 (0.59 to 0.73)* | 0.55 (0.49 to 0.63) | **<0.001** |
| ALS (nmol/l) | 290.2 (244.0 to 338.1) | 145.5 (124.0 to 199.6) | **<0.001** | 424.9 (379.5 to 449.8)* | 287.4 (237.3 to 326.8) | **<0.001** |
| ALS SDS | 3.9 (2.9 to 7.2) | -0.0 (-0.7 to 0.9) | **<0.001** | 4.0 (3.1 to 5.5) | 2.2 (0.9 to 2.9) | **<0.001** |
| Column chromatography | | | | | | |
| 150 kD peak % | 5.83 (4.82 to 6.73) | 4.83 (3.76 to 6.10) | 0.060 | 7.51 (6.75 to 8.96)* | 6.07 (5.48 to 7.31) | **0.006** |
| 150 kD peak SDS | 0.99 (0.59 to 1.43) | 0.46 (-0.17 to 0.93) | **0.022** | 0.85 (0.31 to 1.51) | -0.12 (-0.56 to 0.67) | **0.006** |
| 40-50 kD peak % | 4.35 (3.58 to 5.66) | 4.64 (3.70 to 6.28) | 0.465 | 2.68 (2.11 to 3.39)* | 2.71 (1.91 to 2.94) | 0.933 |
| 40-50 kD peak SDS | -0.88 (-1.74 to 0.42) | -0.35 (-1.47 to 0.67) | 0.407 | 0.15 (-1.41 to 1.34) | 0.05 (-0.69 to 0.59) | 0.955 |
| ratio between peaks | 1.33 (0.97 to 1.71) | 0.95 (0.60 to 1.29) | 0.097 | 2.74 (1.86 to 4.24)* | 2.42 (1.91 to 2.69) | 0.238 |
| ratio between peaks SDS | 1.10 (0.55 to 1.88) | 0.09 (-0.55 to 1.65) | 0.070 | 0.49 (-0.61 to 1.65) | 0.07 (-0.56 to 0.33) | 0.222 |
| IGF-bioactivity (nmol/l) | 1.06 (0.92 to 1.13) | 0.61 (0.49 to 0.65) | **<0.001** | 0.79 (0.42 to 1.03)* | 0.81 (0.71 to 1.04) | 0.675 |
| % IGF-bioactivity | 2.74 (2.56 to 3.31) | 5.08 (3.33 to 6.04) | **<0.001** | 1.07 (0.60 to 1.27)* | 1.87 (1.53 to 2.48) | **<0.001** |

Data are presented as median (IQR)

SDS according to age-and sex-matched reference values[23-25]

*$P<0.05$ PWS Young vs PWS Old

SDS, *P*=0.006). Again, the 150 kD to 40-50 kD ratio was not significantly different from healthy older controls (0.49 (-0.61 to 1.65) vs. 0.07 (-0.56 to 0.33) SDS, *P* = 0.227).

No differences in complex formation were found between the GH-treated young and older PWS children (150 kD: *P* = 0.653 and 150 kD to 40-50 kD ratio: *P* = 0.222).

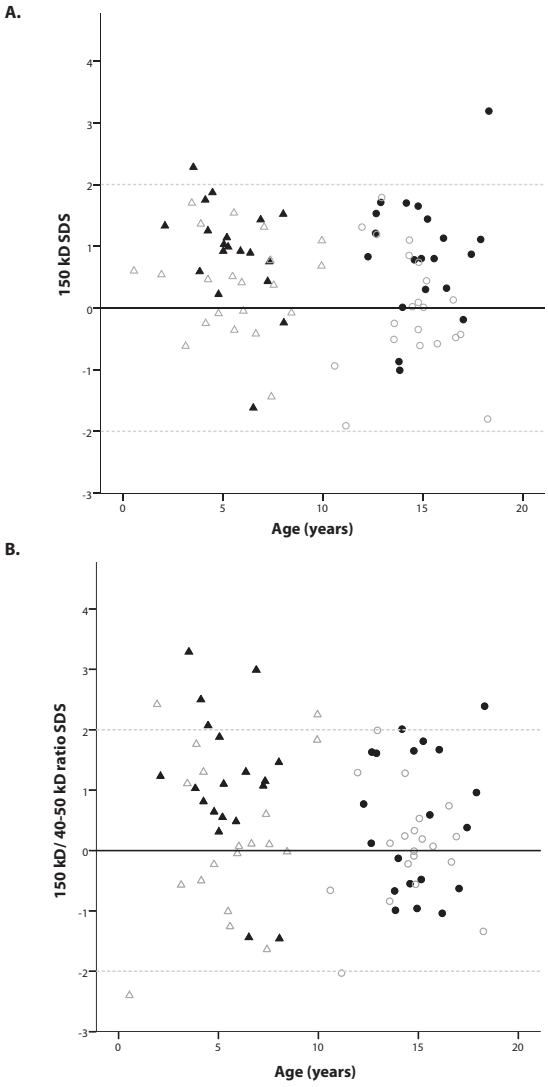


**Figure 1.** A. 150 kD complex SDS in GH-treated children with PWS and controls
B. 150 kD/40-50 kD ratio SDS in GH-treated children with PWS and controls
▲ Young GH-treated PWS children
Δ Healthy young controls
● Older GH-treated PWS children
○ Healthy older controls

## IGF-bioactivity

GH-treated young PWS children had the highest IGF-bioactivity (1.06 (0.92 to 1.13) nmol/l), which was significantly higher than the healthy young controls (0.61 (0.49 to 0.65) nmol/l, $P < 0.001$), and the GH-treated older PWS children (0.79 (0.42 to 1.03 nmol/l, $P = 0.012$) (Figure 2A). The older PWS children had a similar IGF-bioactivity as the healthy older controls (0.81 (0.71 to 1.04) nmol/l).

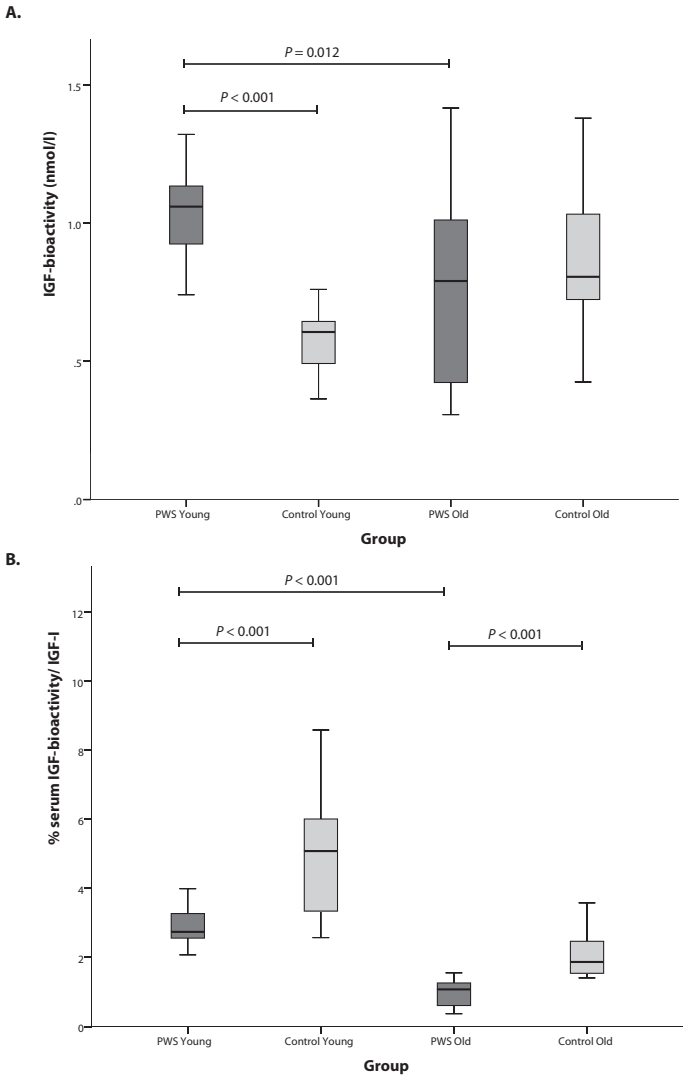


**Figure 2.** A. IGF-bioactivity in GH-treated PWS children and healthy controls
B. % Bioactive-IGF / serum IGF-I in GH-treated PWS children and healthy controls
■ PWS children
■ Controls

The percentage of the IGF-bioactivity of the total serum immunoreactive IGF-I (expressed as % IGF-bioactivity) was, however, significantly lower in both PWS age groups compared to healthy controls (both $P < 0.001$) (Figure 2B). GH-treated young PWS children had a higher % IGF-bioactivity than the older PWS children (2.74 (2.56 to 3.31) vs. 1.07 (0.60 to 1.27), $P < 0.001$). Also in the healthy control group, young children had a significantly higher % IGF-bioactivity than the older ones (5.08 (3.33 to 6.04) vs. 1.87 (1.53 to 2.48), $P < 0.001$) (Table 2).

### Correlations IGF-bioactivity

In healthy controls, serum IGF-bioactivity was strongly correlated with IGF-I SDS, IGF-I/IGFBP3 ratio and ALS SDS ($\rho = 0.745$, $\rho = 0.836$, $\rho = 0.741$, respectively, all $P < 0.0001$) (Figure 3). In contrast, in children with PWS, a lack of correlations was found between these parameters and IGF-bioactivity. Age was positively correlated with IGF-bioactivity in healthy controls ($\rho = 0.770$, $P < 0.0001$), but not in children with PWS ($\rho = -0.282$, $P = 0.078$). In children with PWS, serum IGF-bioactivity did not correlate with the duration of GH treatment and GH dose at time of blood sampling.

As shown in Figure 3A, children with PWS showed a very wide variation in IGF-bioactivity, especially the older PWS children. Almost all children with PWS had a serum IGF-I SDS level above the +2 SDS, but only 1 child had a higher IGF-bioactivity than the highest level in healthy controls, while 4 children had even a very low IGF-bioactivity. This indicates that the serum immunoreactive levels of children with PWS did not discriminate between high and low IGF-bioactivity.

Because we observed a higher IGF-bioactivity in young PWS compared to both age-matched controls and to the older PWS children, we performed a sub-analysis in the young and older PWS children. In young PWS children, IGF-bioactivity was positively correlated with IGF-I/IGFBP3 ratio ($\rho = 0.663$, $P = 0.001$) (Figure 3C), while in the older PWS children there was no correlation.

Despite their high serum IGF-I SDS values, Figure 3D shows that both young and older children with PWS exhibited lower IGF-bioactivity when expressed as proportion of their total serum immunoreactive IGF-I than healthy controls.

### Multiple regression analysis in children with PWS

We performed a multiple regression (MR) analysis to determine associations between IGF-bioactivity and delta height SDS and delta BMI SDS during the previous year in the total group of children with PWS, but no significant associations were found. In the young PWS children, IGF-bioactivity was not associated with delta height SDS, but it was significantly associated with delta BMI SDS ($\beta = 0.644$, $P = 0.005$). IGF-bioactivity was not associated with height SDS and BMI SDS at time of blood sampling. In the older PWS children, no associations were found.

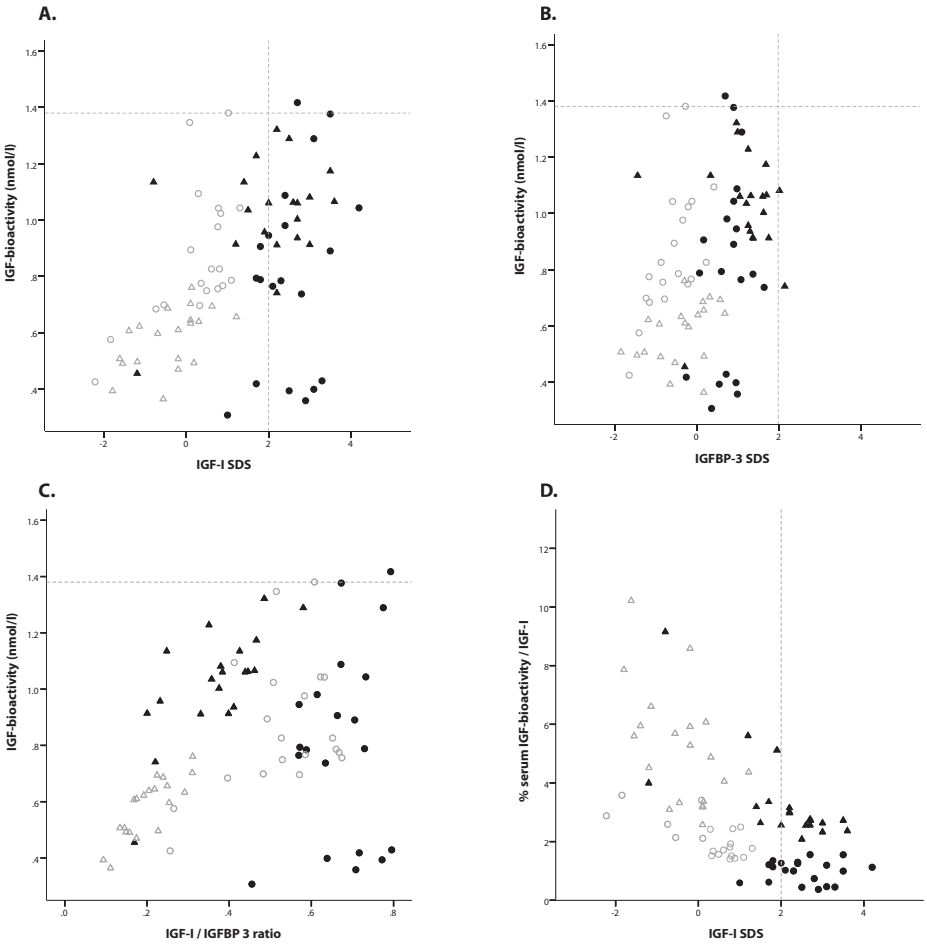


**Figure 3.** A. Correlation between IGF-bioactivity and IGF-I SDS
B. Correlation between IGF-bioactivity and IGFBP3 SDS
C. Correlation between IGF-bioactivity and IGF-I/IGFBP3 ratio
D. Correlations between % serum IGF-bioactivity/IGF-I and IGF-I SDS.
▲ Young GH-treated PWS children
Δ Healthy young controls
● Older GH-treated PWS children
○ Healthy older controls

## DISCUSSION

Our study shows that IGF-I SDS, IGFBP-3 SDS, IGF-I/IGFBP-3 ratio and ALS SDS were significantly higher in GH-treated children with PWS than in healthy controls. Children with PWS had a higher 150 kD ternary complex formation than healthy controls, indicating that most of the serum IGF-I is sequestered in the ternary 150 kD complex with ALS

and IGFBP-3. Young GH-treated PWS children had a higher IGF-bioactivity than healthy young controls and older GH-treated PWS children. Despite the higher immunoreactive IGF-I levels in older PWS children, no difference in IGF-bioactivity was observed compared to healthy older controls, which is reassuring. The % IGF-bioactivity was lower in the GH-treated PWS children than in healthy controls. The most striking finding was the disrupted correlation between serum immunoreactive IGF-I levels and IGF-bioactivity in children with PWS, in contrast to a strong positive correlation in healthy controls.

High serum IGF-I levels during GH treatment are a major problem in children with PWS. In order to shed more light on this phenomenon, this is the first study investigating ALS levels, complex formation and IGF-bioactivity in serum of GH-treated children with PWS compared to age- and sex-matched healthy controls. We observed elevated serum IGF-I levels in GH-treated PWS children in combination with IGFBP-3 levels in the upper normal range. This is in line with previous studies, describing a significant increase in serum IGF-I levels and relatively less increase in IGFBP-3 levels during GH treatment in children with PWS[10, 12]. We additionally found markedly high ALS levels during GH treatment in PWS children. The finding of high ALS levels underlines that children with PWS are very sensitive to GH, because the production of ALS is predominantly regulated by GH[28]. ALS is essential for the stabilization of both circulating IGF-I and IGFBP-3, and restrains the ability of IGF-I to interact with its receptor[18]. In animals, it was shown that ALS is also involved in carbohydrate and fat metabolism[29, 30]. Patients with PWS might benefit from a high serum ALS level, because it may contribute to the improvement of metabolic parameters during GH treatment[31, 32]. On the other hand, it could be that the high ALS levels decrease the IGF-bioactivity through increased 150 kD complex formation. Indeed, we observed a higher degree of 150 kD ternary complex formation in children with PWS than in the controls, indicating that most of the IGF-I is sequestered by ALS and IGFBP-3. This is in accordance with our finding that the % IGF-bioactivity was lower in children with PWS than in the healthy controls.

Whereas we found a strong relationship between serum immunoreactive IGF-I levels and IGF-bioactivity in healthy controls, this appeared not to be the case in children with PWS. Thus serum IGF-I levels in GH-treated PWS children are an inappropriate indicator of the IGF-bioactivity and therefore unreliable for titrating the GH dose. For the young GH-treated PWS children, but not for the older ones, we observed a positive correlation between IGF-bioactivity and the IGF-I/IGFBP3 ratio. In literature, the serum IGFI/IGFBP-3 ratio has occasionally been considered as a surrogate marker for the level of free biologically available IGF-I in the circulation[33, 34]. This may indeed be applicable in the young GH-treated PWS children, but further research is needed to confirm the clinical relevance.

Although they received a lower GH dose, young children with PWS showed a higher serum level of IGF-bioactivity than the older children. The latter had serum

IGF-bioactivity levels that were similar to healthy controls. The fact that we observed a higher IGF-bioactivity only in the young PWS children, might suggest that this is transient, because the older PWS children with more years of GH treatment have a normal IGF-bioactivity. A recent study observed higher IGFBP-3 proteolytic activity in young children compared to older children[25]. IGFBP-3 proteases are capable of cleaving intact IGFBP-3 into smaller fragments with a reduced affinity for IGF-I[35]. This could result in higher IGF-I bioavailability for target tissue to stimulate growth in young children. One explanation could be that GH-treated young PWS children would need a relatively high level of IGF-bioactivity, because they grow fast[13, 36]. However, we observed no correlation between serum IGF-bioactivity and delta height SDS, GH dose and duration of GH treatment. Another explanation could be that young PWS children undergo great changes in body composition. We observed a positive relation between IGF-bioactivity and delta BMI SDS in young GH-treated PWS children, while there was no relation with BMI SDS at time of blood sampling. So the higher the increase in BMI in young PWS children, the higher the IGF-bioactivity. This finding is in line with a previous study in anorexia nervosa patients, who had an increase in IGF-bioactivity during refeeding, when their BMI increased[37].

In children with PWS, different nutritional phases have been described[38] and, in young children, a switch occurs from phase 1b (appropriate feeding with weight increasing at a normal rate) to phase 2a (an increase in body weight without a change in appetite or dietary intake). *Miller at al.* described that the switch to phase 2a was associated with a significant increase in serum IGF-1 levels during GH treatment while on a stable GH dose[38]. In young PWS children, we observed a high IGF-bioactivity while we already had lowered the GH dose more frequently due to supranormal IGF-I levels. It could also be speculated that an increase in IGF-bioactivity is a reaction of the body to compensate for the increase in BMI. We observed a very wide variation in IGF-bioactivity in the older PWS children, despite high immunoreactive IGF-I levels. Based on the high immunoreactive IGF-I levels, physicians might decide to lower the GH dose, while some older patients need in fact a higher GH dose.

