Prader-Willi syndrome:

Advancing knowledge about the effects of growth hormone treatment in children and young adults

Stephany H. Donze

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Prader-Willi syndrome

Advancing knowledge about the effects of growth hormone treatment
in children and young adults

Prader-Willi syndroom

Voortschrijdend inzicht in de effecten van groeihormoonbehandeling bij kinderen en jongvolwassenen

Proefschrift

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Voor alle kinderen en jongvolwassenen met Prader-Willi syndroom en hun ouders

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Chapter 1

General introduction

INTRODUCTION

In 2002, our research group started investigating children with Prader-Willi syndrome (PWS) and the effects and safety of growth hormone (GH) treatment. Today we know that long-term GH treatment during childhood counteracts the clinical course of increasing obesity in children with PWS and has substantially changed their phenotype. Combined with an early diagnosis and multidisciplinary support from a very young age, GH treatment has resulted in a new generation of children with PWS. Even though there has been a remarkable increase in the knowledge about PWS, answering one question generally inspires deeper and more detailed questions for further research. This thesis is the seventh thesis of our PWS research group and contains six new studies about children and young adults with PWS.

This introduction describes the clinical manifestations in different stages of life, the genetic cause, hypothalamic dysfunction and the current knowledge on the effects of GH treatment in children and young adults with PWS. Furthermore, the objectives of the studies described in this thesis are presented.

1.1 PRADER-WILLI SYNDROME

Prader-Willi syndrome is a rare genetic disorder resulting from the lack of expression of the PWS region (locus q11-q13) on the paternally inherited chromosome 15[1-3]. The incidence of PWS is estimated at 1 in every 12.000-15.000 live births and is considered the most common cause of genetic obesity[4,5]. The PWS phenotype is highly variable and clinical features in people with PWS change during their life, the most obvious being the change in appetite and satiety[2,6,7]. Infants with PWS show feeding difficulties and a lack of appetite, while children from the age of 2 years onwards develop an increased interest in food, gradually shifting to an unlimited appetite. The different stages of appetite and satiety are generally subdivided into nutritional phases (Table 1)[7]. These phases are summarized in the following paragraphs.

Table 1. Nutritional phases

Phases	Median ages	Clinical characteristics
0	Prenatal to birth	Decreased fetal movements and lower birth weight
1a	Birth to 9 months	Hypotonia with difficulty feeding and decreased appetite
1b	9 to 25 months	Improved feeding and appetite, growth appropriate
2a	2.1 to 4.5 years	Weight increasing without increase in appetite or calorie excess
2b	4.5 to 8 years	Increased appetite and caloric intake, can feel full
3	8 years to adulthood	Hyperphagia, rarely feels full
4	Adulthood	Appetite no longer insatiable (described in some adults)

Modified from Am J Med Gen[7]

1.1.1 Prenatal period and delivery

Decreased fetal movements, breech position and assisted delivery

Mothers expecting a child with PWS frequently report reduced fetal activity during pregnancy and the prevalence of polyhydramnios is increased[7,8]. Fetal growth is typically normal until 24 weeks of gestation, while intrauterine growth restriction and asymmetrical intrauterine growth are more common thereafter[9,10]. The fetus with PWS is often in breech position and assisted delivery is more common, which might be related to hypotonia and/or hypothalamic dysfunction of the fetus[8,11]. On average babies with PWS are born at a gestational age of 38 weeks, but both preterm and postterm deliveries are regularly reported[7,12].

1.1.2 Neonatal period

Severe hypotonia and feeding difficulties

Birth weight and length are generally about 15-20% lower in babies with PWS than in their siblings, regardless of gestational age[2,13]. In the newborn period, severe hypotonia with feeding difficulties and failure to thrive are clearly present. The pronounced central hypotonia causes decreased movements, lethargy with decreased arousal, a weak or absent cry and diminished reflexes, including the suckling reflex. Almost every newborn with PWS requires some type of assisted feeding for approximately 3-9 months to prevent failure to thrive (Figure 1).

Figure 1. Young infants with PWS. Photos are depicted with permission from parents.

Despite the low-normal weight, an abnormal body composition with an increased fat mass percentage (FM%) and low lean body mass (LBM) is already present and persists throughout life[14,15]. Typical dysmorphic features, that may be present at birth, but generally become more pronounced during infancy and childhood, include a narrow bifrontal diameter, almond-shaped eyes, a narrow nasal bridge and a thin upper vermillion with down-turned corners of the mouth[3,6]. Finally, hypogonadism with genital hypoplasia and improper thermoregulation and breathing occur more often in PWS[2,16,17].