This first study investigated IGF-bioactivity cross-sectionally, thus we were not able to see after which age or duration of GH treatment the IGF-bioactivity had changed and which level of IGF-bioactivity is needed to improve growth and body composition. As our control group consisted of healthy children with an appropriate growth and no GH treatment, we could not compare our results with GH-treated non-PWS patients. Despite high serum IGF-I in all and high IGF-bioactivity levels in young children with PWS, we did not observe side effects or signs of acromegaly. This suggests that other factors, such as tissue specific IGF1R concentrations, IGF1R signal pathways or IGFBP-3 protease activity, might be involved. Based on our data, a large longitudinal follow-up study is warranted in GH-treated children with PWS compared with GH-treated children with GH deficiency.

In conclusion, in GH-treated children with PWS, most of the serum IGF-I is sequestered in the ternary 150 kD complex with ALS and IGFBP-3. High serum IGF-I levels in GH-treated PWS children, result only in a higher IGF-bioactivity in young children, but in a normal IGF-bioactivity in older children compared to healthy controls. In young GH-treated PWS children, the high IGF-bioactivity is related to the increase in BMI and probably transient, but the long-term clinical consequences need further research. In older GH-treated PWS children our data are reassuring. IGF-bioactivity in GH-treated PWS children show a wide variation and a disrupted correlation with immunoreactive IGF-I levels, which makes immunoreactive IGF-I levels an inappropriate indicator of GH dosing in these children.

## ACKNOWLEDGEMENTS

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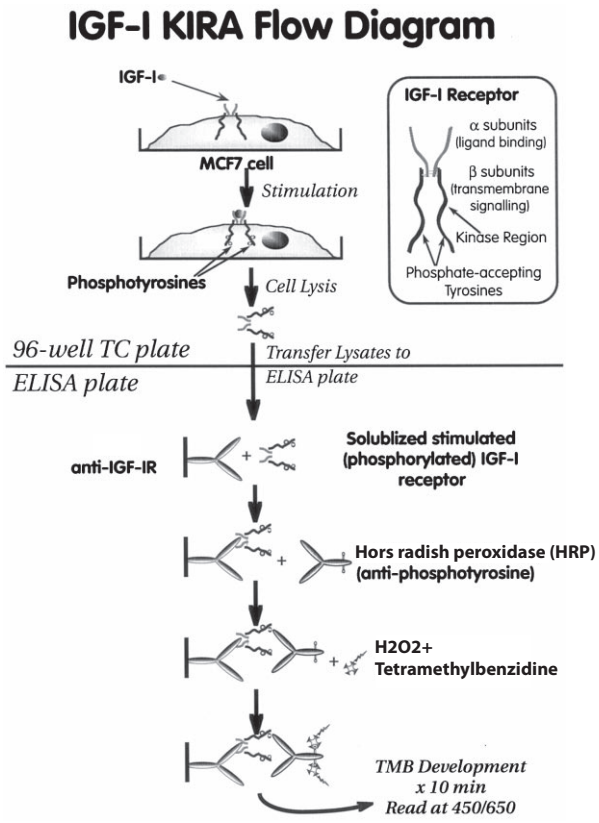
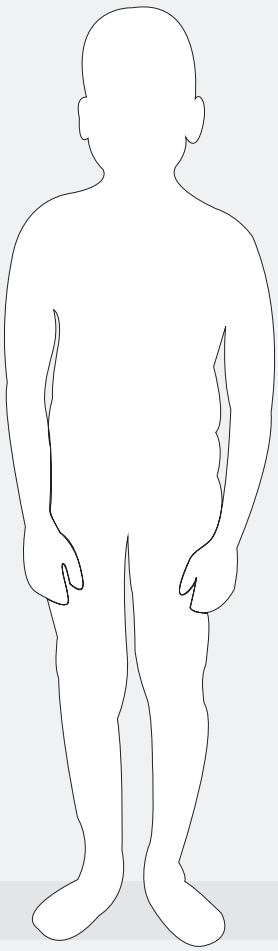
## APPENDIX 1




Adapted from Sadick et al[26]



# CHAPTER 7

# Beneficial effect of growth hormone treatment on health related quality of life in children with Prader-Willi Syndrome: a randomized controlled trial and longitudinal study

N.E. Bakker, E.P.C. Siemensma, M. van Rijn, D.A.M. Festen, A.C.S. Hokken-Koelega

**Submitted**

## ABSTRACT

***Objectives*** Growth hormone (GH) treatment is beneficial for children with Prader-Willi syndrome (PWS), but data about health-related quality of life (HRQOL) and effects of GH treatment are scarce. We therefore evaluated HRQOL in children with PWS and investigated effects of GH treatment.

***Study design*** In a randomized controlled GH trial including 26 children with PWS and during a 11-years longitudinal GH study in 76 children, we annually assessed HRQOL recorded by patients and parents, using a generic questionnaire (DUX25), containing four subdomains (Physical, Home, Social and Emotional) and a PWS-specific questionnaire (DUXPW).

***Results*** At baseline, children with PWS rated their HRQOL on all items similar or higher as healthy and obese children. GH-treated children reported an increase in HRQOL in the Physical ($P < 0.05$) and Social subdomains ($P = 0.05$) and DUXPW ($P < 0.001$), compared to the untreated children. Parents reported an increase in the Physical ($P < 0.01$) and Emotional subdomains ($P < 0.05$) and borderline in the total DUX25 ($P = 0.072$), compared to the parents of the untreated children.

During 11 years of GH treatment, the Physical subdomain continued to improve, according to parents, and the Home, Social and Emotional subdomains of the DUX25 and DUXPW remained similar, according to children and parents.

***Conclusions*** Children with PWS rated their HRQOL equal or even better compared to healthy children and obese children. According to children and parents, HRQOL increased during GH treatment, in contrast to untreated children. This effect sustained during long-term GH treatment. Children with PWS consider themselves quite happy, despite the difficulties related to the syndrome.

## INTRODUCTION

Prader-Willi syndrome (PWS) is a rare neurodevelopmental disorder that occurs in approximately 1:15,000-1:30,000 live births[1, 2]. It results from the absence of expression of paternally expressed genes, located on chromosome 15 at the locus q11-q13 caused by paternal deletion, maternal uniparental disomy (mUPD), imprinting errors (ICD), or by paternal chromosomal translocation[3]. PWS is characterized by a number of symptoms, including muscular hypotonia, hypogonadism, short stature, obesity, psychomotor delay, neurobehavioral abnormalities, and cognitive impairment[4].

Long-term continuous growth hormone (GH) treatment is an effective and safe treatment for children with PWS[5] and has also beneficial effects on cognitive functioning in children with PWS[6]. Two studies showed that GH treatment can improve the physical and psychological aspects of health-related quality of life (HRQOL) in adolescents and adults with PWS[7, 8] but information about HRQOL or the effect of GH treatment on HRQOL in children with PWS is scarce. One study investigated HRQOL in 19 adolescents after 10 years GH treatment and observed a lower HRQOL than in the general population on the items of mobility, breathing, speech, mental function, going to school and getting and having friends[9]. However, the effect of GH treatment on HRQOL could not be investigated because of a lack of an untreated control group with PWS and lack of comparable baseline values.

HRQOL refers to the impact of health and illness on an individual's quality of life[10, 11]. HRQOL can be measured by generic and disease-specific instruments[11]. Generic HRQOL instruments allow comparison with normative data and across disease populations[11]. Disease-specific measurements include domains that are only valid for a specific condition, which maximizes content validity and result in a greater sensitivity and specificity[11].

To determine a child's HRQOL, the opinion of a child itself is probably most important[12]. But, as parents are generally quite able to determine their child's HRQOL, a combination of child reports and parent reports seems to be most useful[12-14].

We hypothesized that children and parents would report lower HRQOL in children with PWS than in a healthy reference population, and that HRQOL in GH-treated children with PWS would be higher compared to untreated children with PWS and would be sustained during long-term GH treatment. The first aim of this study was to describe HRQOL in untreated children with PWS. The second aim was to assess HRQOL in GH-treated children with PWS compared to untreated children. Our final aim was to investigate the effect of 11 years of GH treatment on HRQOL of children with PWS, according to children and their parents.

## MATERIALS AND METHODS

### Design

In April 2002, a multicenter, Randomized Controlled Trial (RCT) was started in children with PWS, investigating the effects of GH treatment versus no GH treatment on growth, body composition, activity level, psychosocial development and quality of life. After stratification for age and body mass index (BMI), children were randomly assigned (1:1) to either the GH treatment group or control group for 2 years. Randomization was performed by the Dutch Growth Research Foundation by using a computer-generated list of random numbers. After the RCT, all children were treated with GH and followed in the Dutch PWS Cohort study. In 2007, the RCT finished and all new patients were directly included in the Dutch PWS Cohort study. HRQOL was annually recorded during the RCT and Cohort study, by children and parents.

All participants fulfilled the following inclusion criteria: genetically confirmed diagnosis of PWS and age 6-12 years (girls) or 6-14 years (boys) at start of study. Both children and their parents had to be able to complete the HRQOL questionnaires.

Biosynthetic GH (Genotropin; Pfizer Inc., New York, NY), 1.0 mg/m$^2$/day, was administered subcutaneous once daily at bedtime in children of the treatment group during the RCT and in all children during the Cohort study. All children were naïve to GH treatment at start of the RCT. During the entire study period, children were seen 3-monthly for anthropometric measurements by the PWS team of the Dutch Growth Research Foundation, in collaboration with Dutch pediatric endocrinologists and pediatricians.

The study protocol was approved by the Medical Ethics Committee of Children's Hospital Erasmus MC- Sophia, Rotterdam, the Netherlands. Written informed consent was obtained from the parents and children older than 12 years and assent in children younger than 12 years of age.

### Measurements

*Health related quality of life*

HRQOL was measured by two questionnaires, the Dutch Children AZL/TNO Questionnaire Quality of Life short form (DUX25) and a PWS-specific questionnaire, the DUX Prader Willi (DUXPW)[15-17]. These questionnaires were constructed for children aged 6 years and older. Children filled out their questionnaire separately from their parents, under supervision of a psychologist experienced in testing children with PWS. The psychologist was blinded for the randomization.

*DUX25*. This generic 25-item questionnaire measures different aspects of daily functioning of children and adolescents. The DUX25 total score is calculated by combining scores of all 25 items and contains four subdomains; Physical, Home, Emotional and Social functioning. Subdomain scores were calculated for each subdomain. The items

**Figure 1.** 5-point Likert Scale by abstract faces with varying expressions (smiley's), ranging ranging from sad (score of 0) to very happy (score of 100)

are scored on a 5-point Likert Scale by abstract faces with varying expressions (smiley's), ranging from sad (score of 0) to very happy (score of 100), thus a higher score indicates a better quality of life (Figure 1). A total score of 60 is classified as the lower limit of normal, because it represents the –2 SDS level of healthy children[18]. When one or two items per subdomain were missing, the value was replaced by the mean of the remaining items. When more than two items per subdomain were missing, the subdomain was removed. The DUX25 is validated and available in a child form and parent form[17-20].

*DUXPW*. Based on the generic DUX25, a PWS disease specific 18-item questionnaire, the DUXPW, was developed in a similar way as the CDDUX for children with celiac disease and BTDUX for children with a bone tumor[21-24]. A DUXPW total score was calculated by combining the scores of all 18 questions.

*Anthropometry and cognition*

Height was assessed by a Harpenden stadiometer and weight was assessed on an accurate scale. BMI (kg/m$^2$) was calculated and converted into SDS, according to Dutch references for age[25, 26]. Growth Analyser version 4.0 was used to calculate BMI and BMI SDS (www.growthanalyser.org).

Cognition was measured by WPPSI-R or WISC-R depending on age, and a total intelligence quotient (IQ) was calculated as described before[6].

## Data analysis

Statistical analyses were performed with SPSS 21.0 (SPSS INC., Chicago, IL). Cronbach's α was calculated to determine internal consistency in the generic DUX25 and the DUXPW, both tests were proven to be internally consistent (Cronbach's $\alpha > 0.70$)[15, 27]. The total and subdomain-correlations appeared to be good, with Spearman correlation coefficients varying between 0.27 and 0.63 in children and 0.41 and 0.75 in parents (all $P < 0.001$).

Differences between children with PWS in the GH treatment group and control group, between children and parents and between children with PWS and various reference groups at baseline were calculated by Mann Whitney U tests.

To analyze the effect of GH treatment during the RCT and longitudinal study, Linear Mixed Modelling for repeated measurements[28] was used with GH treatment and time as factors (GH treatment coded as: 1 = GH treatment group; 0 = control group; time coded as 0 = baseline; 1 = after 2 years of study) in the RCT and time as factor in the longitudinal study.

The effects of age, gender, genotype, anthropometric measurements and total IQ score on HRQOL during GH treatment were determined by using these variables as factors (in case of nominal or ordinal variables) or covariates (in case of scale variables) in the model.

P-values less than 0.05 were considered statistically significant.

## RESULTS

### Baseline characteristics

Hundred-fifty-three prepubertal children started GH treatment between April 2002 and June 2014 in our Dutch PWS Cohort study. Fifty-three children were below the age of 6 years and 23 children and their parents never completed the questionnaires. Twenty-six children with PWS and their parents were enrolled in the RCT and 76 in the long-term study. The 23 children, without completed questionnaires, were not significantly different from the long-term group (age at start $P = 0.06$, IQ $P = 0.131$, gender $P = 0.911$, genetic subtype $P = 0.512$, height SDS $P = 0.859$ and BMI SDS $P = 0.214$).

Table 1 shows the baseline characteristics of 26 participants of the RCT and 76 participants of the long-term group. At baseline, there were no significant differences between the children in the treatment and control group of the RCT.

**Table 1.** Baseline characteristics RCT and long-term group at start of GH treatment

| | Total group RCT | Treatment group | Control group | Long-term group |
|---|---|---|---|---|
| N | 26 | 15 | 11 | 76 |
| Age start study | 8.1 (6.8 to 11.4) | 9.8 (7.3 to 11.6) | 7.4 (6.8 to 8.8) | 5.2 (2.4 to 7.9) |
| Gender (M/F) | 9/17 | 6/9 | 3/8 | 37/39 |
| Genetic subtype | | | | |
| Deletion | 12 | 7 | 5 | 35 |
| mUPD | 9 | 4 | 5 | 33 |
| ICD | 4 | 3 | 1 | 4 |
| Unknown | 1 | 1 | 0 | 4 |
| BMI SDS | 1.5 (0.8 to 2.6) | 1.9 (0.8 to 2.6) | 1.5 (1.1 to 1.8) | 1.2 (0.0 to 1.7) |
| Height SDS | -2.1 (-3.1 to -1.3) | -2.0 (-3.2 to -0.9) | -2.4 (-3.1 to -1.5) | -2.3 (-3.1 to -1.3) |
| IQ[1] | 69 (59 to 81) | 65 (58 to 75) | 76 (66 to 84) | 66 (59 to 80) |

Data is presented as median (IQR).
[1]For details see reference [6].

## Baseline HRQOL in the RCT

*Children and parents*

Table 2 shows baseline HRQOL as reported by the children with PWS and their parents. Children with PWS scored significantly higher than their parents in the Physical subdomain ($P = 0.004$) and tended to score higher in the DUXPW ($P = 0.08$). Total DUX25 scores and the other subdomains did not significantly differ between children with PWS and their parents.

**Table 2.** Baseline HRQOL according to children with PWS at baseline compared to their parents, healthy children, children with a chronic illness, obese children and children with a growth impairment.

| | PWS children | PWS parents | Healthy | Chronic illness | Obese | Growth impairment |
|---|---|---|---|---|---|---|
| **N** | 26 | 26 | 1430 | 76 | 23 | 79 |
| **DUX25** | | | | | | |
| -Total | 81 (13) | 77 (10) | 77 (13) | 81 (13) | 63 (10)[3] | 77 (13) |
| -Physical | 83 (18) | 64 (19)[2] | 75 (18) | 79 (16) | 53 (20)[3] | 74 (17) |
| -Home | 85 (16) | 85 (13) | 84 (15) | 88 (13) | 80 (15) | 82 (16) |
| -Emotional | 80 (15) | 82 (11) | 73 (16)[1] | 78 (15) | 53 (15)[3] | 73 (16) |
| -Social | 80 (18) | 77 (11) | 77 (13) | 80 (15) | 68 (09)[1] | 77 (15) |
| **DUXPW** | 75 (17) | 69 (8) | | | | |

Data are presented as mean (SD), according to normative data of the non-PWS groups[18].
[1]P<0.05, [2]P<0.001 and [3]P<0.0001: significant differences between children with PWS and their parents, healthy children, children with congenital chronic illness, obese children and children with a growth impairment.
Normal between 60-100

*Children with PWS compared to reference groups*

Table 2 shows HRQOL in children with PWS compared to healthy, chronically ill, obese and growth impaired children[18]. Children with PWS scored significantly higher in the Emotional subdomain compared to healthy children ($P < 0.05$) but no differences were found in the DUX25 total score and the Physical, Home and Social subdomains. Children with PWS scored significantly higher in the Physical ($P < 0.0001$), Emotional ($P < 0.0001$) and Social subdomains ($P < 0.05$) and the total DUX25 score, compared to obese children ($P < 0.0001$). No differences were found compared to chronically ill and growth impaired children.

## HRQOL during the RCT

*According to children*

GH-treated children showed a significant improvement in HRQOL during the 2-year-RCT in the Physical subdomain of the DUX25 and the DUXPW, compared to the untreated

ones ($P < 0.05$ and $P < 0.001$) (Figure 2). There was a trend towards a significant improvement in total DUX25 score in GH-treated children compared to the untreated ones ($P = 0.07$) and in the Social subdomain of the DUX25 ($P = 0.05$).

There were no differences between GH-treated and untreated children with PWS in the subdomains Home and Emotional.

*According to parents*

Parents of GH-treated children reported a significant improvement in HRQOL in the 2-year-RCT in the Physical and Emotional subdomains of the DUX25, compared to parents of untreated children ($P < 0.01$ and $P < 0.05$), (Figure 3). There was a trend towards a significant improvement in total DUX25 ($P = 0.07$).

Parents reported no significant differences between GH-treated children and untreated children in the DUXPW, and subdomains Home and Social of the DUX25.

**HRQOL during 11 years of continuous GH treatment**

Figure 4 shows the HRQOL in children with PWS according to children and their parents during 11years of continuous GH treatment. Children reported a significant increase in the DUXPW during the first 2 years of GH treatment (mean (CI 95%) difference compared to baseline 8 (1-15), $P = 0.019$), but thereafter it remained similar. Parents reported no significant change in the DUXPW.