1.1.3 Infancy

Improved feeding and steady growth along a growth percentile

The feeding difficulties gradually improve during infancy and most infants with PWS grow steadily along a growth percentile with weight increasing at a normal rate[7]. There is a significant mental and motor developmental delay and developmental milestones are typically reached at double the normal age[18-20]. Physical therapy and speech therapy are necessary to improve muscle strength and facilitate achievement of developmental milestones[20].

1.1.4 Toddler

Weight gain without a substantial change in appetite or caloric intake

Usually body weight starts to increase between 18 and 36 months of age without an increase in calorie intake or interest in food[7]. Due to the abnormal body composition, there is an imbalance in energy intake and energy expenditure and children with PWS generally need 60% of the calories that healthy individuals without PWS need[15,21]. To prevent children with PWS from becoming morbidly obese, timely start of a well-balanced low-calorie diet and regular exercise are necessary[20,22].

Figure 2. Young children with PWS. Photos are depicted with permission from parents.

1.1.5 Early childhood

Weight gain with an increased interest in food

In early childhood the interest in food increases and the child will become overweight if there is no parental interference (Figure 2). Most children begin to develop food-related behavior, which includes frequent asking and talking about food and reading cookbooks or watching cooking shows on television. The child is typically very preoccupied with the next meal and reminds others not to forget. If allowed, the child will eat more than he or she is supposed to and becomes obese[7]. In addition to the timely start of a well-balanced low-calorie diet and regular exercise, strict supervision and restriction of access to food are necessary from early childhood onwards[20,22].

Children with PWS grow poorly, resulting in short stature and small hands and feet. However, due to an early diagnosis, multidisciplinary support and GH treatment from a very young age, obesity and short stature are nowadays less common in children with PWS[23]. Most children with PWS have a psychomotor delay and cognitive impairment, resulting in a mild to moderate learning disability. Total IQ scores vary widely, but are around 65 points on average[24-26]. Other common symptoms during early childhood are strabismus[27], scoliosis[28,29], sleep-related breathing disorders[30,31] and stress-related central adrenal insufficiency[32].

Figure 3. Children with PWS. Photos are depicted with permission from parents.

1.1.6 Childhood and puberty

Hyperphagia accompanied by food-seeking behaviour

Childhood and adolescence are characterized by hyperphagia, typically accompanied by food seeking and a lack of satiety (Figure 3). The obsession towards food consumption combined with a reduced metabolic rate and physical activity easily leads to extreme obesity in children with PWS[33]. They are at risk of extreme overeating, which might result in acute life-threatening gastric rupture. Continuous parental guidance and supervision are crucial for children with PWS. Simultaneously with the change in eating behaviour, the majority of children with PWS start developing a variety of challenging behavioural and psychiatric symptoms, including temper tantrums, stubbornness, difficulties with changing routines and manipulative behaviour[34-36]. One third of children with PWS fulfil the criteria for Autism Spectrum Disorders[37,38]. The characteristics of PWS can be difficult to cope with and are likely to cause significant and long-term caregiver burden[39].

Puberty generally starts spontaneously, but the progression of pubertal development is delayed or incomplete. Both primary hypogonadism as well as hypothalamic dysfunction have been reported to cause the abnormal pubertal development[17,40-42]. Sex steroid replacement therapy might be needed to attain complete pubertal development and achieve adequate bone mineral density in adulthood[43].

1.1.7 Adulthood

Diminishing hyperphagia

Occasionally, the insatiable appetite fades and some adults with PWS are able to feel full. The transition from childhood to adulthood is complex and ethical issues regarding autonomy of persons with PWS are challenging. Pervasive food seeking behaviour, insistence on sameness, mood disorders and inactivity make it difficult for adults with PWS to live independently. Most adults require continuous assistance in daily life and many live in group homes. Since a few years, there are group homes that specialize in PWS, providing structure and clarity with regard to food, which significantly improves quality of life.

The adult population of today was diagnosed later in life, when obesity (and its complications) was generally already present. Most adults were not treated with GH during childhood and median adult height in untreated women is 145-150 cm and in men 155-160 cm. Until recently there were no reports about the phenotype of adults with PWS who were diagnosed in the first weeks of life and treated with GH from infancy onwards. It is generally believed that, if severe obesity and its complications can be prevented, adults with PWS may have an improved life expectancy (Figure 4).

Figure 4. Young adults with PWS. Photos are depicted with permission from patients and parents.