Children reported a significant increased during the first 2 years of GH in the Physical subdomain of the DUX25 (mean (CI 95%) difference compared to baseline 15 (6-24), $P = 0.001$), while it was not significantly different after 11 years compared to baseline (difference 3 (-11-17)). Parents reported a significant increase in the Physical subdomain during 2 years of GH treatment (difference 9 (0-18), $P = 0.044$) and this continued to increase during 11 years of GH (mean (CI 95%) difference after 11 years compared to baseline 17 (4-30), $P = 0.009$). Scores in the Home subdomain increased significantly during 4 years of GH treatment (mean (CI 95%) difference compared to baseline 12 (4-20), $P = 0.002$) reported by the children, but it was not significantly different after 11 years compared to baseline (difference 1 (-11-12)). Reported by the parents, scores in the Home subdomain remained similar compared to baseline.

Total DUX25 scores and the Emotional and Social subdomains remained similar compared to baseline according to both children and parents.

During the first 4 years of GH treatment, children with a higher IQ had a significantly higher total DUX25 score ($\beta = 0.28$, $P = 0.031$) and higher scores in the Home ($\beta = 0.27$, $P = 0.038$) and Emotional ($\beta = 0.38$, $P = 0.026$) subdomains than children with a lower IQ, but after 11 years of GH treatment there was no significant effect of IQ anymore. Parents of older children indicated a significantly lower HRQOL than parents of younger children based on the total DUX25, its subdomains and the DUXPW ($\beta = -1.7$, $P < 0.0001$

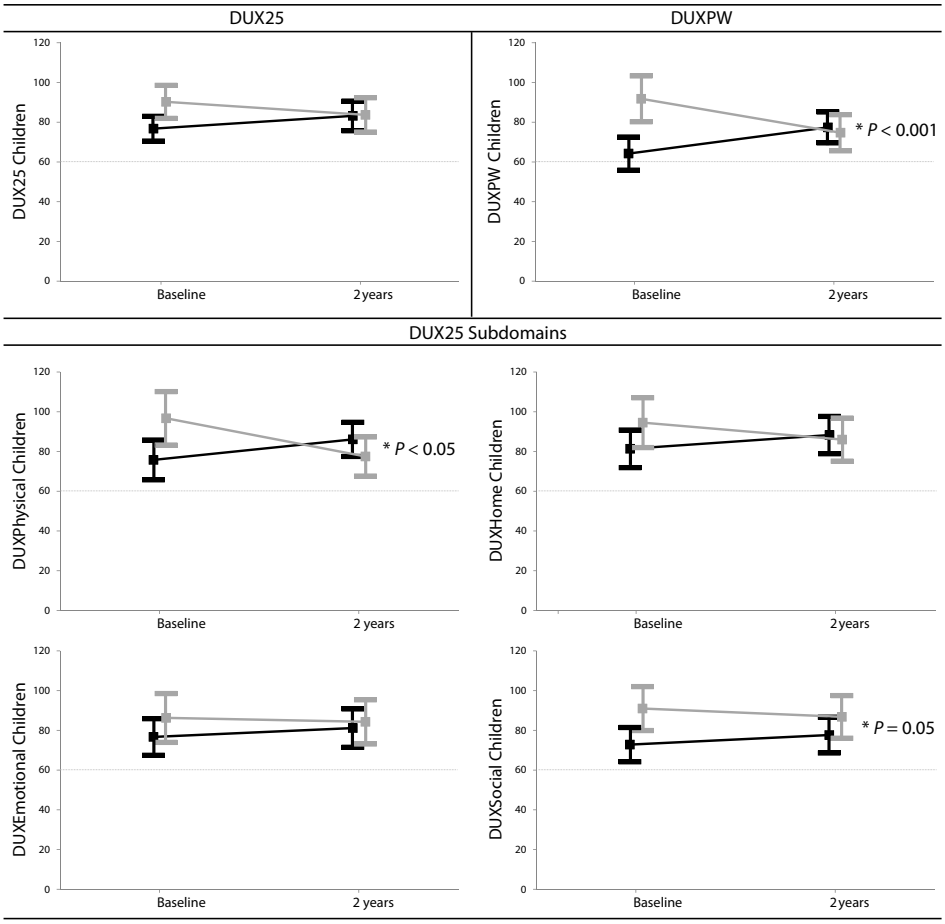



**Figure 2.** This figure presents mean scores and their 95% CI on the DUX25, subdomains of the DUX25, and DUXPW according to children in GH-treated (in black) and untreated (in grey) children with PWS at baseline and after 2 years of study during the RCT. Significant differences between GH-treated and untreated children are indicated in the figure. The lower limit of normal is indicated by a dashed line.

■ GH group

■ untreated group

for the DUX25, β = -1.3, $P = 0.009$ for the Physical subdomain, β = -1.6, $P < 0.001$ for the Home subdomain, β = -2.0, $P < 0.001$ for the Emotional subdomain, β = -1.7, $P < 0.001$ for the Social subdomain and β = -1.0, $P = 0.002$ for the DUXPW score). Age had no significant effect on HRQOL according to the children. Children with a higher BMI SDS, had a significantly higher score in the Social subdomain, β = 2.5, $P = 0.045$ and tended to have a lower score in the Physical subdomain (β = -1.9, $P = 0.056$). Parents of children with a higher BMI SDS, indicated a significantly lower score in the Physical subdomain than parents of children with lower BMI SDS (β = -3.2, $P = 0.007$).

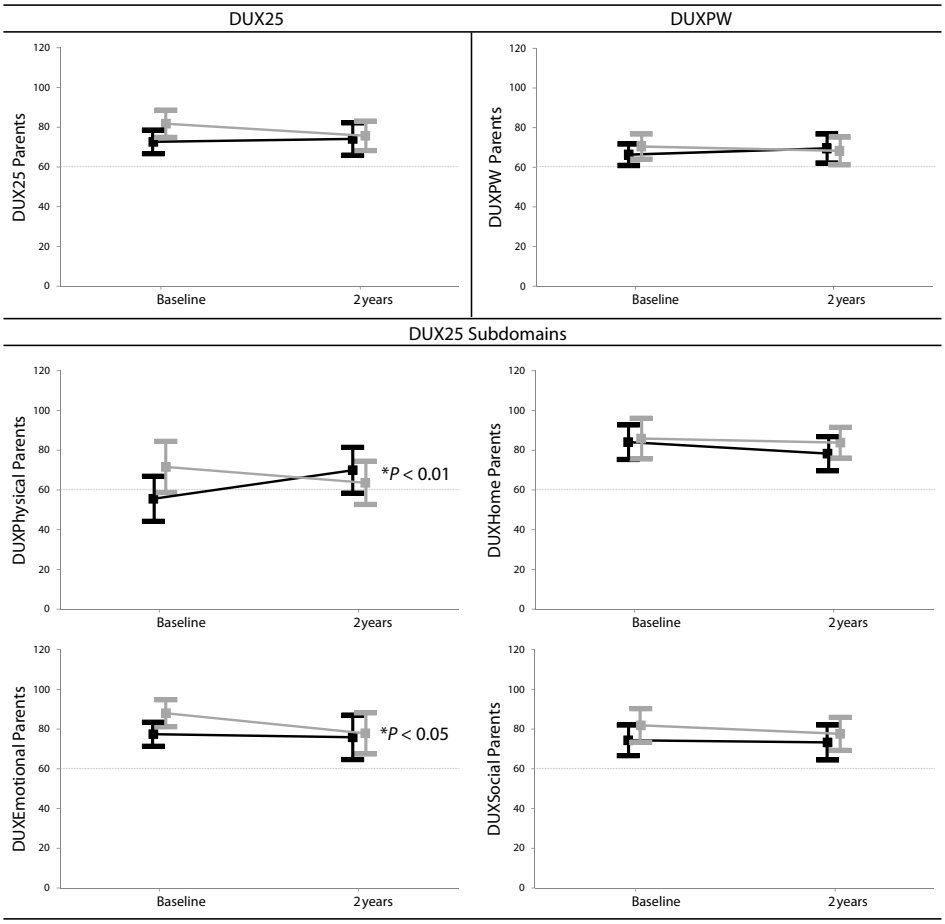



**Figure 3.** This figure presents mean scores and their 95% CI on the DUX25, subdomains of the DUX25, and DUXPW according to parents in GH-treated (in black) and untreated (in grey) children with PWS at baseline and after 2 years of study during the RCT. Significant differences between GH-treated and untreated children are indicated in the figure. The lower limit of normal is indicated by a dashed line.

■ GH group

■ untreated group

Parents of children with a deletion rated their children's HRQOL higher than parents of children with other genotypes with regard to the Emotional and Social subdomains of the DUX25 (Emotional subdomain: mean (CI 95%) difference between children with a deletion and an ICD 9 (1-18), $P = 0.032$; Social subdomain: mean (CI 95%) difference between children with a deletion and an mUPD 5 (1-9), $P = 0.039$, and between children with a deletion and an ICD 9 (1-17), $P = 0.026$).

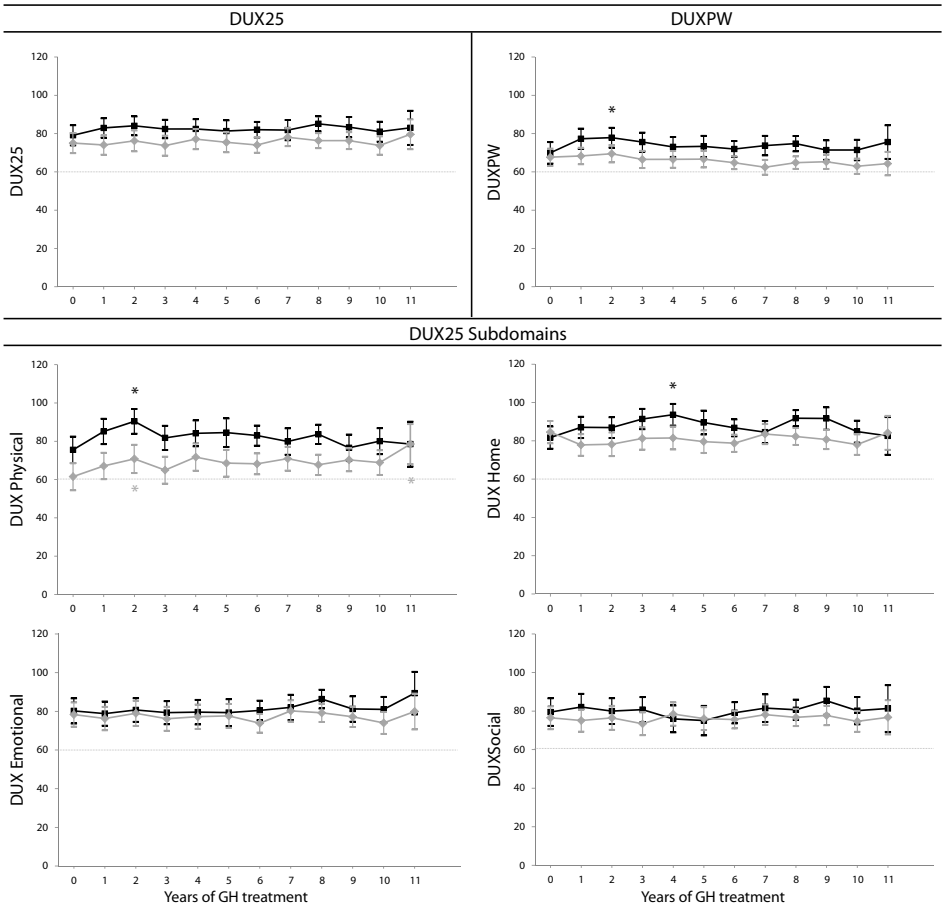



**Figure 4.** This figure presents mean scores and their 95% CI on the DUX25, subdomains of the DUX25, and DUXPW according to children (in black) and their parents (in grey) at baseline and during 11 years of GH treatment. The lower limit of normal is indicated by a dashed line.

■ according to children with PWS

◆ according to their parents

* significantly higher than baseline $P < 0.05$

## DISCUSSION

Our study assessed HRQOL in children with PWS according to both children and parents. In addition, it assessed the effects of GH treatment on HRQOL during a 2-year GH RCT and during 11 years of continuous GH treatment. We showed that prior to start of GH treatment, children with PWS rated their HRQOL similar as their parents, they scored only higher in the Physical subdomain. Compared to healthy children and children with various other diseases, children with PWS rated their HRQOL equal or even better.

According to children and parents, HRQOL improved in GH-treated children with PWS, while it decreased or remained similar to baseline in untreated controls with PWS. After the improvement during the first 2 years in the DUXPW and the Physical and Home subdomains of the DUX25, the HRQOL remained similar during 11 years of GH treatment, according to both children and parents.

In contrast to our expectation, children with PWS rated their HRQOL equal or higher than children with other diseases and healthy children, already at baseline. In a study investigating HRQOL in 9 untreated children with PWS (age range 5-14 years)[8], HRQOL was found to be lower in children with PWS compared to a reference population. However, in that study HRQOL was rated by parents only. A possible explanation for the high HRQOL reported by the children in our study is the mild to moderate intellectual impairment in people with PWS[29], while the HRQOL reference values are based on children without intellectual impairment. Comparison of HRQOL in children with PWS to a normal reference population may be less reliable due to the inequality in intelligence.

Our results showed that during the first years of GH treatment, the child's IQ had a significant positive relation with the child-reported HRQOL, which means that children with PWS with an IQ level closer to normal reported a higher HRQOL than those with a lower IQ. During long-term GH treatment this effect disappeared, probably because long-term treatment significantly improved cognition in children with PWS, particularly in those with lower a cognitive functioning at start[6]. It is therefore likely that the positive association between IQ and HRQOL which was found during the first years of GH treatment, had disappeared after 11 years of GH treatment, when IQ levels had become more similar.

The improvement of HRQOL during GH treatment is in line with findings of another study, in which HRQOL was assessed during 2 years of GH treatment in 13 adults with PWS[7]. Two years of GH treatment improved HRQOL, according to both patients and parents. Improvement of HRQOL during long-term GH treatment was also observed in short children born small for gestational age[30, 31].

At baseline, children rated their HRQOL similar as their parents, they scored only higher on the Physical subdomain than their parents. During long-term GH treatment, HRQOL reported by children and parents was very stable, on average slightly higher according to the children. Parents reported a lower HRQOL in older children with PWS, while this was not reported by the children. Disagreement between parent and child reports on HRQOL was found in other studies in children with other disorders than PWS[12, 14]. Parents may score lower because they are negatively influenced by the burden of care-giving, their own well-being and concerns about their child's future perspectives[32, 33] and children might be positively influenced by adaptation to their illness[34]. The reason that children rated their HRQOL slightly better than their parents might also be due to their delay in development of Theory of Mind (ToM), i.e. the ability to infer in the

mental state of oneself and others. We observed in a previous study that children with PWS were able to recognize emotions, the first stage of the development of the ToM[35], but it might be more challenging to reflect their own feelings and emotions in abstract figures like a happy or a sad smiley. This, in combination with our experience that the children enjoyed completing questionnaires, might result in more positive scores.

There were no effects of GH treatment on HRQOL in the Social subdomain according to children and parents. Social impairment and symptoms of an autism spectrum disorder is a characteristic for most patients with PWS[35, 36]. This typical behavior is therefore not likely to change. It is in line with a recent study, describing neither improvement nor deterioration of behavioral problems in children with PWS during long-term GH treatment[37]. So, it might be that GH treatment stabilizes HRQOL in the Social subdomain. However, despite the fact that social impairment is a common finding in PWS, HRQOL in the Social subdomain was not extremely low according to children and parents, suggesting that both seem to cope with this social impairment.

Interestingly, HRQOL in the Social subdomain was higher in children with a deletion than children with an mUPD or ICD, according to parents. In the same line, a recent study showed that the total QOL of caregivers of adolescents with a deletion was higher than in caregivers of adolescents with an mUPD[38]. The fact that psychiatric problems, like psychosis and autism spectrum disorder, are more common in children and adolescents with an mUPD than with a deletion, could contribute to these findings[39, 40].

Not surprisingly, a higher BMI SDS was associated with a lower HRQOL on the Physical subdomain, according to parents. However, this association was not significant in self-reports of the children. They reported a higher HRQOL on the Physical subdomain than their parents, indicating that children with PWS have little sense of the consequences of their higher BMI on their Physical health. Remarkably, during 11 years of GH treatment, our results showed that the child's BMI SDS had a significant positive relation with the child-reported HRQOL on the Social subdomain. *Dykens et al*. observed that adults with a higher BMI had less maladaptive behavior relative to the lean subjects[29]. It might be that children with better social skills, are in a better position to acquire food. Or children with less food restriction experience less stress and rate their HRQOL higher on the Social subdomain.

Our study shows that children with PWS report a normal HRQOL. Both children and parents report improvement in HRQOL during GH treatment, while this progression was not found in the randomly assigned untreated children with PWS. During long-term GH treatment, HRQOL remained very stable. Based on our results, we conclude that GH treatment in children with PWS is not merely an effective treatment for normalizing height and improving body composition, but also has beneficial effects on their HRQOL. Children with PWS consider themselves quite happy children, despite the difficulties related to the syndrome.

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# CHAPTER 8

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

## GENERAL DISCUSSION

Thirteen years ago, in April 2002, the Dutch randomized controlled growth hormone trial for children with Prader-Willi syndrome (PWS) was initiated, to improve knowledge and care for children with PWS. Different aspects of the PWS phenotype and the effects of growth hormone (GH) on these aspects were unknown. All pediatric-endocrinologists in the Netherlands were informed about our study and patients were included by collaborating pediatric endocrinologists. After stratification for age and body mass index, young children (age < 3.5 years) were randomly assigned to either the GH treatment group or control group for 1 or 2 years, depending on age. After the RCT, all children continued GH treatment and are, nowadays, followed in the Dutch PWS Cohort study. Since 2007, all new patients are directly included in the Dutch PWS Cohort study. Children are followed during GH treatment in the Dutch PWS Cohort study until they reach final height, to study long-term effects and safety aspects of GH treatment. The Dutch PWS studies have markedly improved the knowledge about PWS and have led to better care for children and adolescents with PWS.

In the studies presented in this thesis, we investigated various aspects of PWS, such as cryptorchidism, growth and body composition, bone health and puberty, dietary management, IGF-I levels and health-related quality of life during GH treatment, in the large Dutch cohort of children with PWS.

In this chapter, our results are discussed, also in view of the literature. Subsequently, clinical implications of our results are presented and recommendations for future research are provided.

### 8.1 Cryptorchidism

Fetal life is a critical phase in the development of important organ systems, including the gonads. Hypogonadism with unilateral or bilateral cryptorchidism is a major diagnostic feature in boys with PWS[1]. Literature on the optimal treatment of cryptorchidism in boys with PWS is scarce. We therefore evaluated the effects of hCG treatment on testis position in our Dutch PWS Cohort study and performed testis histology in case of orchidopexy.

Our study showed that 81% of the testes descended to a lower position after hCG treatment. Twenty-three percent of the testes reached a stable scrotal position and, after 1.4 years of follow-up, did not require further surgery and 76% required an additional orchidopexy to ensure stable positioning in the scrotum. In general, the orchidopexy was more easy to perform than in the past, because most patients had, after the hCG treatment, an adequate length of the spermatic cord for successful scrotal fixation. Boys with PWS have a higher anesthetic risk, thus a lower position is desirable to ease the orchidopexy procedure and reduce the operation time.

Studies investigating the effect of hCG treatment in healthy boys with cryptorchidism are contradictive[2, 3]. In line with our results, a recent paper investigated testicular descent in a cohort of healthy boys and reported that hCG treatment induced testicular descent, at least one level down, in 81.8% of the testes[4]. Boys with bilateral cryptorchidism and a younger age at start of hCG treatment responded better to hormonal treatment[5, 6].

The major concern of hCG treatment are the potential side effects and the fact that long-term effects on spermatogenesis are unknown. One study reported that HCG given for testicular descent in healthy young boys with cryptorchidism may suppress the number of germ cells[7]. While another study showed that the number of germ cells per tubule in contralateral testes of men with cryptorchidism is already lower than the number of germ cells in testes of men with spontaneously descended testes. And, in addition, hCG treatment did not have any adverse effect on the histology of the contralateral testis, but in fact improved it without harming the germ cells[8]. We observed in our study in infants who underwent orchidopexy after hCG treatment a wide variation in histology, from a normal number of germ cells to Sertoli cell only (SCO), in combination with a relation with age: the younger the better. A reversed relation with histology outcome and age was also observed in non-PWS boys who underwent an orchidopexy with biopsy[9]. Our findings are in line with Belgian data, showing a wide variation in germ cell number, in eight older boys, without hCG treatment[10]. Our earlier research in boys with PWS without hCG treatment showed that they have a failure of Sertoli-cell function, which becomes apparent after the onset of puberty[11]. Data in small groups of adult males with PWS showed total absence of spermatogonia[12, 13]. As far as we know no man with PWS has fathered a child. This might suggest that the number of germ cells in PWS declines over the years, independently of hCG treatment or not. Semen analysis should give further information about fertility in young man with PWS, but because of ethical constraints, this has never been done.

Gonadal failure is also described in other syndromes, like Down syndrome and Noonan syndrome, suggesting that chromosomal abnormalities are related to gonadal dysfunction[14, 15]. Genes located in the PWS critical region 15q11-13 might play a role, directly or indirectly, in this process of early gonadal failure. The MAGEL2 gene is situated in the critical PWS region, and in Magel2-null mice, a reduced fertility with early reproductive decline and termination was observed[16]. Perhaps more important is the C15orf2 gene, located next to MAGEL2. Biallelic transcription of C15orf2 is observed and occurs exclusively in the testis. C15orf2 seems to play an important role in the spermatogenesis[17]. We were not able to test our infant biopsies for C15orf2 expression, but it seems likely that the C15orf2 gene is not expressed in PWS testes. Thus, it might be that the lacking information of these paternally imprinted genes in the PWS region contribute to the inadequate testicular development and function.

*In conclusion, our study demonstrates that most infants with PWS had testes in a lower position after hCG treatment, with 23% of the testes in a stable scrotal position and 76% requiring additional orchidopexy to ensure stable scrotal position. In general the orchidopexy was more easy to perform than in the past, because of an adequate length of the spermatic cord. A younger age at orchidopexy was associated with a better histological outcome but the effect of hCG treatment at an early age on testicular function in and after puberty has to be awaited.*

*Based on our results it is worth giving hCG treatment to PWS infants with cryptorchidism (Appendix 1).*

## 8.2 Growth and body composition

*8.2.1 Effects*

The first report about effects of GH treatment on height velocity and weight gain in children with PWS was published in 1996[18]. Rapidly thereafter randomized controlled trials demonstrated improved growth and body composition during GH treatment in children with PWS[19-22]. Nowadays, the most important reason for treating these children with GH is to optimize their body composition. Without GH treatment, body composition tends to deteriorate over time[23], resulting in a mean BMI of 35 (+3.5 SDS) at the age of 18 years[24]. We therefore investigated the course of PWS during 8 years of continuous GH treatment over a long period from childhood to adolescence.

Our study demonstrated that GH treatment is able to maintain body composition, and thus inhibits the natural course of PWS. Lean body mass (LBM) SDS, BMI $SDS_{PWS}$, height SDS and head circumference SDS improved significantly during the first year of GH treatment and remained at the same level over the subsequent years of treatment.

Our study showed that long-term GH treatment in combination with a strict diet and moderate exercise has a continued beneficial effect on LBM. After 8 years of treatment, LBM SDS was still higher than at baseline. This improvement in LBM is very important for children with PWS with regard to psychomotor development and against the development of scoliosis[25-28].

Fat percentage (fat%) SDS improved significantly during the first year of GH treatment, but after 8 years of GH treatment, it had returned to baseline SDS. Thus the natural course of fat mass in PWS is not fully suppressed by GH treatment, but GH is able keep fat% SDS around the 2 SDS. As fat% SD scores for untreated PWS children do not exist, we could not compare with untreated PWS children. Such a comparison was possible for BMI SDS. Compared to untreated PWS references, BMI SDS decreased significantly during the first year of treatment and remained in the low normal range during the subsequent years of GH treatment. BMI SDS according Dutch healthy reference children was in the upper normal range after 8 years of GH treatment, which is very acceptable for children with PWS.

The question remained whether a high fat% in PWS is harmful. It is already known that, in patients with PWS, body fat is predominantly located subcutaneously[29] and therefore protects them from complications of obesity[30, 31]. A recent study showed that patients with PWS had a specific distribution of adipose tissue, characterized by a decreased percentage of abdominal fat and increased fat mass in the limbs, which is associated with a better metabolic profile[32]. In addition, they found a higher adipocyte volume relative to the total amount of fat mass in PWS patients. So it could be speculated that subcutaneous adipose tissue has functional features that favor its expansibility, without adverse consequences on metabolic health[32]. This mechanism was previously described as the adipose tissue expandability hypothesis and states that a failure in the capacity for adipose tissue to expand, rather than obesity *per se* is the key factor linking positive energy balance and metabolic syndrome[33].

*In conclusion, our study demonstrated positive long-term effects of GH treatment in children with PWS, with an initial improvement and then maintenance of their body composition, height SDS and head circumference SDS. In combination with a healthy lifestyle, GH treatment can thus counteract the natural course of PWS, which is very important, because development towards morbid obesity is a major threat to these patients. Thus long-term GH treatment contributes to a better future health for these children.*

### *8.2.2 Safety*

Mortality rates in children with PWS are increased, 3% under the age of 30 years is reported[34]. Most common cause of death in children is a respiratory infection, in both GH-treated and untreated children with PWS[35]. Various causes of mortality are mentioned in literature: sleep apnea[36], tonsillar and adenoid hypertrophy[35], central adrenal insufficiency[37] or a combination[38]. Since the implementation in 2008 of stress doses hydrocortisone treatment during illness and surgery[37], no sudden death is reported in the Netherlands.

Because of the physiologic effects of GH on insulin sensitivity, the risk for diabetes mellitus (DM) has always been a topic. GH might increase the propensity to develop DM, because it is known as diabetogenic agent[39], but circulating levels of IGF-I are reported to be inversely correlated with the risk of cardiovascular diseases[40]. Increased in insulin sensitivity during GH treatment in PWS is found[41], which is in contrast to findings in non-PWS children with GH treatment. This was, however, never investigated during long-term GH treatment in PWS.

In our 8 years GH follow-up study, in a large group 60 of children with PWS, we showed that GH treatment had no adverse effects on glucose parameters, lipid profile and blood pressure. Some safety parameters such as total cholesterol, LDL levels and the systolic blood pressure significantly improved. No cases of non-insulin dependent DM occurred.

It is known that adults with PWS have a higher incidence of DM II[42], but the adult population is on average overweight and has never been treated with GH. Thus, GH treatment may have a favorable effect on the cardiovascular risk profile in children with PWS.

Our results showed a rapid increase in IGF-I levels in the first year of treatment to just above +2.0 SDS. High IGF-I levels in response to GH treatment are frequently found in children with PWS[41, 43, 44]. We found that the IGF-I/IGFBP-3 molar ratio increased during the first year of treatment but then stabilized around 0.4, a normal value for healthy children[45]. Therefore, GH-dose titration based on the IGF-I/IFGBP-3 ratio might be more adequate in children with PWS. In paragraph 8.5, we discuss our research data on the high IGF-I levels in more detail.

*In conclusion, our study demonstrates no adverse effects of long-term GH treatment on glucose parameters and lipid profile. Moreover, total cholesterol, LDL levels and systolic blood pressure improved during long-term GH treatment in children with PWS.*

### 8.3 Bone health and puberty

Puberty is a crucial stage in bone mass acquisition; skeletal mass approximately doubles between the start and the end of adolescence[46]. Peak bone mass is normally attained between 18-20 years in girls and 18-23 years in boys[47]. Endocrine factors are involved in this process, including parathyroid hormone, thyroxine, GH and sex steroids[48, 49]. Because of the low GH levels, hypogonadism and a sedentary lifestyle, adults with PWS are prone to have a lower bone mineral density (BMD). Indeed more osteoporosis and a high fracture risk has been observed in adults with PWS[50-52]. In our previous study in prepubertal children with PWS, a normal BMD of the total body ($BMD_{TB}$) and lumbar spine ($BMD_{LS}$) was found, also when adjusted for height (lumbar spine bone mineral apparent density, $BMAD_{LS}$)[53]. We also showed that children with PWS have a normal onset of puberty, but a delay in pubertal development beyond Tanner stage 3 compared to age- and sex-matched controls[11, 54]. These findings together suggested that there could be a decline in BMD in adolescents with PWS, in contrast to age-and sex-matched peers. We therefore investigated $BMD_{TB}$, $BMD_{LS}$ and $BMAD_{LS}$ during puberty and long-term GH treatment in children and adolescents with PWS.

Our study showed that $BMD_{TB}$SDS and $BMD_{LS}$SDS increased and $BMAD_{LS}$SDS remained stable during 4 years of GH treatment in prepubertal children with PWS. This finding is in line with a 3-year GH study, which also found a stable $BMD_{LS}$SDS in children with PWS[41], in accordance with data in GH-treated children with GH deficiency[55], and demonstrating that GH treatment has a positive effect on BMD in children with PWS. In addition, we observed that lean body mass (LBM) SDS was the most powerful predictor of $BMD_{TB}$SDS and $BMD_{LS}$SDS in adolescents with PWS, indicating that GH treatment is also indirectly able to improve BMD by increasing the LBM[56]. Thus, it seems that increasing LBM by GH

treatment, and probably in combination with physical activity, is important to optimize the BMD, especially in PWS patients.

Each 1 year earlier start of GH treatment resulted in a 0.20 SDS increase in $BMD_{TB}$ and $BMD_{LS}$ at the age of 14 years, indicating that early GH treatment has a protective effect on BMDs in children with PWS. It was already shown that an early start of GH treatment had positive effects on physical and neurodevelopmental aspects[21, 57]. Infants with PWS, who were treated with GH before their first birthday spoke their first words at a mean age of 14 months and walked independently at 23 months[21].

The $BMD_{TB}$SDS decreased significantly during adolescence, after the age of 11 years in girls and 14 years in boys. $BMAD_{LS}$SDS decreased significantly from the age of 11 years in girls and 16 years in boys. It is known that there are gender differences in bone density during adolescence due to differences in the timing of growth and puberty, resulting in an earlier peak bone mass in girls than boys[46]. We observed very low serum levels of estradiol and testosterone and a lack of pubertal progression beyond Tanner stage 3. Remarkably, a few adolescents progressed to Tanner stage 5. In contrast to our expectations, a higher Tanner stage was associated with a trend to a lower $BMD_{TB}$SDS and in a significantly lower $BMAD_{LS}$SDS. One explanation of their pubertal progress could be possibly related to elevated androgen levels[58-60] in combination with a high fat%, but we were not able to study this in our small group of patients with Tanner stage 5. Thus in patients with PWS, Tanner stages are less reliable than hormonal levels of testosterone and estradiol. In our opinion, low doses of sex hormones should be considered when girls are 11 and boys are 14 years old, unless there is a normal increase of serum sex hormone levels. Our results underline the importance of sex hormone substitution. Children aged 17 to 19 years, had a low BMD, which will most likely result in osteopenia and osteoporosis at an early age if not timely intervened. Parents and physicians often hesitate to start sex hormone replacement therapy, especially in boys with cognitive impairment, but a recent study observed positive effects of testosterone replacement therapy on body fat percentage and BMD without an increase in behavioral problems or aggression in 16-48 years old males with PWS[61].

*In conclusion, during long-term GH treatment of prepubertal children with PWS, the $BMD_{TB}$SDS, $BMD_{LS}$SDS and $BMAD_{LS}$SDS remained completely within the normal range compared to age- and sex-matched reference values. However, during adolescence a decline in BMDs is observed to low-normal values. To avoid a higher risk for osteoporosis in later life, we recommend to stimulate physical activity to improve lean body mass and to monitor BMD from the age of 11 years in girls and 14 years in boys with PWS. To prevent the decline in $BMD_{TB}$ or $BMAD_{LS}$, clinicians should be aware of this problem and start sex hormone therapy when serum sex hormone levels remain low in girls from the age of 11 and in boys from the age of 14.*

## 8.4 Dietary management

PWS is the most common cause of syndromic obesity. Dietary management is one of the most challenging aspects in PWS. The management involves a multidisciplinary lifelong approach to prevent complications, prolong life expectancy and improve quality of life. Prevention and treatment of obesity is essential and depend on a low energy and well-balanced diet with rigorous supervision and restriction of food access, combined with regular meals and exercise[36].

It is known that GH treatment improves body composition in PWS and other syndromes. But especially in children with PWS, it is important to obtain insight into the effects of GH treatment on energy balance, so as to determine what lifestyle interventions might be possible. Children born small for gestational age, children with Turner and Silver Russell syndrome have an increased energy intake during GH treatment[62, 63], but randomized controlled trials comparing energy intake in children with PWS with and without GH treatment were lacking. We therefore investigated in our randomized controlled GH study, the effect of GH treatment on dietary energy intake, body composition, adiponectin levels and resting energy expenditure in children with PWS.

Our results showed that energy intake, expressed as percentage of the daily energy requirements for age- and sex-matched healthy children, was 20% lower in young infants and 50% lower in prepubertal children above the age of 10 years. Our data indicate that the parents of children with PWS are very well capable to restrict the diet of their child, also in the older ones. The BMI SDS was, however, higher in the older prepubertal children, despite a reported lower energy intake to 50% of normal. This could be due to the switch in nutritional phases from the hypotonic infant phase to the toddler phase, which is associated with weight gain without a significant change in appetite or caloric intake[64]. On the other hand, older children with PWS might obtain access to food without knowledge of their parents. When they are faced with tempting food, even children who are otherwise well behaving, might steal food. At the same time, we observed a decrease in fat% and an increase in serum adiponectin levels during GH treatment in the prepubertal children. Adiponectin is an adipocytokine that is inversely related to adiposity and insulin resistance, and is thought to be protective with regard to cardiovascular disease and type 2 diabetes mellitus[65, 66]. So despite their higher BMI, adiponectin levels increased suggesting a protective effect of GH treatment with regard to the development of cardiovascular risk factors.

Both in the infant group and in the prepubertal children, we observed a higher energy intake in the GH-treated children than in the untreated children, but this did not reached significance, probably because of the large variation in energy intake. During GH treatment, there was an increase in LBM and decrease in fat% in both infants and prepubertal children, suggesting that GH treatment is able to improve body composition, while at the same time the energy intake increased. This is in line with a previous

study observing that prepubertal GH-treated children with PWS consumed 226 kcal/d more than untreated children[67], in accordance with other groups of children receiving GH treatment[62, 63].

Maintenance of body weight requires energy balance. Two important components of the energy balance equation are energy intake and REE[68, 69]. We found no difference in REE between the GH-group and the untreated group, which is in line with a previous study[70]. The ratio of energy intake to REE estimates the physical activity level when assuming energy balance. The baseline physical activity level was lower compared to estimations of physical activity level in a previous study[71]. As physical agility is known to increase during GH treatment in PWS[21, 70], we expected an increase in physical activity level in GH-treated children. To our surprise, GH treatment did not change the physical activity level. It is important to conclude that stimulating physical activity remains an important focus to reach energy balance in these children.

*In conclusion, children with PWS have a low to very low energy intake compared to daily energy requirements for age- and sex-matched children. In infants, the energy intake increased during GH treatment compared to baseline, but it was not significantly different from the untreated ones. In prepubertal children, the energy intake did not significantly increase. In contrast to the energy intake, the children had a significant decrease in fat% and an increase in adiponectin levels, suggesting a protective effect of GH treatment with regard to the development of obesity and diabetes mellitus type II. The focus of attention for physicians and parents is to also include physical activity in daily routines to keep energy balance in children with PWS.*

## 8.5 High IGF-I levels

### *8.5.1 Serum IGF-I, IGFBP-3, ALS levels and complex formation*

The insulin-like growth factor (IGF) system, consists of 2 ligands (IGF-I and IGF-II), 2 receptors (IGF1 receptor and IGF2 receptor) and 6 high-affinity IGF binding proteins (IGFBPs), that all have greater affinity for binding to the IGFs than to the IGF-IR[72, 73]. Under normal circumstances, most of the circulating serum IGF-I is sequestered in 150 kD ternary complexes with one molecule of IGFBP-3 or IGFBP-5 and one molecule of acid-labile subunit (ALS). ALS is an 85 kDa glycoprotein that is produced almost exclusively by the liver and secreted into the circulation, after which it plays an important role in the maintenance of normal serum IGF-I and IGFBP-3 levels[74]. In children with PWS, there were no data on IGF-I complex formation. We therefore investigated ALS levels and complex formation in serum of GH-treated children with PWS compared to age- and sex-matched healthy controls.

In line with previous studies, we observed elevated serum IGF-I levels in GH-treated PWS children in combination with IGFBP-3 levels in the upper normal range[43, 44] and markedly increased ALS levels. As ALS is predominantly regulated by GH[75], our data

indicate that children with PWS are very sensitive to GH. ALS is essential for the stabilization of both circulating IGF-I and IGFBP-3[76]. Indeed, we observed a higher degree of 150 kD ternary complex formation in children with PWS than in the controls, indicating that during GH treatment most of the IGF-I is sequestered by ALS and IGFBP-3, which is reassuring. There is little evidence of biological functions of ALS, although patients with ALS deficiency have impaired growth, delayed puberty and insulin insensitivity[72]. Recently it was shown in animals that ALS is positively involved in carbohydrate and fat metabolism, as well as growth[77, 78]. GH-treated patients with PWS have an improvement of metabolic parameters[79, 80], which might be partially explained by their high serum ALS levels.

*In conclusion, in GH-treated children with PWS, most of the serum IGF-I is sequestered in the ternary 150 kD complex with ALS and IGFBP-3.*

*8.5.2 IGF-bioactivity*

In children with PWS, it appears difficult to find the right balance between the positive effects of GH on body composition and possible risks of higher serum IGF-I levels. Nowadays, the standard GH dose for children with PWS is 1 mg/m$^2$/day (≅ 0.035 mg/kg/day)[81]. A GH-dose response study showed that children with PWS need a GH dose of at least 1 mg/m$^2$/day to improve and maintain their body composition[44]. A lower GH dose resulted in a lower LBM and higher fat%, while a higher dose improved body composition even more, but also resulted in higher IGF-I levels. High IGF-I levels are observed during standard GH-dose treatment in children with PWS world-wide, without a total normalization of body composition. So there seems to be a disparity between the high serum IGF-I levels and the effects at tissue level. There is, however, a debate whether or not to accept these high immunoreactive IGF-I levels, because of the unknown long-term consequences. In older, non-GH-treated patients with different types of cancer, a relation with the IGF axis was found, but the exact underlying mechanisms are unknown[73]. On the other hand, in adult patients with GH deficiency treated with GH, no increased incidence of cancers has been found[82].