1.2 GENETIC CAUSE

1.2.1 History

Prior to the availability of genetic testing, the diagnosis of PWS was based on a combination of clinical signs and symptoms, also known as the Holm's criteria[6]. The first genetically diagnosed patient with PWS was described in 1976 and had a 15/15 Robertsonian translocation[44]. In the 1980s deletions of the long arm of the paternally inherited chromosome 15 were added to the genetic causes of PWS[45,46]. Maternal uniparental disomy (mUPD) of chromosome 15 was reported to lead to PWS a few years later[47]. We now know that PWS is caused by a lack of expression of the paternally inherited genes of the Prader-Willi region, located on chromosome 15q11-13, caused by either a deletion, an mUPD, an imprinting center defect (ICD) or a translocation[3].

1.2.2 Genomic imprinting

Deoxyribonucleic acid (DNA) is a molecule consisting of two chains that coil around each other to form a double helix. The long thread of DNA consists of smaller threads, called chromosomes, which contain many different genes that carry the genetic instructions for the growth, development and functioning of all known

organisms (Figure 5). Humans have 46 chromosomes in the nucleus of each cell in their body, 23 chromosomes inherited from the father and 23 from the mother. These chromosomes form 23 pairs in each cell, labelled 1 to 23.

Genomic imprinting is an epigenetic mechanism through which particular genes are imprinted or silenced during gametogenesis, causing genes to be expressed in a parent-of-origin-specific manner[48]. In healthy subjects, the PWS region on the paternally inherited chromosome 15 is expressed, while this region is silenced by imprinting on the maternally inherited chromosome 15. An abnormal or absent expression of paternally derived genes on the PWS region causes PWS. The PWS region contains numerous imprinted genes of which the exact function remains to be elucidated (Figure 6).

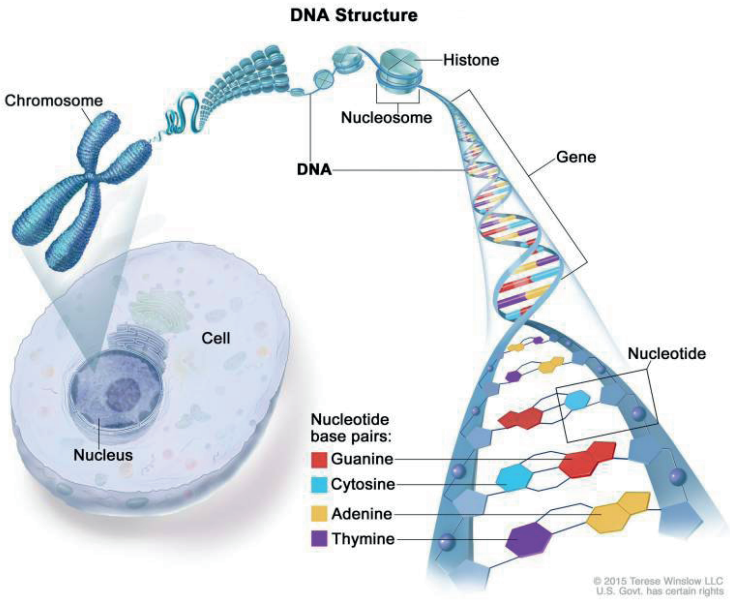

Figure 5. Cell with nucleus, chromosomes and DNA. *Reproduced with permission from Terese Winslow LLC*, *Medical and Scientific Illustration*.

1.2.3 Genetic subtypes

Either a deletion of the paternally derived chromosome 15, or an mUPD, ICD or translocation of chromosome 15 may lead to PWS[3] (Figure 7). The deletion subtype was previously described the most common cause of PWS, causing approximately 70% of PWS cases. Nowadays, the frequency of deletions and mUPD are similar, most probably due to older maternal age at pregnancy and possibly an increase in the use of assisted reproductive technologies[12].

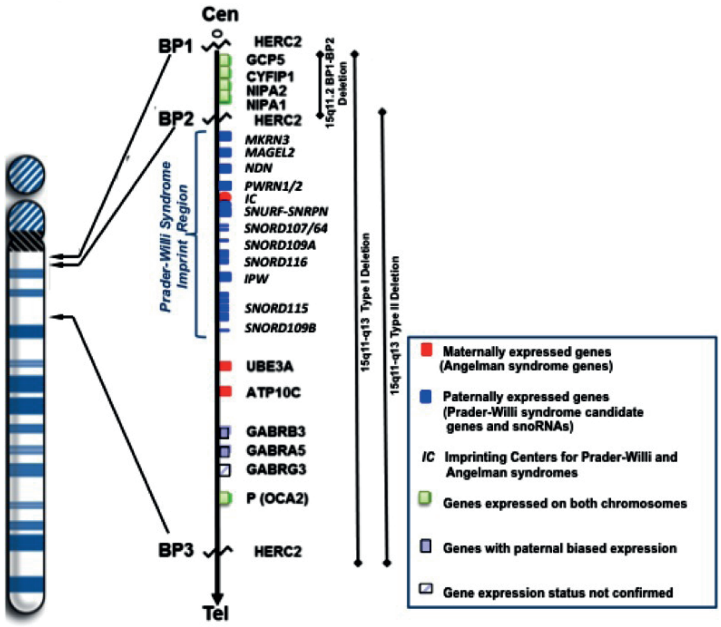

Figure 6. PWS region on chromosome 15, locations of breakpoints BP1, BP2 and BP3 and position of imprinted and non-imprinted genes. Three deletion subtypes and their locations in the 15q11-q13 region are shown. Cen = centromere, Tel = telomere. *Reproduced with permission from Angulo et al, Journal of Endocrinological Investigation, 2015.*