Despite the high serum IGF-I levels during GH treatment in children with PWS, no signs of acromegaly are reported[83, 84], suggesting that IGF-bioactivity is not very high. In children with PWS, as well as in healthy children, there are no data on the ability of serum IGF-I to phosphorylate the IGF1 receptor, i.e. a measure for IGF-bioactivity. In order to assess IGF-bioactivity of serum of children with PWS on GH treatment, we developed an IGF1 receptor kinase activation assay (KIRA)[85, 86], to investigate IGF-bioactivity in GH-treated children with PWS and healthy controls.

Our study showed that young GH-treated children with PWS had higher IGF-bioactivity than age-matched controls and older GH-treated children with PWS. Older GH-treated

children with PWS had a similar IGF-bioactivity as age-matched controls, which is reassuring.

At this moment, we cannot explain the difference in IGF-bioactivity between young and older GH-treated children with PWS. A recent study in healthy children observed higher IGFBP-3 proteolytic activity in young children compared to older children[87]. This phenomenon was also described in pregnant women[88, 89]. IGFBP-3 proteases are capable of cleaving intact IGFBP-3 in smaller fragments with a reduced affinity for IGF-I[90]. This could result in higher IGF-I bioavailability of IGF-I to target tissues in young children and pregnant women. It could be that GH-treated young PWS children would need a relatively high level of IGF-bioactivity during the first years of GH treatment, when they grow fast and undergo great changes in their body composition[70, 83]. Our results showed a positive relation between IGF-bioactivity and delta BMI SDS in young GH-treated PWS children. It is well know that in young children with PWS, a switch occurs from appropriate feeding and growth to an increase in body weight associated with a significant increase in serum IGF-I levels during GH treatment, while on a stable GH dose[64]. This is in line with a small study in pregnant women, that observed a different IGF axis in women with excessive weight gain during pregnancy compared to those with normal weight gain[91]. Another study found a significant increase of IGF-I levels in pregnant women in the second trimester when there is an apparent increase in body mass and hyperplasia of adipose tissue[92]. So there might be a relation between weight gain and the IGF axis, in line with our finding that young children with PWS who gained more weight had a higher IGF-bioactivity.

*In conclusion, high serum IGF-I levels in GH-treated PWS children, were only associated with a higher IGF-bioactivity in young children, and normal IGF-bioactivity in older children compared to healthy controls. In young GH-treated children, the physiological significance of high serum IGF-I levels and clinical consequences need further research, but in older GH-treated PWS children our data are reassuring.*

*8.5.3 Relation between serum IGF-I, IGFBP-3, ALS levels and IGF-bioactivity*

Serum IGF-I levels are often used to estimate IGF-bioactivity, and a large study in adults observed that circulating total IGF-I levels were significantly related to IGF-bioactivity[93]. It has also been suggested that the serum IGFI/IGFBP-3 ratio is a surrogate marker for the level of free biologically available IGF-I in the circulation[94], also in GH-treated children with PWS[43]. We observed no correlation between serum IGF-I levels and IGF-bioactivity in children with PWS, in contrast to a strong positive relation in healthy controls. In the young GH-treated PWS children and healthy controls, we observed a positive correlation between IGF-bioactivity and the IGF-I/IGFBP3 ratio. Thus serum IGF-I levels in GH-treated PWS children are an inappropriate indicator of IGF-bioactivity and are therefore unreli-

able for titrating the GH dose. In young GH-treated children with PWS, the IGF-I/IGFBP3 ratio might be a proxy of IGF-bioactivity, although more research is needed to confirm the clinical relevance.

*GH-treated PWS children showed a wide variation in IGF-bioactivity and a disrupted correlation between serum IGF-I levels and IGF-bioactivity, in contrast to controls where there was a strong positive correlation. Serum immunoreactive IGF-I in GH-treated PWS children is thus not an appropriate indicator of IGF-bioactivity.*

### 8.6 QOL

Studies investigating health-related quality of life (HRQOL) in children with an intellectual disability are scarce. Because of a different time perspective, dominance of short-term memory and recent incidents, HRQOL questionnaires are not easy to interrogate. One study investigated the quality of life in intellectually impaired adolescents and their parents and found that the combination of self-report and parent-report provided a comprehensive insight into functioning and the different aspects of quality of life in these adolescents[95]. So to determine a child's HRQOL, the opinion of a child itself in combination with a parent-report about their child's HRQOL seemed to be most useful[96-98]. Information about HRQOL in children with PWS was very limited. Furthermore, nothing was known about the effect of GH treatment versus no treatment on HRQOL in children with PWS, and the HRQOL during a long period of GH treatment. We, therefore, investigated HRQOL in children with PWS according to both children and parents. In addition, we assessed the effects of GH treatment on HRQOL in children with PWS during a 2-year RCT and during 11 years of continuous GH treatment.

Prior to start of GH treatment, children with PWS rated their HRQOL similar as their parents, children scored only higher on the Physical subdomain. Children with PWS might be positively influenced by adaptation to their illness and may lack the long-term view of events[98, 99]. Our study also showed that children with PWS rated their HRQOL equal or higher than children with other diseases and even healthy children, already at baseline, indicating that children with PWS consider themselves quite happy children. Remarkably, HRQOL rated by their parents was almost equal, despite the difficulties related to the syndrome. In a small study investigating HRQOL in 9 untreated children with PWS[100], HRQOL was found to be lower in children with PWS than in a reference population. However, in that study HRQOL was rated by parents only. It is known that parents may be negatively influenced by the burden of care-giving, their own well-being and concerns about their child's future perspectives[101, 102], therefore a combination of self-report and parent-report is most valuable.

GH treatment in children with PWS improves body composition, growth, physical strength, and has beneficial effects on cognitive functioning in children with PWS[20, 83, 103].

Our study showed that HRQOL improved during GH treatment in children with PWS according to children and parents, while it decreased or remained similar to baseline in untreated controls with PWS. The improvement of HRQOL during GH treatment is in line with a GH study in 13 adults with PWS, in which HRQOL was assessed during 2 years[104].

In addition, our study showed that HRQOL remained very stable during the 11 years of GH treatment, according to both children and parents. One study investigated HRQOL after 10 years of GH treatment in 20 children with PWS[105] and found a lower HRQOL in patients with PWS than in a reference population. However, in that study, they had no untreated control group with PWS and HRQOL was only measured in the children after 10 years of GH treatment.

Although the relationship between height and quality of life is inconclusive, children born small for gestational age and children with chronic renal disease showed an improvement in health-related quality of life during GH treatment[106, 107]. These children have, however, a normal intelligence. Children and adolescents with PWS have a cognitive impairment and reduced social skills. Adult phase is characterized by temper tantrums, impulsivity, mood fluctuations, difficulty with change in routine, stubbornness and aggression[108-110]. Up to date, we observed no improvement but also no deterioration of behavioral problems in children with PWS during long-term GH treatment[111]. So in our opinion the proven positive effects of GH treatment outweigh the presumed negative effects.

*In conclusion, our study showed that children with PWS report a normal HRQOL. Both children and parents reported improvement in HRQOL during GH treatment, while this progression was not found in the randomly assigned untreated children with PWS. During long-term GH treatment, HRQOL remained very stable. Children with PWS consider themselves quite happy children, despite the difficulties related to the syndrome.*

## 8.7 General conclusions and clinical implications

Since 2002, our longitudinal study assessed height, weight, body composition, bone mineral density, dietary intake, safety and quality of life in a large group of children with PWS during GH treatment. In addition, many other therapies and features of PWS have been studied over the years, such as hCG treatment for cryptorchidism as described in this thesis (Appendix 2).

We showed that hCG treatment resulted in an anatomical lower testis position in most infants with PWS and 23% of the testes reached a stable scrotal position. Seventy-six percent required an additional orchidopexy to ensure a stable scrotal position. In our opinion, it is worth giving hCG treatment to PWS infants with cryptorchidism.

During long-term GH treatment, height SDS and head circumference SDS normalized and GH turned out to be a potent force to counteract the clinical course of obesity in

children with PWS. Bone mineral density remained stable during GH treatment in prepubertal children with PWS but decreased during adolescence due to incomplete pubertal development. Based on our findings, we recommend clinicians to start sex hormone therapy from the age of 11 in girls and 14 in boys, unless there is a normal increase of serum levels of sex hormones.

Furthermore, our study demonstrated that parents of children with PWS are very well capable to restrict energy-intake up to 50% compared to daily energy requirements for age- and sex-matched healthy children. Although GH treatment was associated with a slight increase in energy intake, body composition and adiponectin levels improved, which suggests a protective effect of GH treatment with regard to the development of diabetes mellitus type II. To keep energy balance, the focus of attention for parents and physicians remains to stimulate physical activity.

GH treatment with 1 mg/m$^2$/day revealed no adverse effects on glucose homeostasis, serum lipids, blood pressure and bone maturation, however, it results in high IGF-I levels. Further to this, we found that most of the serum IGF-I is sequestered in the 150 kD complex. High immunoreactive IGF-I levels in GH-treated PWS children, were associated with a higher IGF-bioactivity in young children, but normal IGF-bioactivity in older children. Our data showed that serum immunoreactive IGF-I levels in GH-treated children with PWS, are an inappropriate indicator of the IGF-bioactivity and therefore an unreliable tool to adjust GH dose.

Finally, our results showed that children with PWS and their parents rated their health-related quality of life equal or even better compared to healthy children and children with various other diseases, and it increased during GH treatment, in contrast to untreated ones. This positive effect sustained during long-term GH-treatment. Children with PWS consider themselves quite happy (Figure 1).

## 8.8 Directions for further research

In the present thesis, we described long-term effects of GH treatment in children and adolescents with PWS. GH has positive effects on many different aspects, as it improves and maintains body composition, height, weight, bone mineral density and quality of life. Because GH treatment is only registered until patients with PWS reach adult height, we were not able to study the effect of GH treatment in young adults after final height. To maintain lower visceral adipose tissue and prevent cardiovascular disease risk factors, GH treatment may be advisable for adult patients with PWS[112]. A randomized controlled GH trial in young adults is, therefore, warranted. Further research should also study BMD during long-term GH treatment in combination with sex hormone replacement therapy in adolescents and young adults with PWS, in order to find the optimal treatment combination. Besides, continuation of GH treatment during transition from late adolescence

to early adulthood in patients with PWS must be studied, because in young adults with GHD, BMDs deteriorate after discontinuation of GH treatment[113-115].

The cause of hyperphagia in PWS is still unknown, but hypothalamic dysfunction might be involved. An increased neuronal reward circuitry activation in response to food, especially high energy foods, both pre- and post-meal was observed[116-118]. In addition, differences in various gut hormones, including high levels of ghrelin (an orexogenic hormone) and lower levels of insulin (an anorexogenic hormone), are considered to contribute to their appetite abnormalities[119, 120]. Adipokines, produced by the adipose tissue, in individuals with PWS are also assumed to play a role in regulating food intake in this syndrome[121, 122]. It is therefore important to longitudinally investigate orexogenic and anorexogenic hormones both in a fasting state and in a fed state, preferable combined with brain function tests and brain imaging.

We are the first who investigated IGF-bioactivity in GH-treated children with PWS. We measured serum levels of ALS and IGF-bioactivity cross-sectionally, but to understand more about the etiology of high serum IGF-I levels, ALS levels and IGF-bioactivity, especially in young children with PWS, a large longitudinal follow-up study is recommended. Despite high serum IGF-I levels and IGF-bioactivity levels, side effects or signs of acromegaly were not reported, which might suggest that other factors as tissue specific IGF1R concentrations, IGF1R signal pathways or IGFBP-3 protease activity could be involved. Further research is needed to investigate the IGF1R function in PWS.

Finally, health-related quality of life in children with PWS during GH treatment is very reassuring. But during puberty and young adulthood, patients with PWS might face more difficulties with respect to their social participation, living environment and work. It is therefore important to keep track of quality of life during these phases of life.

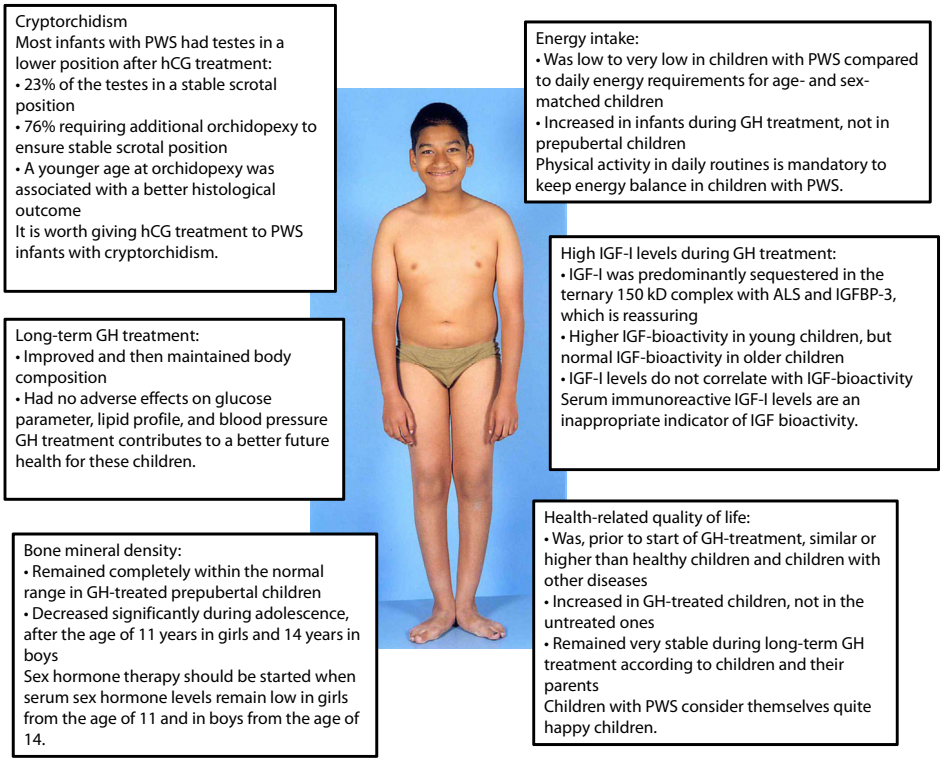
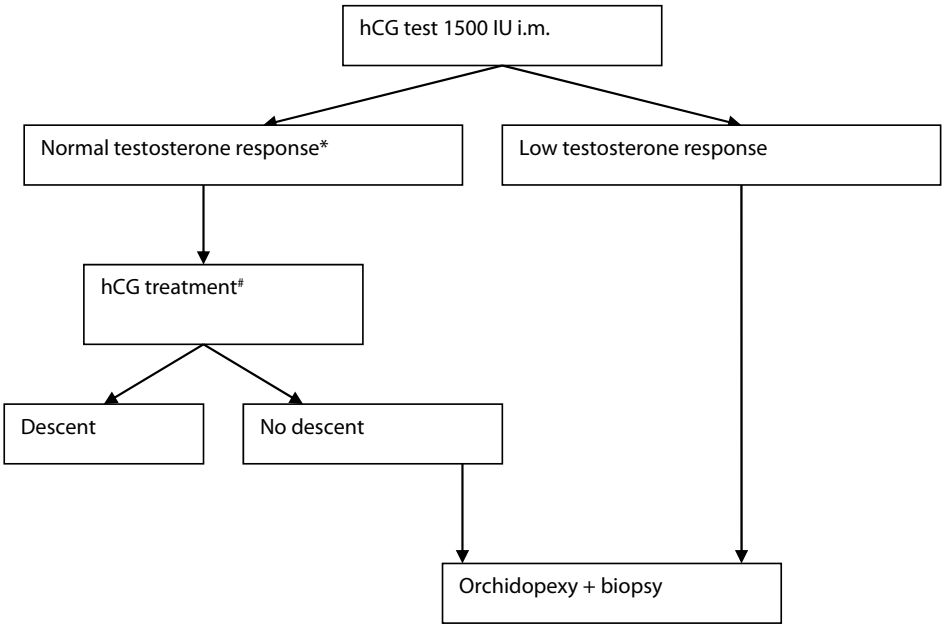



**Figure 1.** Conclusions of this thesis
13-year old boy with PWS with 9 years of GH treatment. Photo is depicted with permission from parents and the child

## APPENDIX 1: RECOMMENDATION FOR HCG TREATMENT




*** Normal testosterone response after hCG stimulation test**

age 3-12 months - 2 to 20 times greater than at baseline

age 1-4 years - 5 to 10 times greater (2.5 to 9.0 nmol/l)