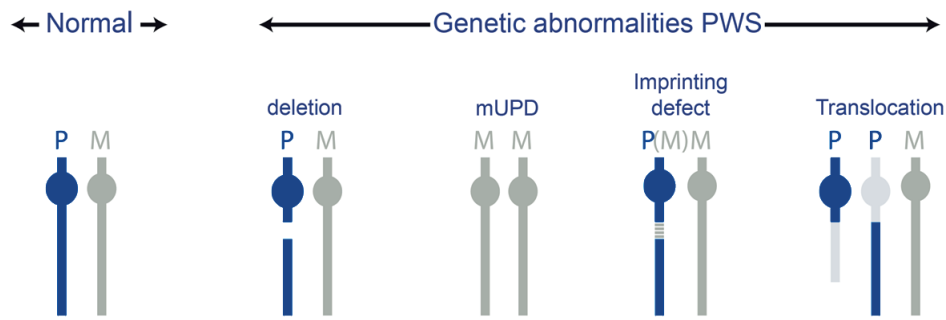

Figure 7. Schematic overview of the genetic abnormalities causing PWS. P=paternally inherited chromosome 15. M=maternally inherited chromosome 15. mUPD=maternal uniparental disomy.

Deletions generally occur de novo and manifest as either a large type I deletion or a smaller type II deletion. The two proximal breakpoints (BP1 and BP2) and the distal breakpoint (BP3) seem to predispose to these typical deletions seen in PWS (Figure 6)[1,3]. In mUPD, both chromosomes 15 are inherited from the mother due to gamete completion 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[49].

Phenotypic differences between the deletion and mUPD subtypes have been reported. People with the deletion subtype of PWS tend to have more typical PWS facial features and hypopigmentation, while people with mUPD are reported to have a higher verbal IQ and an increased risk of developing psychiatric problems, including psychosis and autism spectrum disorders[1,50-52]. The clinical phenotype of people with PWS is, however, highly variable, and there is no clear association between genotype and phenotype.

Imprinting center defects explain less than 5% of all PWS cases[53]. A mutation in the imprinting control region of chromosome 15 results in a maternal imprint of the paternally derived chromosome, leading to a complete loss of the paternal expression of the genes of the PWS region. Less than 1% of the patients with PWS have an unbalanced Robertsonian translocation, where part of the paternally inherited chromosome 15 is transferred to another chromosome, leading to chromosomal deletions or additions[3,54]. The recurrence risk is typically very low in the case of a deletion or an mUPD, but if the father carries a translocation or if PWS is caused by an imprinting center mutation, the recurrence risk is considerably higher, i.e. up to 50%[3].

1.3 HYPOTHALAMIC DYSFUNCTION

The hypothalamus is a coordinating center in the brain consisting of several small nuclei with a variety of functions. Its most important function is to link the nervous system to the endocrine system via the pituitary gland by synthesizing and secreting neurohormones that stimulate the pituitary, e.g. growth hormone-releasing hormone (GHRH), gonadotropin-releasing hormone (GnRH), corticotropin-releasing hormone (CRH) and thyrotropin-releasing hormone (TRH). These neurohormones are released into the blood stream and stimulate the anterior pituitary to secrete GH, gonadotropins (LH/FSH), prolactin (PRL), corticotropin (ACTH) and thyrotropin (TSH), respectively. Besides, the hypothalamus is involved in circadian rhythm, sleep, hunger, and body temperature regulation.

Hypothalamic dysfunction can explain several symptoms of PWS, including excessive daytime sleepiness, sleep-related breathing disorders, insatiable hunger, temper tantrums, abnormal temperature control and hormonal dysregulation, such as hypogonadotropic hypogonadism, functional growth hormone deficiency and stress-related central adrenal insufficiency[11].

1.4 GROWTH HORMONE TREATMENT

At birth, children with PWS show a low median birth weight standard deviation score (SDS) of -1.4 and a low-normal median birth length of -0.50 SDS[2,13]. If not treated with GH, most children with PWS have a decreased growth velocity and a reduced pubertal growth spurt, resulting in a mean adult height in women of 145-150 cm and

155-160 cm in men[13,55]. These and other features, like small hands and feet, increased fat mass (FM) and reduced LBM, support the presence of GH deficiency[56]. However, most children do not fulfil the criteria for GH deficiency when they are tested for GH deficiency[57]. Functional GH deficiency due to hypothalamic dysfunction might underlie the clinical features of GH deficiency in children with PWS.

The first studies investigating GH treatment in children with PWS were published in the 90s[58-60] and GH treatment was approved for children with PWS by the Food and Drug Administration and by the European Medicines Agency soon thereafter. In 2002, our research group started investigating the effects and safety of GH treatment in the Dutch national GH trial. After a randomized controlled trial, lasting 1 year for infants and 2 years for prepubertal children, all children were followed during continuous GH treatment in the Dutch PWS Cohort study until attainment of adult height (see Appendix for study designs).

Today we know that long-term GH treatment is effective and safe in children with PWS[23,61-63], improving body composition, bone mineral density, cognition, adaptive functioning, linear growth and adult height[23,24,43,64]. Combined with an early diagnosis and multidisciplinary support from a very young age, GH treatment counteracts the clinical course of increasing obesity in children with PWS and has substantially changed their phenotype. Currently, most Dutch infants with PWS start GH treatment soon after diagnosis before the age of 6 months and are followed until attainment of adult height in the Dutch PWS Cohort study.

1.5 TOPICS OF THIS THESIS

1.5.1 Psychomotor development during infancy

During the first years of life, mental and motor development are delayed and children with PWS reach developmental milestones on average at double the normal age. Brain development during infancy and early childhood is very important for cognitive functioning on the long term[65]. We have shown that children with PWS have a trend towards smaller white matter volume, indicating reduced structural connectivity or aberrant myelinisation. This may reflect a delay in brain maturation and underlie cognitive deficits in children with PWS[66].

GH receptors are expressed throughout the brain and GH and IGF-I are expected to be involved in brain growth, development and myelinisation[67,68]. Short-term studies suggest a positive effect of GH on mental and motor development in infants and children with PWS[19,69,70]. We previously found a significant improvement in mental and motor development in infants with PWS after 1 year of GH treatment compared to randomized untreated controls[19]. There are currently no studies on the longer-term effects of GH on mental and motor development in infants with PWS. In the second chapter of this thesis we therefore investigated the effects of 3 years of GH on

psychomotor development in 63 children with PWS who started GH treatment at a very young age. We hypothesized that psychomotor development would not only improve during the first year of GH, but would continue to improve during the second and third year, thereby reducing the disparity compared with non-PWS peers.

1.5.2 Cognitive development during childhood

People with PWS typically have mild to moderate cognitive impairment with an average IQ between 60 and 70[24,71]. The physical benefits of GH treatment have been thoroughly investigated and include, among others, an improvement in body composition, linear growth and physical strength[23]. GH treatment is also associated with cognitive benefits, which are attributed to the effects of GH and insulin-like growth factor (IGF)-I on brain growth, development and myelinisation[67,72]. A recent review about the effects of GH treatment on cognition concluded that GH could stimulate GH receptors in brain areas involved in learning and memory, thereby improving cognitive functioning[68].

We previously demonstrated, during a 2-year randomized controlled trial, that cognitive functioning in GH-treated children with PWS developed at the same pace as cognitive functioning in healthy references, while in untreated controls, there was a significant deterioration in abstract verbal reasoning and vocabulary[24]. Furthermore, in 50 children with PWS, we found a significant improvement in abstract verbal reasoning and visuospatial skills during 4 years of GH treatment, indicating that 4 years of GH had reduced the gap between children with PWS and healthy controls with regard to these skills[24]. There are currently no studies investigating the effects of more than 4 years of GH in children with PWS. In the third chapter of this thesis we therefore investigated cognitive functioning during 8 years of GH treatment in 43 children with PWS. We also investigated whether starting GH treatment during infancy, i.e. before 2 years of age, would result in improved cognitive functioning after 8 years of GH. We hypothesized that cognitive functioning would not deteriorate during long-term GH treatment and would progress at the same rate as healthy references. Additionally, we hypothesized that starting GH during infancy would benefit cognitive functioning on the long term.

1.5.3 Growth hormone deficiency in adulthood

Some features of people with PWS resemble those seen in growth hormone deficiency (GHD), such as short stature and an abnormal body composition with a low LBM and an increased FM. The benefits of GH treatment in children with PWS are well established as it improves body composition, bone mineral density (BMD), adaptive functioning and linear growth[23,43,64]. Furthermore, we and others have shown that GH treatment is also beneficial for adults with PWS, with a sustained improvement in FM and LBM when GH is continued after attainment of adult height, and a deterioration of body composition when GH treatment is discontinued[73,74].