**# hCG treatment**

age 3-12 months - 250 IU i.m. twice a week during 6 weeks

age 1-4 years - 500 IU i.m. twice a week during 6 weeks

## APPENDIX 2: SUMMARY OF THE RESULTS FROM THE DUTCH COHORT STUDY IN CHILDREN AND ADOLESCENTS WITH PWS

| Body composition | Baseline | Effects of GH treatment |
|---|---|---|
| Fat mass | Fat mass is higher compared to healthy peers<br>*Infants:* Fat% is 28.4<br>*Children:* Fat% is 36.9, which is higher than in healthy peers and above the normal range, fat% is elevated in 95% of the children[22, 83]. | Fat% decreases during GH treatment on the short term, but remains higher compared to healthy peers during 4 years of GH treatment[22, 83]. |
| | | **After 8 years of GH treatment, fat% is not significantly different than at baseline, around the +2 SDS[123].** |
| LBM | LBM is lower compared to healthy peers[22, 83]. | LBM increases during GH treatment, while it further decreases in untreated children on the short term. During 4 years GH treatment LBM stabilizes, but does not normalize and remains lower than in healthy peers[22, 83]. |
| | | **After 8 years of GH treatment, LBM is still in the low to normal range, -1.5 SDS, and significantly higher than at baseline[123].** |
| BMI | *Infants:* have a normal BMI<br>*Children:* have a higher BMI compared to healthy peers. BMI is elevated in 24% of children[22, 83]. | BMI remains stable during GH treatment in infants, but decreases in children on the short term. During 4 years of GH treatment, BMI increases compared to healthy peers, but decreases compared to PWS reference values, BMI decreases and remains lower than at baseline during 4 years[22, 83]. |
| | | **After 8 years of GH treatment, BMI is not significantly different than at baseline, around the +1 SDS. Compared to PWS references, the BMI is significantly lower than at baseline, -1 SDS[123].** |
| **Anthropometrics** | **Baseline** | **Effects of GH treatment** |
| Height | Height is lower compared to healthy peers[22, 83]. | Height normalizes during GH treatment on the short term. During 4 years GH treatment height normalizes in most children[22, 83]. |
| | | **After 8 years of GH treatment, height is not significantly different from that in healthy peers[123].** |
| Head circumference | Head circumference is smaller compared to healthy peers[22, 83]. | Head circumference normalizes during GH treatment on the short term. During 4 years GH treatment, head circumference increased during the first 2 years of treatment and stabilizes thereafter[22, 83]. |
| | | **After 8 years of GH treatment, head circumference is not significantly different from that in healthy peers[123].** |

| Growth factors | Baseline | Effects of GH treatment |
|---|---|---|
| IGF-I | IGF-I levels are lower compared to healthy peers[22, 83]. | IGF-I levels increase rapidly during GH treatment to significantly higher levels than in healthy peers, but stay within the normal range in most children. The IGF-I/IGFBP-3 ratio increased during GH treatment, suggesting that more unbound IGF-I is present in the circulation[22, 83]. |
| | | **On average, the IGF-1 levels are just above +2 SDS during 8 years of treatment[123].** |
| IGFBP-3 | IGFBP-3 levels are similar as in healthy peers[22, 83]. | IGFBP-3 levels increase during GH treatment but not to the same extend as IGF-I levels[22, 83, 124]. |
| ALS | | **ALS levels are significantly higher in GH-treated children with PWS than in healthy peers[124].** |
| Complex formation | | **In children with PWS, the 150 kD ternary complex formation is significantly higher than in healthy peers, indicating that most of serum IGF-I is sequestered in the ternary 150 kD complex with ALS and IGFBP-3[124].** |
| IGF-bioactivity | | **Young GH-treated PWS children, median age 5.2 years, have higher serum IGF-bioactivity than healthy peers, but no difference is observed in IGF-bioactivity between older GH-treated PWS children, median age 14.9 years, and healthy peers. In children with PWS, IGF-bioactivity during GH shows a wide variation and there is a disrupted correlation with IGF-I levels[124].** |
| **Puberty** | **Baseline** | **Effects of GH treatment** |
| Adrenarche | Children with PWS have significantly higher DHEAS levels from 3 to 10 years of age, compared to healthy peers. They are younger at onset of pubarche and have a higher prevalence of premature pubarche[125]. | |
| Boys | The majority of male patients with PWS have primary testicular failure, which becomes apparent after onset of puberty. No classical hypogonadotropic hypogonadism is observed[11]. | GH treatment has no significant effect on inhibin B levels[11]. |
| | **The median ages at attaining Tanner genital stage 2 and 3 in boys are not significantly different than in healthy peers, whereas the progression to Tanner stage 4 and 5 is significantly delayed. Remarkably, despite an increase in Tanner stages, almost no increase in serum testosterone levels was observed[126].** | |

| Puberty | Baseline | Effects of GH treatment |
|---|---|---|
| Girls | The primordial follicle pool and number of small antral follicles are conserved in girls and female adolescents with PWS. No classical hypogonadotropic hypogonadism is observed. However, maturation of follicles and progression of pubertal development are impaired, which might be due to dysregulation of LH secretion. Because these impairments are not absolute, ovulation and thus conception cannot be ruled out in individual female adolescents with PWS[54].<br><br>**The median ages at attaining Tanner mammae stages 2 and 3 in girls are not significantly different than in healthy peers, whereas the progression to pubertal Tanner stage 4 and 5 is delayed. Remarkably, despite an increase in Tanner stages, almost no increase in serum estradiol levels is observed[126].** | |
| **Thyroid hormone levels** | **Baseline** | **Effects of GH treatment** |
| FT4 | FT4 levels are lower than in healthy peers, but the majority (93.7%) has FT4 levels within the normal range (above -2 SDS), only 6.3% of patients have FT4 below the normal range (-2 SDS), but without abnormalities in TSH and T3 levels[127]. | FT4 levels decrease during 1 year of GH treatment, but stay in the normal range in the majority (91.2%) and are unchanged in untreated children. The change in FT4 over 1 year of GH treatment is not associated with change in IGF-I or clinical signs[127]. |
| TSH | TSH levels are similar as in healthy peers[127]. | TSH levels do not change and are within the normal range during 1 year of GH treatment[127]. |
| T3 | T3 levels are higher than in healthy peers, 5.5% have levels above the normal range (+2 SDS)[127]. | T3 levels do not change and are within the normal range during 1 year of GH treatment[127]. |
| **Adipocytokine levels** | **Baseline** | **Effects of GH treatment** |
| Adiponectin | Adiponectin levels are higher compared to healthy peers[128]. | Adiponectin levels increase during 2 years of GH treatment, while they remain similar to baseline in untreated children. This increase in adiponectin levels is related to the decrease in body fat percentage[128]. |
| Acylation stimulating protein (ASP) | *Infants:* ASP levels are elevated in 68%. *Children:* 94% have higher ASP levels than healthy peers[79]. | GH treatment has no effect on ASP levels[79]. |

| Glucose homeostasis | Baseline | Effects of GH treatment |
|---|---|---|
| Insulin (fasting) | *Infants:* Insulin levels are within the normal range in all infants.<br>*Children:* have higher insulin levels than infants, although within the normal range[79, 83, 128]. | Insulin levels remain within the normal range during GH treatment, while in untreated children, levels increase. During long-term treatment, insulin levels increase with age and GH has no effect on insulin levels after correction for age[79, 83, 128]. |
| Glucose (fasting) | Glucose levels are within the normal range in most infants[79, 83, 128]. | Glucose levels remain similar to baseline during GH treatment and are within the normal range. During long-term treatment glucose levels increase with age, GH has no effect on glucose levels after correction for age[79, 83, 128]. |
| **Cardiovascular risk-factors** | **Baseline** | **Effects of GH treatment** |
| | *Infants:* 63% have at least 1 and 33% have at least 2 of the cardiovascular risk factors, defined as hypertension or dyslipidemia.<br>*Children:* 73% have at least 1 and 49% have at least 2 of the cardiovascular risk factors[79]. | |
| Total Cholesterol (fasting) | *Infants:* 26% have elevated total cholesterol levels<br>*Children:* 35% have elevated total cholesterol levels[79, 83] | Total cholesterol levels do not change during 4 years of GH treatment[79, 83].<br>**After 8 years of GH treatment, total cholesterol levels decrease significantly compared with baseline[123].** |
| LDL-cholesterol (fasting) | *Infants:* 33% have elevated LDL levels<br>*Children:* 46% have elevated LDL levels[79, 83] | The HDL/LDL ratio improves during GH treatment. LDL levels decreases during 4 years of GH treatment[79, 83].<br>**After 8 years of GH treatment, LDL levels decrease significantly compared with baseline[123].** |
| HDL-cholesterol (fasting) | *Infants:* 11% have decreased HDL levels<br>*Children:* 20% have decreased HDL levels[79, 83] | HDL levels do not change during GH treatment[79, 83]. |
| Triglycerides (fasting) | Triglycerides are above the normal range in 15% of the children[128]. | Triglycerides do not change during GH treatment[128]. |
| Lipoprotein (fasting) | *Infants:* 31% have elevated Lipoprotein levels<br>*Children:* 11% have elevated lipoprotein levels[79]. | |
| Blood pressure | *Infants:* have a normal systolic and diastolic blood pressure.<br>*Children:* 12% of the children have an elevated systolic blood pressure, diastolic blood pressure is in the normal range in all children[79, 83]. | There are no changes in systolic and diastolic blood pressure during 4 years of GH treatment[79, 83]. |

| Cardiovascular risk-factors | Baseline | Effects of GH treatment |
|---|---|---|
| | | **After 8 years of GH treatment, systolic blood pressure decreases significantly compared with baseline. Diastolic blood pressure does not change during 8 years of treatment[123].** |
| **Psychological aspects** | **Baseline** | **Effects of GH treatment** |
| Mental development: infants | *Infants*: mental development is 71.6% of expected development. Mental development is not associated with severity of SRBD. Mental development in infants with OSAS is lower than in those without[25, 129]. | In infants, mental development improves 9.3% during 1 year of GH treatment[25]. |
| Mental development: children | *Children*: have significantly lower scores compared to healthy peers on 4 subtests of WIPPSI-R or the WISC-R. 60% of children have higher verbal than performance scores, 24% have higher performance than verbal scores and 16% of children have a verbal and performance score within the same range[25]. | |
| | Sleep efficiency index is associated with better performance on the Picture arrangement /completion Wechsler subscales. There are no associations between SRBD and cognition[130]. | |
| Motor development | *Infants*: motor development is 56.8% of the expected motor development. There are no associations between motor development and severity of SRBD[25, 129]. | In infants motor development improves 11.2% during 1 year of GH treatment. Infants with lower developmental age show the greatest improvement[25]. |
| Cognition | At baseline, children have a mean Total IQ score of 66[103]. | GH treatment prevents deterioration of certain cognitive skills in children with PWS on the short term and significantly improves abstract reasoning and visuospatial skills during 4 years of GH treatment. Furthermore, children with a greater deficit have more benefit from GH treatment. The younger the children were at baseline, the higher they score on these subtests and the higher their TIQ scores[103]. |
| Behavior | Children with PWS have less anxiety-related problems and more emotional and social related problems when compared to references with a comparable IQ. Neurobehavioral abnormalities are related with daytime sleepiness. There are no associations between SRBD and neurobehavioral abnormalities[25, 130]. | |

| Psychological aspects | Baseline | Effects of GH treatment |
|---|---|---|
| | Children with PWS show similar problem behavior as a reference population with a comparable intellectual disability. Social problems are the most pronounced within problem behavior in PWS[111]. | There are no significant effects of GH treatment during the RCT and 8 years of GH treatment[111]. |
| Theory of Mind (ToM) | Children with PWS, aged 7-12 years, have a delay in ToM development of 4 (3-5) years. They have mainly difficulties in abstract language and second-order belief[131]. | |
| Adaptive functioning | The maximal developmental age in adaptive functioning is 8 years in children with PWS till the age of 14. | The earlier growth hormone is started during infancy, the better the adaptive functioning on the long-term. |
| Visual-motor integration (VMI) | Children with PWS aged 7-17 years score -3 SD in VMI compared to a healthy peers, but -1 SD for visual perception and motor coordination individually. | |
| Health-related quality of life (HRQOL) | **Children with PWS rate their HRQOL on all items similar or higher as healthy and obese children[132].** | **GH-treated children report an increase in HRQOL in the Physical and Social subdomains and DUXPW, compared to the untreated children. Parents report an increase in the Physical and Emotional subdomains compared to the parents of the untreated children.**<br>**During 11 years of GH treatment, the Physical subdomain continue to improve, according to parents, and the Home, Social and Emotional subdomains of the DUX25 and DUXPW remain similar, according to children and parents[132].** |
| **Psychiatry** | **Baseline** | **Effects of GH treatment** |
| Psychiatric disorders | Psychosis and depression are rare in children with PWS aged 7-17 years. 20% of children with PWS score positive for oppositional defiant disorder[133]. | |
| Autism Spectrum Disorder (ASD) | 36% of children with PWS score positive for ASD. Most prominent symptoms are symptoms in maladaptive behavior and routines[131]. | |
| Precursors and metabolite of the dopamine and serotonin system | Tyrosine-ratio and plasma HVA-level decrease significantly in case more depressive symptoms are present. Tryptophan-ratio is significantly higher in children with PWS than healthy peers. Plasma levels of the aromatic amino acids are significantly increased compared to healthy peers, but levels of branched-chain amino acids are similar[134]. | |

| Bones | Baseline | Effects of GH treatment |
|---|---|---|
| Bone mineral density (BMD) | Total body BMD is comparable to healthy peers, lumbar spine BMD is lower than in healthy peers but within the normal range. Lumbar spine bone mineral apparent density (BMAD) is higher compared to healthy peers, but within the normal range; BMI is positively associated with BMD. IGF-I levels were not associated with BMD[53]. | Total body BMD does not change during 2 years of GH treatment. IGF-I is positively associated with total body- and lumbar spine BMD. After correction for BMI, the increase in lumbar spine BMD is higher in GH-treated than in untreated children. There is no effect of GH treatment on BMAD[53]. |
| | | **In prepubertal children, total body BMD and lumbar spine BMD significantly increase during 4 years of GH treatment while BMAD remains stable.** |
| | | **During adolescence, total body BMD and BMAD decrease significantly, in girls from the age of 11 years and in boys from the age of 14 and 16 years, resp., but all BMDs remain within the normal range. After 9 years of GH treatment, lean body mass SDS is the most powerful predictor of total body BMD and lumbar spine BMD in adolescents with PWS[126].** |
| Bone maturation | The BA/CA ratio is lower than in healthy peers[83]. | BA shows catch-up with CA during GH treatment. GH treatment has no effect on the ΔBA/ΔCA ratio[83]. |
| | | **After 8 years GH treatment, the BA/CA ratio is not significantly different compared with 1[123].** |
| Scoliosis | The total prevalence of scoliosis is 36% (2.7% in the non-PWS population); 50% of children with scoliosis need referral to an orthopedic surgeon; 13% of children need conventional or surgical treatment. Scoliosis is present in 9% of infants, 15% of juveniles and 80% of adolescents. Children with scoliosis have a higher BMI than those without and children with severe scoliosis have lower IGF-I levels compared to children with less severe scoliosis[28, 135]. | GH treatment has no effect on the onset or severity of scoliosis, the curve progression and the start of scoliosis treatment. IGF-I levels have no significant effect on the progression of scoliosis[28]. |
| **Remaining areas** | **Baseline** | **Effects of GH treatment** |
| Sleep related breathing disorders (SRBD) | Mean apnea hypopnea index (AHI) is 5.1/hr, of which 2.8 are central apneas (normal range: 0-1/hr). 9% of non-obese and 50% of obese children have obstructive sleep apnea syndrome (OSAS)[136]. | AHI remains comparable to baseline after 6 months of GH treatment[136]. |
| Central adrenal insufficiency (CAI) | 60% of the children have CAI during stress. All children have normal morning cortisol levels and a normal diurnal rhythm of cortisol. Children with stress related CAI have a higher central apnea index. SRBD is worse in children with CAI. A central apnea index of 4.15 per hour and higher is indicative for having CAI[37, 38]. | |

| Remaining areas | Baseline | Effects of GH treatment |
|---|---|---|
| Cryptorchidism | **Human chorionic gonadotropin administration results in an anatomically lower testis position in 81% of our infant boys with PWS, and 23% of the testes reach a stable scrotal position. Of the cases 76% require an orchidopexy to ensure a stable position in the scrotum[137].** | |
| Energy intake | ***Infants:* Baseline energy intake of infants with PWS was 16% lower than normal daily energy requirements.** | **Energy intake in infants increases after 1 year GH treatment, this tends to be higher in the GH treated infants than in the untreated ones.** |
| | ***Children:* Baseline energy intake of prepubertal children with PWS is till 50% lower than normal daily energy requirements[138].** | **The increase in energy intake is higher in the GH-treated children, but this is not different compared to the untreated ones[138].** |
| Resting energy expenditure (REE) | **At baseline, the median energy intake to REE ratio is 1.17[138].** | **REE is not different between GH-treated children and untreated ones[138].** |

LBM: Lean body mass; SDS: Standard deviation score; BMI: Body mass index; IGF-I: insulin like growth factor-I; IGF-BP3: IGF-binding protein 3; ALS: Acid-labile subunit; DHEAS: dehydroepiandrosterone sulfate; TSH: Thyroid stimulating hormone; fT4: Free thyroxin; T3: Triiodothyroxin; ASP: Acylation Stimulating Protein; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; WIPPSI-R: Wechsler Preschool and Primary Scale of Intelligence- revision; WISC-R: Wechsler Intelligence Scale for Children- revision; SRBD: Sleep related breathing disorders; IQ: intelligence quotient; ToM: Theory of mind; VMI: Visual-motor integration; HRQOL: Health-related quality of life; DUX25: Dutch Children AZL/TNO Questionnaire Quality of Life short form; DUXPW: Based on the generic DUX25, a PWS disease specific questionnaire; ASD: Autism Spectrum Disorder; HVA: Homovanillic acid; BMD: Bone mineral density; BMAD: Bone mineral apparent density (BMD corrected for height); BA: bone age; CA: Calendar age; AHI: Apnea hypopnea index; OSAS: obstructive sleep apnea syndrome; CAI: Central adrenal insufficiency; REE: Resting energy expenditure
**Bold**: described in this thesis

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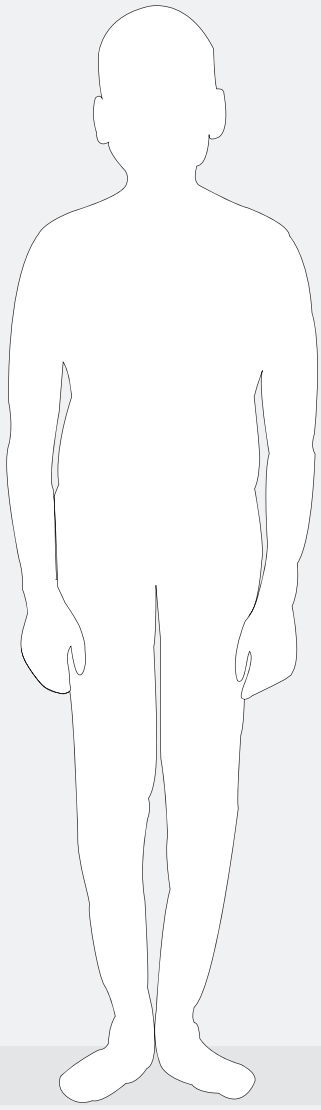


# CHAPTER 9

## Summary / Samenvatting

## SUMMARY

This doctoral dissertation provides a detailed account of the various studies performed to improve knowledge of Prader-Willi syndrome (PWS) and to optimize care for patients and parents. Studies were embedded in the Dutch PWS Cohort study in children and adolescents with PWS. General aspects of children with PWS were studied, such as cryptorchidism, body composition, height, bone mineral density, energy intake, IGF-bioactivity and health-related quality of life. Furthermore, we evaluated the long-term effects and safety of growth hormone (GH) treatment. This chapter summarizes these studies and their most important outcomes.

### Chapter 1

This chapter provides an introduction in PWS by presenting the characteristics of the different phases of life, the genetic background and GH treatment. Furthermore, it introduces unresolved issues, such as treatment of cryptorchidism and long-term effects of GH treatment on body composition, height, bone mineral density, energy intake, IGF-bioactivity and health-related quality of life. Finally, this chapter presents the aims and outline of this thesis.

### Chapter 2

Boys with PWS have often undescended testes and the penis may be small, but most characteristic is the hypoplastic scrotum, which is also poorly rugated and poorly pigmented. In general, a sufficient hypothalamic-pituitary-gonadal axis (HPG-axis) in childhood and primary testicular failure in adulthood are reported. This might suggest that early treatment of cryptorchidism is desirable in boys with PWS. Surgery is the most common treatment of cryptorchidism. Human chorionic gonadotropin (hCG) is a hormone produced by the placenta during pregnancy. The pituitary analog of hCG is luteinizing hormone (LH), which has a stimulating effect on the testes. HCG has been shown to induce testicular descent, presumably by increasing weight and vascularity of the testis and also by stimulating testosterone production. Literature on the optimal treatment of cryptorchidism in boys with PWS was scarce. We, therefore, evaluated the effects of hCG administration on testis position in 16 infant boys, median (IQR) age 1.6 (1.2 to 1.8) years, with Prader-Willi syndrome. In those who subsequently underwent orchidopexy, a biopsy was taken and testis histology was evaluated. First, the boys received a hCG stimulation test, to ensure that their testes responded to hCG. After a positive test, hCG treatment was initiated. The boys received 250 to 500 IU (depending on age) intramuscularly twice weekly for 6 weeks. At baseline, we found 1 testis in a stable scrotal position, 1 vanished testis and 1 atrophic abdominal testis. During hCG

treatment, 23% of 29 testes reached a stable scrotal position, 62% a lower position and 14% did not change position. Thus, 22 testes required orchidopexy.