The fact that both children and adults with PWS respond very well to GH treatment, with a significant improvement in body composition, health profile, normalization of stature in children, and a significant increase in serum IGF-I and IGFBP-3 levels strongly supports the likelihood of GHD in these patients. However, GH treatment for adults with PWS is currently not reimbursed if they do not fulfil the consensus criteria of adult GHD. The reported prevalence of GHD in PWS is variable depending on the diagnostic test and the chosen cut-off points. In the fourth study of this thesis we investigated the prevalence of adult GHD using GHRH-Arginine tests in 60 young adults with PWS who had attained adult height after GH treatment during childhood, to have an opportunity to treat them.

1.5.4 Bone mineral density in young adulthood

BMD is influenced by endocrine factors such as GH, insulin-like growth factor and sex steroids and by body composition and body mass index (BMI)[75,76]. Both sex steroids and GH are known to play an important role in the accrual of peak bone mass[77]. Recent studies have shown that long-term GH treatment optimizes BMD in prepubertal children with PWS[43,78], leading to a normal BMD compared to healthy peers[43,79]. During puberty, however, we found a decline in BMD in parallel to incomplete pubertal development and low sex hormone levels[41-43]. This could explain the reported increased prevalence of osteopenia and osteoporosis resulting in a high fracture risk in adults with PWS[80-82].

When young adults with PWS without adult GHD have attained adult height, they have to stop GH treatment. In GH-deficient young adults without PWS, BMD deteriorates after cessation of GH[83-85]. How BMD develops after cessation of GH was, however, not known in young adults with PWS. In chapter 5 of this thesis we therefore investigated the effects of GH versus placebo on BMD in young adults with PWS who had attained adult height and were treated with GH during childhood in a two-year, randomized, double-blind, placebo-controlled cross-over GH study. Secondly, we investigated the effects of no sex steroid replacement therapy (SSRT) versus SSRT on BMD in hypogonadal young adults with PWS.

1.5.5 Sleep-related breathing disorders in young adulthood

Sleep-related breathing disorders (SRBD) are common in patients with PWS, causing poor sleep quality and excessive daytime sleepiness[86-88]. There have been some reports on SRBD in PWS discussing the safety of GH treatment in children with PWS. We, however, described a non-significant decline in apnea hypopnea index (AHI) after 6 months of GH treatment in 35 prepubertal children with PWS and a recent review concluded that GH can be safely administered, provided that SRBD is monitored and treated appropriately[30,89].

When young adults with PWS have attained adult height, they have to stop GH treatment, unless they have adult GHD. We have previously shown that young adults with PWS benefit from continuation of GH by maintaining the improved body composition obtained during childhood, without safety concerns regarding their metabolic health profile[73,90]. There were, however, no studies about the effects of GH on SRBD in young adults with PWS who were treated with GH during childhood. We therefore investigated the effects of one year of GH versus one year of placebo on SRBD in young adults with PWS who had attained adult height and were treated with GH during childhood in a two-year, randomized, double-blind, placebo-controlled cross-over GH study. We hypothesized that GH would not negatively influence SRBD. Secondly, we investigated the prevalence of obstructive sleep apnea (OSA) in our cohort of young adults with PWS.

1.5.6 Leukocyte telomere length in young adulthood

Studies in adults with PWS who were not treated with GH describe an increased risk of developing age-associated diseases at a relatively young adult age, e.g. diabetes mellitus type 2 (T2DM) and cardiovascular disease (CVD)[81,91]. The mortality rate of people with PWS was estimated to be 3% per year across all ages, rising to 7% in those aged over 30[5]. As described in other syndromes, the ageing process might be accelerated in PWS[92-95], which may partly explain the increased mortality rate and risk of T2DM and CVD. Ageing is characterized by a progressive time-dependent decline of normal tissue and organ function and recent studies have shown that telomere shortening is involved[96,97]. Telomere length shortens during proliferation and declines as a function of chronological age. When telomere length becomes critically short, the cell enters either senescence or apoptosis. The accumulation of senescent cells might drive the process of organismal ageing[98].

Chapter 7 of this thesis investigated leukocyte telomere length (LTL) in young adults with PWS and compared LTL to healthy young adults of similar age. As all young adults who participated in this study were treated with GH, we also investigated LTL in young adults born short for gestational age who were also treated with GH. We hypothesized that LTL would be shorter in young adults with PWS compared to both groups, independent of GH treatment, and that accelerated biological ageing could partly explain the increased mortality rate and risk of T2DM and CVD in adults with PWS.

1.6 AIMS AND OUTLINE OF THIS THESIS

Chapter 1 gives an introduction on the topics described in this thesis.

Chapter 2 describes the effects of 3 years of GH on mental and motor development in 63 infants and toddlers with PWS.

Chapter 3 provides the results of our prospective study about cognitive functioning during 8 years of GH treatment in 43 children with PWS.

Chapter 4 presents the prevalence of adult growth hormone deficiency in 60 young adults with PWS who were treated with GH during childhood.

Chapter 5 describes the effects of 1 year of GH versus 1 year of placebo on bone mineral density in 27 young adults with PWS who were treated with GH during childhood.

Chapter 6 describes sleep-related breathing disorders in 27 young adults with PWS during 1 year of GH and 1 year of placebo.

Chapter 7 presents leukocyte telomere length of 47 young adults with PWS.

Chapter 8 discusses the results and conclusions of this thesis taking recent literature and clinical implications of our findings into account.

Chapter 9 contains an English and Dutch summary of the results described in this thesis.

Chapter 10 lists an overview of publications by the PWS-team of the Dutch Growth Research Foundation.

Chapter 11 contains a list of abbreviations, a list of publications, and a list of co-authors affiliations. It further contains the PhD portfolio, acknowledgements, and curriculum vitae.

APPENDIX: DUTCH PRADER-WILLI SYNDROME STUDIES

Dutch Growth Research Foundation

Since 2002, Dutch PWS studies are performed and coordinated by the Dutch Growth Research Foundation (Stichting Kind en Groei). The PWS team consists of MD-researchers, research nurses, a dietician, physical therapists and a psychologist. The Dutch randomized controlled GH trial (Dutch GH RCT) and follow-up study (Dutch PWS Cohort study) are multicenter studies investigating the effects and safety of long-term GH treatment in children with PWS. Every three months, MD-researchers and research nurses visit the 14 participating centers throughout the Netherlands and examine children and young adults with PWS who are treated with GH, in collaboration with local pediatricians and pediatric endocrinologists (Figure 8). At start of GH treatment, after 6 months, 12 months and yearly thereafter, various measurements are performed at the Erasmus University Medical Center – Sophia Children's Hospital in Rotterdam.

The PWS Transition study and the Young Adult PWS (YAP) study are single center studies investigating the effects and safety of GH in young adults with PWS. Young adults with PWS visit the Erasmus University Medical Center – Sophia Children's Hospital once or twice a year for various measurements.

Figure 8. Participating centers Dutch GH trial and PWS Cohort Study.

PWS Reference center

Since 2015, there is one Dutch Prader-Willi Reference Center accredited by the Dutch Ministry of Health in 2 locations:

Reference Center for children and (young) adults, Rotterdam

Children and young adults
S.H. Donze, L. Damen, L. Grootjen, A. Juriaans, E.F. Mahabier, P.M.M.C. van Eekelen, E. Piso, G.C.B. Bindels-de Heus, A.C.S. Hokken-Koelega

Previously: R.J. Kuppens, N.E. Bakker, S.T. Lo, E.P.C. Siemensma, R.F.A. Tummers-de Lind van Wijgaarden, D.A.M. Festen, A. Lukoshe, L. Schafthuizen, B. Kerkhof

Dutch Growth Research Foundation and Erasmus University Medical Center, Sophia Children's Hospital

Adults
L.C.G. de Graaff-Herder, K. Davidse

Erasmus University Medical Center Rotterdam

Reference Center for children, Nijmegen

A.A.E.M. van der Velden, J. Geelen

Radboud University Medical Center, Amalia Children's Hospital

Participating centers and pediatricians

Amsterdam	J. Rotteveel, M.J.J. Finken, VU University Medical Center
Amsterdam	N. Zwaveling-Soonawala, Academic Medical Center
Apeldoorn	L. Lunshof, Gelre Hospitals
Den Bosch	P.E. Jira, E.G.A.H. van Mil, Jeroen Bosch Medical Center
Den Haag	E.C.A.M. Houdijk, Haga Hospital
Eindhoven	R.J.H. Odink, V. van Tellingen, St. Catharina Hospital
Enschede	M.E.J. Wegdam-den Boer, Medical Center Twente
Groningen	G. Bocca, University Medical Center Groningen
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, C. de Bruijn, Leiden University Medical Center
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)

Dutch PWS Studies in children

Patients
Until July 2019, 220 Dutch children with PWS were included in the randomized controlled GH study and/or the PWS cohort study (Figure 9). For both studies, children had to meet the following criteria:

Inclusion criteria
- Genetically confirmed diagnosis of PWS;
- Under 16 years of age;
- 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 or 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
From 2002 to 2008, 61 infants were included in the randomized controlled GH study: 47 prepubertal children and 7 pubertal children. Infants and prepubertal children were randomized into either a GH-treated group or a control group for respectively 1 or 2 years. Subsequently all were treated with GH 1 mg/m^2 per day. 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 attainment of adult height. All were prospectively followed in the Dutch PWS cohort study in collaboration with pediatricians and pediatric endocrinologists throughout The Netherlands.