Of 17 obtained biopsies in 12 patients, 2 showed germ cells in more than 60% of seminiferous tubules, 3 in 30% to 60% and 7 in less than 30%. In addition, 4 boys had Sertoli cell only syndrome and 1 had a vanished testis. In patients undergoing orchidopexy, a younger age and increased testosterone levels after hCG stimulation were associated with a greater number of germ cell containing tubules.

We conclude that hCG treatment resulted in an anatomically lower testis position in most boys with PWS. Twenty-three percent of testes reached a stable scrotal position and 76% required an orchidopexy to ensure a stable position in the scrotum. A younger age at orchidopexy was associated with a better histological outcome but the effect of hCG treatment at an early age on adult testicular function has to be awaited.

**Chapter 3**

The main reason for treating children with PWS with GH is to optimize their body composition. Without GH treatment, body composition tends to deteriorate over time, resulting in a mean BMI of 35 (+3.5 SDS) at the age of 18 years. Short-term GH treatment has beneficial effects on height and body composition in children with PWS, but it was unknown whether long-term GH treatment could counteract the clinical course of increasing obesity in PWS by maintaining the improved body composition over the years. We, therefore, investigated the course of PWS during 8 years of continuous GH treatment over a long period from childhood to adolescence. We followed 60 prepubertal children for 8 years of GH treatment (1 mg/m$^2$/day). After an increase during the first year of GH treatment, lean body mass (LBM) remained stable for 7 years at a level above baseline. After a decline in the first year, fat percentage SD score (SDS) and body mass index (BMI) SDS remained stable at a level comparable to baseline. However, BMI $SDS_{PWS}$ was lower after 8 years of GH treatment than at baseline. After 8 years of treatment, height SDS and head circumference SDS had completely normalized. IGF-I SDS increased during the first year of treatment and remained stable since then. GH treatment did not adversely affect glucose homeostasis, serum lipids, blood pressure, and bone maturation.

We conclude that, in combination with a healthy lifestyle, GH treatment is a potent force for counteracting the clinical course of obesity in children with PWS. This is very important, because development towards morbid obesity is a major threat to these patients. Thus GH treatment may improve the future health of these children.

**Chapter 4**

Bone mineral density (BMD) is influenced by activity and endocrine factors, including parathyroid hormone, thyroxine, GH and sex steroids. BMD increases during childhood and peak bone mass is normally attained between the age of 18-20 years in girls and

18-23 years in boys. Bone strength later in life depends largely on this attained peak bone mass. Children with PWS might be prone to a lower BMD during childhood and adolescence because of low GH levels, hypothyroidism, hypogonadism and a sedentary lifestyle. Longitudinal data on bone mineral density (BMD) in children and adolescents with PWS during long-term GH treatment were not available. We, therefore, investigated the effects of long-term GH treatment and puberty on BMD of total body ($BMD_{TB}$), lumbar spine ($BMD_{LS}$) and bone mineral apparent density of the lumbar spine ($BMAD_{LS}$) in 77 children with PWS who remained prepubertal during GH treatment for 4 years and 64 children with PWS who received GH treatment for 9 years. The same dual-energy X-ray absorptiometry (DXA) machine was used for all annual measurements of $BMD_{TB}$, $BMD_{LS}$ and $BMAD_{LS}$. We found that in the prepubertal group, $BMD_{TB}$SDS and $BMD_{LS}$SDS significantly improved during 4 years of GH treatment while $BMAD_{LS}$SDS remained stable. During adolescence, $BMD_{TB}$SDS and $BMAD_{LS}$SDS decreased significantly, in girls from the age of 11 years and in boys from the age of 14 and 16 years, respectively, but the BMDs remained within the normal range. After 9 years of GH treatment, lean body mass SDS was the most powerful predictor of $BMD_{TB}$SDS and $BMD_{LS}$SDS in adolescents with PWS.

This long-term GH study demonstrates that $BMD_{TB}$, $BMD_{LS}$ and $BMAD_{LS}$ remains stable in prepubertal children with PWS but decreases during adolescence due to incomplete pubertal development. Based on our findings, clinicians should be aware of this problem and start sex hormone therapy when serum sex hormone levels remain low in girls from the age of 11 and in boys from the age of 14.

**Chapter 5**

PWS is the most common cause of syndromic obesity and dietary management is one of the most challenging aspects in PWS. GH treatment is known to increase energy intake in children born small for gestational age, children with Turner or Silver Russell syndrome, but randomized controlled trials comparing energy intake in children with PWS with and without GH treatment were very limited. Especially in children with PWS, it is important to obtain insight into the effects of GH treatment on energy balance, so as to determine what lifestyle interventions might be beneficial. We, therefore, investigated in our randomized controlled GH trial (RCT), the effect of GH treatment on dietary energy intake, body composition, adiponectin levels and resting energy expenditure (REE) in children with PWS.

Our results showed that baseline energy intake, expressed as percentage of the daily energy requirements for age- and sex-matched healthy children, 20% lower in young infants and 50% lower in prepubertal children above the age of 10 years. Energy intake in infants increased after 1 year of GH treatment and this tended to be higher in the GH-group than untreated group. In prepubertal children, the increase in energy-intake was higher in the GH-group, but this was not different compared to the untreated group.

REE was not different between the GH-group and the untreated group. The increase in energy intake during 2 years of GH treatment was correlated with lower fat% SDS, and higher adiponectin levels.

This RCT demonstrates that parents of children with PWS are very well capable to restrict energy intake with 20-50% compared to daily energy requirements for age- and sex-matched healthy children. GH treatment was associated with a slight increase in energy intake, but also improved body composition and adiponectin levels, which suggests a protective effect of GH treatment with regard to the development of obesity and diabetes mellitus type II.

**Chapter 6**

In children with PWS, elevated serum IGF-I levels during standard dose GH treatment are a major concern and often subject of debate. However, to maintain an acceptable body composition with a fat mass percentage at max 2 SDS, children with PWS require relatively high IGF-I levels. There is, however, still debate whether or not to accept these high immunoreactive IGF-I levels, because of the unknown long-term consequences. Despite the high serum IGF-I levels, no signs of acromegaly have been observed, indicating no increased IGF-bioactivity. In children with PWS, there were no data on IGF-I complex formation and the ability of serum IGF-I to phosphorylate its receptor, a measure of IGF-bioactivity. We, therefore, investigated serum IGF-I, IGFBP-3 and acid-labile subunit (ALS) levels, complex formation and IGF-bioactivity in 20 young GH-treated children with PWS, median (IQR) age 5.2 (4.3 to7.2) years, and 20 older ones, age 14.9 (13.8 to 16.2) years, compared with 41 age- and sex-matched healthy controls. Complex formation was studied by $^{125}$I-hIGF-I column chromatography and IGF-bioactivity by IGF1 receptor kinase activation assay (KIRA). We observed that serum IGF-I, IGFBP-3, ALS levels and IGF-I/IGFBP-3 ratio were significantly higher in GH-treated PWS children than in healthy controls. The 150 kD ternary complex formation was, however, also significantly higher than in controls, indicating that most of the serum IGF-I was sequestered in the ternary 150 kD complex with ALS and IGFBP-3. Young GH-treated PWS children had a higher serum IGF-bioactivity than controls, but no difference was observed in IGF-bioactivity between older GH-treated PWS children and controls. The proportion of IGF-bioactivity of total serum IGF-I was, however, lower in GH-treated PWS children than in controls. Serum immunoreactive IGF-I levels did not correlate with IGF-bioactivity in GH-treated children with PWS, in contrast to a strong positive correlation in healthy controls.

In conclusion, our study shows that in GH-treated PWS children, most of the serum IGF-I is sequestered in the 150 kD complex. A higher IGF-bioactivity was only found in young GH-treated PWS children and not in the older ones. IGF-bioactivity during GH treatment showed a wide variation and there was a disrupted correlation between im-

munoreactive IGF-I levels and IGF-bioactivity, which makes immunoreactive IGF-I levels an inappropriate indicator of GH-dosing in PWS children.

**Chapter 7**

It was already known that GH treatment is beneficial for children with PWS, but information about health-related quality of life (HRQOL) was lacking. Furthermore, nothing was known about the effect of GH treatment on HRQOL in children with PWS, or HRQOL during a long period of GH treatment. Studies investigating HRQOL in children with an intellectual disability are scarce. Because of a different time perspective, dominance of short-term memory and recent incidents, HRQOL questionnaires are not easy to interrogate. So to determine a child's HRQOL, the opinion of a child itself in combination with parents determining their child's HRQOL are most useful. We, therefore, investigated HRQOL in children with PWS according to both children and parents. We included 26 children with PWS children in our RCT and, in addition, 76 children from the Cohort study during 11-years of GH treatment. HRQOL recorded by patients and parents were annually assessed, using a generic questionnaire with 25 questions (DUX25), containing four subdomains (Physical, Home, Social and Emotional) and a PWS-specific questionnaire (DUXPW). Prior to start of GH treatment, children with PWS rated their HRQOL similar as their parents, children scored only higher on the Physical subdomain. Children with PWS rated their HRQOL on all items similar or higher as healthy and obese children. GH-treated children reported an increase in HRQOL in the Physical and Social subdomains and DUXPW, compared to the untreated children. Parents reported an increase in the Physical and Emotional subdomains, compared to the parents of the untreated children. During 11 years of GH treatment, the Physical subdomain continued to improve, according to parents, and the Home, Social and Emotional subdomains of the DUX25 and DUXPW remained similar, according to children and parents.

In conclusion, our RCT and long-term GH study show that children with PWS rated their HRQOL equal or even better compared to healthy children and obese children. According to children and parents, the HRQOL increased during GH treatment, in contrast to untreated children with PWS. This effect sustained during long-term GH treatment. So, despite the difficulties related to the syndrome, children with PWS consider themselves quite happy.

**Chapter 8**

In the general discussion, we discuss our results in the context of the literature. We present our general conclusions and suggestions for further research.

## SAMENVATTING

Dit proefschrift geeft een gedetailleerd overzicht van de verschillende studies die zijn uitgevoerd om de kennis over kinderen met het Prader-Willi syndroom (PWS) te verbeteren en de zorg te optimaliseren. De studies zijn onderdeel van de Nederlandse PWS Cohort studie in kinderen en adolescenten met PWS. Algemene aspecten van kinderen met PWS zijn onderzocht, zoals cryptorchisme, lichaamssamenstelling, lengtegroei, botdichtheid, energie-inname, IGF bioactiviteit en kwaliteit van leven. Tevens hebben we de lange-termijn effecten en veiligheid van groeihormoon (GH) behandeling onderzocht. Dit hoofdstuk geeft een samenvatting van deze studies en de belangrijkste resultaten.

### Hoofdstuk 1

Dit hoofdstuk geeft achtergrond informatie over PWS. De karakteristieke kenmerken per levensfase worden beschreven, de genetische oorzaken en een inleiding in GH-behandeling. Daarna volgt er een korte inleiding van alle onderzochte onderwerpen; behandeling van cryptorchisme en de lange termijn effecten van GH-behandeling op lichaamssamenstelling, lengte, botdichtheid, energie inname, IGF bioactiviteit en kwaliteit van leven. Het eind van dit hoofdstuk geeft een overzicht van de vraagstellingen in dit proefschrift.

### Hoofdstuk 2

Jongens met PWS hebben meestal unilateraal of bilateraal cryptorchisme en soms een kleine penis. Het scrotum is hypoplastisch en nauwelijks gerimpeld en gepigmenteerd. In de literatuur wordt beschreven dat de hypothalamus-hypofyse-gonade as op jonge leeftijd goed werkt en dat er op volwassen leeftijd vooral sprake is van primair testiculair falen. Dit kan erop wijzen dat cryptorchisme bij jongens met PWS op een jonge leeftijd behandeld moet worden. Meestal worden niet-ingedaalde testes met een operatie vastgezet in het scrotum, door middel van orchidopexie. Humaan choriongonadotrofine (hCG) is een hormoon dat in de placenta geproduceerd wordt. Het hypofysaire analoog van hCG is luteïniserend hormoon (LH). LH heeft een stimulerende werking op testesweefsel. Ook van hCG is het bekend dat het een stimulerende werking op testesweefsel heeft. Het verhoogt de doorbloeding en stimuleert de productie van testosteron, waardoor de testes zwaarder worden en soms alsnog indalen. Naar de optimale behandeling van cryptorchisme in PWS is nauwelijks onderzoek gedaan. We hebben daarom bij 16 jongens met PWS, mediane (IQR) leeftijd 1.6 (1.2-1.8) jaar, onderzocht of hCG-behandeling effect heeft. Bij de jongens die ook een orchidopexie ondergingen, werd een testes biopt genomen en de histologie bestudeerd. Voorafgaand aan de hCG-behandeling kregen de jongens een hCG stimulatietest, om te kijken of de testes gevoelig waren voor hCG. Na een positieve hCG-test, kregen ze 250-500 IE hCG (afhankelijk van hun leeftijd) 2 maal per week, gedurende 6 weken intramusculair toegediend. Bij start vonden we

dat 1 testis al scrotaal zat, 1 jongen had een "vanished" testis en 1 testis was abdominaal gelegen en atrofisch. Na de hCG behandeling was 23% van de testes ingedaald in het scrotum, 62% was wel gedaald maar niet tot in het scrotum en 14% was niet van positie veranderd. Dus 22 testes hadden een aanvullende orchidopexie nodig.

In 17 testes biopten van 12 jongens vonden we in 2 testes kiemcellen in meer dan 60% van de zaadbuisjes, in 3 testes 30-60% en in 7 testes minder dan 30%. Vier jongens hadden alleen maar Sertoli cellen en 1 biopt was van de "vanished" testis. Een jongere leeftijd en een hogere testosteron oploop na de hCG stimulatietest waren gecorreleerd aan een hoger aantal kiemcel bevattende zaadbuisjes.

Uit dit onderzoek concluderen we dat hCG-behandeling bij de meeste jongens met PWS leidt tot een lagere testes positie. Drieëntwintig procent van de testes zaten stabiel in het scrotum na hCG-behandeling en 76% hadden een aanvullende orchidopexie nodig. Een jonge leeftijd ten tijde van de orchidopexie was geassocieerd met een betere histologische uitkomst. Het effect van de hCG-behandeling op jonge leeftijd op de functie van de testes op oudere leeftijd moet worden afgewacht.

**Hoofdstuk 3**

De belangrijkste reden om kinderen met PWS te behandelen met GH is om hun lichaamssamenstelling te verbeteren. Zonder GH worden kinderen met PWS steeds dikker, met uiteindelijk een gemiddelde body mass index (BMI) van 35 (+3.5 SDS) op de leeftijd van 18 jaar. Het is bekend dat kortdurende GH-behandeling een positief effect heeft op de lengtegroei en lichaamssamenstelling van kinderen met PWS, maar het was niet bekend of de positieve effecten die eerder gevonden waren, ook blijven bestaan tijdens de puberteit en of het veilig is om GH voor langere tijd te gebruiken. Daarom hebben we bij 60 kinderen de GH effecten (1 mg/m$^2$/dag) tijdens 8 jaar (of langer) onderzocht. De groep was gemiddeld 5,5 jaar oud bij het starten van de behandeling en prepubertair. In het eerste jaar steeg de spiermassa standaard deviatie score (SDS) en bleef daarna stabiel. Na 8 jaar GH-behandeling was de spiermassa SDS significant verbeterd, maar gemiddeld lager dan bij leeftijdsgenoten. Na een daling in het eerste jaar van de GH-behandeling, bleven het vetpercentage SDS en de BMI SDS stabiel op hetzelfde niveau als bij start GH. De BMI $SDS_{PWS}$ was na 8 jaar significant lager dan bij start. Na 8 jaar GH-behandeling waren de lengte en de hoofdomtrek volledig genormaliseerd. IGF-I SDS waarden stegen significant gedurende het eerste jaar van de GH-behandeling en bleven daarna stabiel. GH-behandeling had geen negatief effect op de glucose huishouding, lipiden (vetten), bloeddruk en botleeftijd.

We concluderen dat GH-behandeling, in combinatie met een gezonde leefstijl, het vetpercentage en de BMI ook op de lange termijn goed onder controle kan houden. Dit is zeer belangrijk, omdat de ontwikkeling van obesitas een groot probleem is bij kinderen met PWS.

**Hoofdstuk 4**

Botdichtheid wordt beïnvloed door lichamelijke activiteit en hormonen, zoals parathyreoïdhormoon, thyroxine, GH en geslachtshormonen. Gedurende de kinderleeftijd stijgt de botdichtheid en de piek wordt normaal gesproken bereikt op de leeftijd van 18-20 jaar in meisjes en 18-23 jaar in jongens. De stevigheid van de botten later in het leven wordt met name bepaald door de hoogte van de piek die bereikt is. Kinderen en adolescenten met PWS hebben een hoger risico op een lagere botdichtheid, omdat ze vaak lage hormoonspiegels hebben, zoals GH, thyroxine en geslachtshormonen, en een inactieve leefstijl. Gegevens over botdichtheid in kinderen en adolescenten met PWS gedurende langdurig GH gebruik waren niet bekend. Daarom hebben we het effect van langdurig GH gebruik en puberteitsontwikkeling op de botdichtheid onderzocht. In 77 prepubertaire kinderen gedurende 4 jaar GH-behandeling en in 64 kinderen die in de puberteit kwamen tijdens 9 jaar GH-behandeling hebben we de botdichtheid van het hele lichaam ($BMD_{TB}$), de lumbale wervelkolom ($BMD_{LS}$) en de $BMD_{LS}$ gecorrigeerd voor de lengte ($BMAD_{LS}$) onderzocht. De metingen werden elk jaar verricht met hetzelfde dual-energy X-ray absorptiometry (DXA) apparaat. In de prepubertaire kinderen verbeterden de $BMD_{TB}$SDS en de $BMD_{LS}$SDS significant gedurende 4 jaar GH en de $BMAD_{LS}$SDS bleef stabiel. Gedurende adolescentie daalden de $BMD_{TB}$SDS en de $BMAD_{LS}$SDS significant, in meisjes vanaf 11 jaar en in jongens vanaf 14. De BMD waarden bleven wel in het normale gebied. Na 9 jaar GH-behandeling, was de spiermassa SDS de belangrijkste voorspeller van de $BMD_{TB}$SDS en de $BMD_{LS}$SDS in adolescenten met PWS.

Deze lange-termijn GH studie laat zien dat $BMD_{TB}$, $BMD_{LS}$ en $BMAD_{LS}$ stabiel blijven in prepubertaire kinderen met PWS, maar dalen in adolescentie door een onvolledige puberteitsontwikkeling. Gebaseerd op onze bevindingen, adviseren we behandelaars alert te zijn op dit probleem en tijdig geslachtshormonen te starten als de testosteron- of estradiolspiegels laag blijven, bij meisjes vanaf de leeftijd vanaf 11 jaar en in jongens vanaf 14 jaar.

**Hoofdstuk 5**

PWS is de meest voorkomende oorzaak van syndromale obesitas. Het dieet is dan ook een van de belangrijkste aspecten in de behandeling van PWS. Kinderen die te klein blijven nadat ze te klein geboren zijn, meisjes met Turner syndroom en kinderen met Silver Russell syndroom, gaan tijdens GH-behandeling meer eten. Bij kinderen met PWS was nauwelijks onderzoek gedaan of er een verschil in energie-inname is tussen kinderen met GH en zonder GH-behandeling. Wij hebben daarom in onze gerandomiseerde en gecontroleerde GH studie (RCT) bij kinderen met PWS de effecten van GH-behandeling onderzocht op energie-inname, lichaamssamenstelling, serum adiponectinespiegel (een hormoon afgescheiden door de vetcellen en een maat voor insuline gevoeligheid) en het energieverbruik in rust (REE). Onze resultaten laten zien dat bij start van

de studie de energie-inname, uitgedrukt als percentage van de dagelijkse aanbevolen hoeveelheid voor gezonde kinderen van dezelfde leeftijd en geslacht, in kinderen met PWS jonger dan 3.5 jaar 20% lager was en in kinderen ouder dan 10 jaar 50% lager. De energie-inname in jonge kinderen met PWS steeg significant in het eerste jaar GH gebruik. Deze stijging was groter dan bij onbehandelde kinderen, maar niet significant. In prepubertaire kinderen was de stijging ook het grootst in de GH groep, maar ook dit was niet significant verschillend. REE was niet significant verschillend tussen kinderen die GH kregen en onbehandelde kinderen. De stijging in energie-inname was positief gecorreleerd aan de afname in vetpercentage en de toename van de serum adiponectinespiegels.

Deze studie laat zien dat ouders van kinderen met PWS heel goed in staat zijn om de energie-inname te beperken met 20-50%. GH-behandeling is geassocieerd met een kleine toename in energie-inname, maar verbetert tegelijkertijd de lichaamssamenstelling en adiponectinespiegels, c.q. de insulinegevoeligheid. Dit suggereert dat GH-behandeling een beschermend effect heeft op de ontwikkeling van obesitas en diabetes mellitus type II.

**Hoofdstuk 6**

Bij kinderen met PWS worden tijdens GH-behandeling hoge serum IGF-I spiegels gemeten. Echter, om de lichaamssamenstelling acceptabel te houden, met een vet percentage rond de +2 SDS, hebben kinderen met PWS deze hoge IGF-I spiegels nodig. Toch is er discussie of de hoge serum IGF-I spiegels acceptabel zijn of niet, omdat de lange-termijn gevolgen onbekend zijn. Ondanks de hoge IGF-I spiegels, hebben kinderen met PWS gedurende de GH-behandeling geen tekenen van acromegalie, dus klinisch is er geen sprake van te hoge bioactiviteit. In kinderen met PWS was geen informatie beschikbaar over de IGF complex vorming en het vermogen van IGF-I om zijn receptor te fosforyleren, een maat voor de IGF bioactiviteit. Daarom hebben we onderzoek verricht in 20 jonge GH-behandelde kinderen met PWS, mediane (IQR) leeftijd 5.2 (4.3 - 7.2) jaar, en 20 oudere, 14.9 (13.8 - 16.2) jaar, en vergeleken met 41 gezonde controle kinderen van dezelfde leeftijd en geslacht. Complexvorming is gemeten met een $^{125}$I-hIGF-I kolomchromatografie en IGF bioactiviteit met een IGF1 receptor kinase activatie assay (KIRA). We vonden dat de serum IGF-I, IGFBP-3 en ALS spiegels en IGF-I/IGFBP-3 ratio significant hoger waren bij GH-behandelde kinderen met PWS dan in gezonde controle kinderen. De 150 kD complexvorming was echter ook significant hoger dan in gezonde controle kinderen, wat erop wijst dat het IGF-I voornamelijk in het 150 kD complex gebonden zit met IGFBP-3 en ALS. Jonge GH-behandelde kinderen met PWS hadden een hoger IGF bioactiviteit dan controle kinderen, maar oudere GH-behandelde kinderen met PWS hadden dezelfde IGF bioactiviteit als controle kinderen. Het percentage IGF bioactiviteit van het totaal aanwezige IGF-I was echter significant lager in GH-behandelde kinderen

met PWS dan in controle kinderen. De serum IGF-I spiegels bij kinderen met PWS correleerden totaal niet met de IGF bioactiviteit, dit in tegenstelling tot de sterke correlatie die we vonden bij gezonde controle kinderen.

Uit onze studie kunnen we concluderen dat het serum IGF-I in GH-behandelde kinderen met PWS grotendeels gebonden is in het 150 kD complex. Alleen jonge kinderen met PWS hebben een hogere bioactiviteit gedurende de GH-behandeling dan controle kinderen, oudere kinderen met PWS niet. De IGF bioactiviteit in kinderen met PWS gedurende GH-behandeling varieert sterk en vertoont geen correlatie met de serum IGF-I spiegels. Serum IGF-I spiegels zijn dus geen goede maat voor de bioactiviteit of om GH te doseren in kinderen met PWS.

**Hoofdstuk 7**

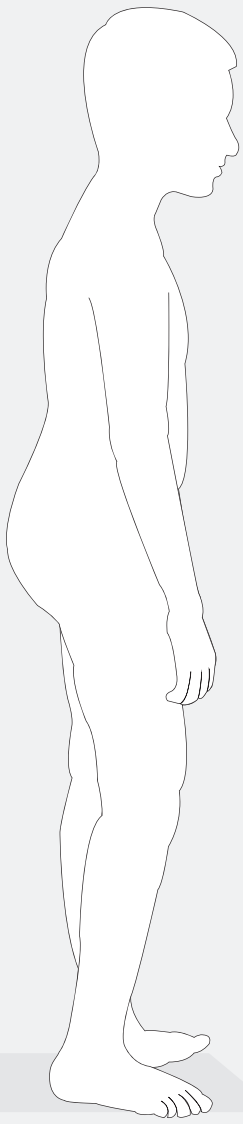
GH-behandeling heeft een positief effect op kinderen met PWS, maar informatie naar de kwaliteit van leven (HRQOL) was er nauwelijks. Verder was er geen onderzoek verricht naar het effect van GH-behandeling op de HRQOL bij kinderen met PWS. Studies naar HRQOL bij kinderen met een verstandelijke beperking in het algemeen zijn er vrijwel niet. Jonge kinderen en kinderen met een verstandelijke beperking hebben vaak een ander tijdsperspectief en een dominant korte termijn geheugen. Hierdoor is het lastig om HRQOL vragenlijsten af te nemen. Om HRQOL te meten bij deze kinderen is de mening van het kind zelf in combinatie met de mening van de ouders, de beste manier om de HRQOL te onderzoeken. Daarom hebben we 26 kinderen en hun ouders uit de gerandomiseerde en gecontroleerde GH studie (RCT) onderzocht, en 76 kinderen en hun ouders uit de Cohort studie gedurende 11 jaar GH-behandeling. Jaarlijks vulden de kinderen en hun ouders een algemene vragenlijst (DUX25) in met 25 vragen, onder te verdelen in 4 onderdelen: Fysiek, Thuis, Sociaal en Emotioneel, en een PWS specifieke vragenlijst (DUXPW). Voordat kinderen met PWS aan de GH-behandeling begonnen, scoorden zij hun HRQOL hetzelfde als hun ouders. Alleen op het onderdeel Fysiek scoorden ze hoger dan hun ouders. Kinderen met PWS scoorden op alle HRQOL onderdelen gelijk of hoger dan gezonde kinderen en kinderen met obesitas. Tijdens GH-behandeling rapporteerden de kinderen met PWS een stijging in de onderdelen Fysiek en Sociaal en in de DUXPW, vergeleken met de onbehandelde PWS kinderen. De ouders van GH-behandelde kinderen met PWS rapporteerden een stijging in de onderdelen Fysiek en Emotioneel, in vergelijking met de ouders van de onbehandelde kinderen. Gedurende 11 jaar GH-behandeling, rapporteerden ouders een continue stijging in het onderdeel Fysiek, maar de onderdelen Thuis, Emotioneel en Sociaal van de DUX25 en de DUXPW bleven gelijk, zowel gerapporteerd door ouders als de kinderen zelf.

We concluderen dat kinderen met PWS hun HRQOL als gelijk of zelfs als beter beoordelen dan gezonde kinderen en kinderen met obesitas. Volgens de kinderen en hun ouders stijgt de HRQOL gedurende de GH-behandeling, in vergelijking met kinderen

met PWS die niet behandeld worden. Het positieve effect van GH-behandeling blijft stabiel gedurende 11 jaar GH-behandeling. Dus ondanks de moeilijkheden die gepaard kunnen gaan met PWS, vinden kinderen met PWS zichzelf best gelukkig.

**Hoofdstuk 8**

In de algemene discussie bediscussiëren we de resultaten van onze studie in het kader van de huidige literatuur. We geven onze algemene conclusies en suggesties voor verder onderzoek.



# CHAPTER 10

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACTH | corticotropin |
| ALS | acid-labile subunit |
| AMH | anti-Mullerian hormone |
| AR(1) | first-order autoregressive |
| BA | bone age |
| $BMAD_{LS}$ | bone mineral apparent density of the lumbar spine |
| BMD | bone mineral density |
| $BMD_{LS}$ | BMD of lumbar spine |
| $BMD_{TB}$ | BMD of total body |
| BMI | body mass index |
| CA | calendar age |
| CI | confidence interval |
| CRH | corticotropin-releasing hormone |
| CV | coefficients of variation |
| DDX4 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 4 |
| DER | daily energy requirements |
| DM | diabetes mellitus |
| DMEM | Dulbecco's modified eagle's medium |
| DNA | deoxyribonucleic acid |
| DUX25 | Dutch child AZL TNO quality-of-life 25 item questionnaire |
| DUXPW | PWS-specific questionnaire about quality-of-life |
| DXA | dual-energy X-ray absorptiometry |
| EMA | European Medicines Agency |
| EMM | estimated marginal means |
| FDA | Food and Drug Administration |
| FFM | fat free mass |
| FM | fat mass |
| FSH | follicle stimulating hormone |
| GH | growth hormone |
| GHRH | growth hormone-releasing hormone |
| GRH | gonadotropin-releasing hormone |
| hCG | human chorionic gonadotropin |
| HDL | high density lipoprotein |
| HHHO | hypotonia-hypomentia-hypogonadism-obesity |
| HOMA-IR | homeostatic model assessment of insulin resistance |
| HPG-axis | hypothalamic-pituitary-gonadal axis |
| HRQOL | health-related quality of life |

| | |
|---|---|
| ICD | imprinting center defect |
| IGFBP-3 | IGF binding protein 3 |
| IGF-I | insulin-like growth factor type I |
| IQ | intelligence quotient |
| IQR | inter quartile range |
| KIRA | receptor kinase activation assay |
| LBM | lean body mass |
| LDL | low density lipoprotein |
| LH | luteinizing hormone |
| mUPD | maternal uniparental disomy |
| PBS | phosphate-buffered saline |
| % | percentage |
| PRL | prolactin |
| PV | processus vaginalis |
| PWS | Prader-Willi syndrome |
| RCT | randomized controlled trial |
| REE | resting energy expenditure |
| SCO | Sertoli cell only |
| SDS | standard deviation score |
| SEM | standard error of the mean |
| SF | skinfold |
| TFI | tubular fertilization index |
| TRH | thyrotropin-releasing hormone |
| TSH | thyrotropin |
| TSPY | testis specific protein on Y chromosome |
| WHO | World Health Organization |

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**Bakker NE**, van Doorn J, Renes JS, Donker GH, Hokken-Koelega AC 2015 IGF-I levels, complex formation and IGF-bioactivity in growth hormone treated children with Prader-Willi Syndrome. *Submitted*

**Bakker NE**, Siemensma EP, van Rijn M, Festen DA, Hokken-Koelega AC 2015 Beneficial effect of growth hormone treatment on health related quality of life in children with Prader-Willi Syndrome: a randomized controlled trial and longitudinal study. *Submitted*

Kuppens RJ, Diène G, **Bakker NE**, Molinas C, Faye S, Nicolino M, Bernoux D, Delhanty PJ, van der Lely AJ, Allas S, Julien MD, Delale T, Tauber M, Hokken-Koelega AC 2015 Elevated ratio of acylated to unacylated ghrelin in children and young adults with Prader-Willi Syndrome. *Endocrinology, in press*

Growth Hormone Treatment in Children with PWS. **Bakker NE**, Hokken-Koelega ACS. Prader-Willi Syndrome by Höybye C. Nova Science Publishers, Inc New York, 2013. ISBN: 978-1-62618-993-5

## PhD PORTFOLIO

**Name**: Nienke Eline Bakker **PhD period**: March 2011 – March 2015
**Promotor:** Prof.dr. A.C.S. Hokken-Koelega
**Affiliations:** Dutch Growth Research Foundation, Rotterdam, The Netherlands/ Department of Pediatric Endocrinology, Children's Hospital Erasmus MC- Sophia, Rotterdam, The Netherlands

### Summary of PhD training

| General Courses | Year | Workload (ECTS) |
|---|---|---|
| Good clinical practice, Erasmus MC | 2012 | 1.0 |
| Introduction to Clinical Research, NIHES, Erasmus MC | 2012 | 0.9 |
| Biostatistics for Clinicians, NIHES, Erasmus MC | 2012 | 1.0 |
| Regression Analysis for Clinicians, NIHES, Erasmus MC | 2012 | 1.9 |
| Courses for the Quantitative Researcher, NIHES, Erasmus MC | 2012 | 1.4 |
| Integrity in Medical Research, Medical Ethics and Philosophy, Erasmus MC | 2012 | 2.0 |
| Biomedical English Writing and Communication, MolMed, Erasmus MC | 2013 | 4.0 |
| Specific Courses | | |
| Basic Human Genetics Course, MolMed, Erasmus MC | 2011 | 0.5 |
| PubMed and Endnote, Medical Library, Erasmus MC | 2011 | 0.3 |
| Photoshop & Illustrator CS5, Molmed, Erasmus MC | 2012 | 0.3 |
| Radiation protection 5A, Zorgacademie, Erasmus MC | 2013 | 1.0 |
| Seminars and workshops | | |
| Weekly research meeting, department of Pediatric Endocrinology | 2011-15 | 4.0 |
| Weekly patient presentation, department of pediatrics | 2011-12 | 2.0 |
| Annual PhD day | 2012 | 0.3 |
| Young investigators day, TULIPS/ NVK | 2012 | 0.3 |
| PWS Patient Care Day, Diegem/Brussel, Belgium | 2013 | 0.2 |
| Research Day, Sophia Children's Hospital (oral presentation) | 2013 | 1.0 |
| Minisymposium 'Gewetensbezwaren op de werkvloer: waar ligt de grens?', Commissie Medisch Ethische Vraagstukken, Erasmus MC, Rotterdam | 2014 | 0.2 |
| International and national conferences | | |
| 50th Annual Meeting of the European Society of Paediatric Endocrinogy (ESPE), Glasgow, UK | 2011 | 1.0 |
| 4e Northern-European Neuro-Endocrine Group (NENEG) meeting, Amsterdam, NL (oral presentation) | 2011 | 1.0 |
| 51th Annual Meeting of the European Society of Paediatric Endocrinogy (ESPE), Leipzig, Germany (2 poster presentations) | 2012 | 1.0 |
| IPWSO 8th International PWS conference, Cambridge, UK (oral presentation) | 2013 | 1.0 |

| | | |
|---|---|---|
| 9th Joint Meeting of the European Society of Paediatric Endocrinogy (ESPE), Milan, Italy (poster presentation) | 2013 | 1.0 |
| Congress Dutch society for pediatrics (NVK), Veldhoven (oral presentation) | 2013 | 1.0 |
| PWS expert meeting, Toulouse, France | 2014 | 0.6 |
| 53rd Annual Meeting of the European Society of Paediatric Endocrinogy (ESPE), Dublin, Ireland (poster presentation) | 2014 | 1.0 |
| Lecturing | | |
| PWS Parents Information Day, Capelle a/d IJssel (oral presentation) | 2014 | 1.0 |
| PWS Parents Information Day, Antwep, Belgium (oral presentation) | 2014 | 1.0 |
| Annual IMC Weekendschool day "Growth and Development", Rotterdam | 2013-14 | 1.0 |
| Education lecture study day of the Dutch society for physicians for people with intellectual disability (NVAVG), Vianen | 2014 | 1.0 |
| Research proposals | | |
| Young Adult Prader-Willi study: Effects of growth hormone after final height: A clinical care to the optimal dosage of growth hormone in young adults with PWS. | 2012 | 5.0 |
| Grants | | |
| Pfizer: Prolongation Transition Study | 2013 | 1.0 |
| Miscellaneous | | |
| Co-author Landelijke richtlijn "Diagnostiek en Behandeling van kinderen met het Prader-Willi syndroom" | 2013 | 4.0 |
| Co-author "Wegwijzer PWS" | 2014 | 3.0 |
| Growth Hormone Treatment in Children with PWS N.E. Bakker, A.C.S. Hokken-Koelega. *Prader-Willi Syndrome*, by C. Höybye. Nova Science Publishers, Inc, 2013. ISBN: 9781626189935 | 2014 | 2.0 |

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*Nienke*

## CURRICULUM VITAE

Nienke Eline Bakker was born on 28th of January in Langedijk, the Netherlands. She attended secondary school at The Willem de Zwijger college in Papendrecht, where she received her gymnasium certificate in 2003. Following this, she started her basic medical training at the Erasmus University of Rotterdam. She became enthusiastic about scientific research after doing a research rotation in the fourth year at the department of Obstetrics and Gynecology under supervision of prof.dr. J.S.E. Laven. This research resulted eventually in the completion of her doctoral thesis, which was awarded to her in 2007.

As a part of her clinical training, she did an elective rotation in tropical medicine at Macha Mission Hospital in Zambia together with her future husband Martijn Boon. She received her medical degree cum laude in 2010.

Being enthusiastic about nearly every aspect of medicine, she decided to dig into pediatrics by working as a pediatric resident (AGNIO) at the Albert Schweitzer Hospital. After a year of hard but gratifying work, she wanted to do something different. Via a collegue, she was introduced to prof.dr. Anita Hokken-Koelega, who offered her a PhD project on children with Prader-Willi syndrome (PWS). She more than happily accepted this offer and worked with much enthusiasm on her PhD thesis at Dutch Growth Research Foundation (Stichting Kind en Groei) for the coming four years. The result of this hard work now lays bundled in front of you.

Having zoomed in on a small area of pediatrics hasn't erased Nienke's broad interest for people and medicine as a whole. She decided to zoom out again and recently started her training as a general practitioner at Erasmus University Medical Center. But although she continued her career in a different area of medicine, children with PWS will always keep a special place in her heart.