Since 2009, infants are directly included in the PWS cohort study for follow-up during long term GH treatment until attainment of adult height. All children are treated with GH 1 mg/m^2 per day and are prospectively followed in collaboration with pediatricians and pediatric endocrinologists throughout The Netherlands.

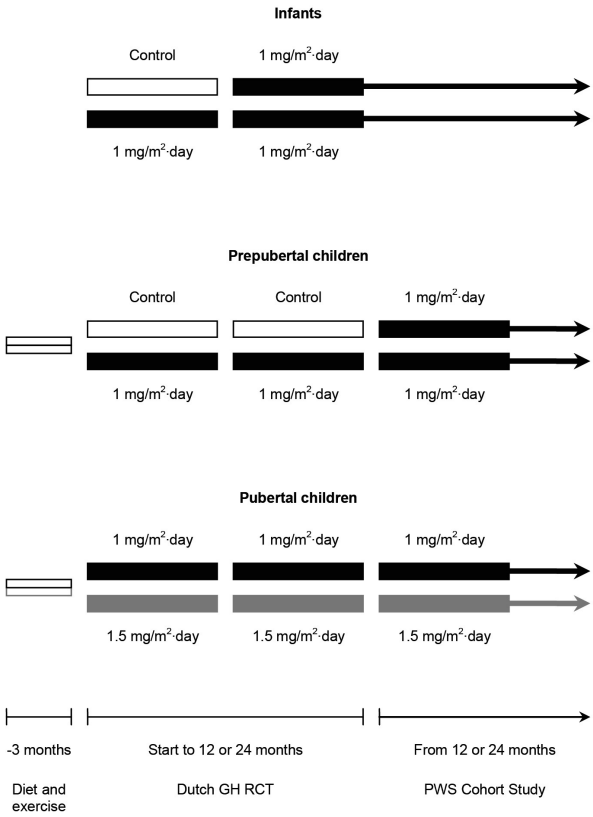

Figure 9. Design of the multicenter randomized controlled GH study (Dutch GH RCT) and the PWS cohort study. GH dose 1.0 mg/m^2·day≈0.035 mg/kg/day, GH dose 1.5 mg/m^2·day≈0.052 mg/kg/day.

PWS Transition Study

Patients
Until January 2014, 28 Dutch young adults with PWS who were treated with GH during childhood and had attained adult height, were included in the PWS Transition study (Figure 10).

Inclusion criteria
- Genetically confirmed diagnosis of PWS;
- GH treatment during childhood for at least 2 years and being on GH at time of inclusion;
- Adult height attainment, defined as a height velocity less than 0.5 cm per 6 months and complete epiphyseal fusion.

Exclusion criteria
- Non-cooperative behavior;
- Medication to reduce weight or fat.

Design
Two-year, randomized, double-blind, placebo-controlled, cross-over study investigating the effects of 1 year placebo versus 1 year GH on body composition, metabolic health profile, cognition, bone mineral density and sleep-related breathing disorders. Young adults were stratified according to gender and BMI (below or above 25 kg/m^2) and then randomly and blindly assigned to receive 1 year of subcutaneous injections once daily at bedtime of either 0.67 mg/m^2/day GH or 1 year of identical appearing placebo, after which they crossed-over to the alternative treatment for another year. Investigators were blinded for the allocation.

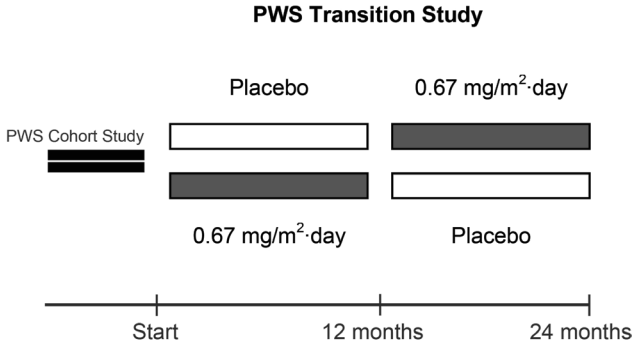

Figure 10. Design of the 2-year randomized, double-blinded, placebo-controlled cross-over GH trial. GH dose 0.67 mg/m^2·day≈0.023 mg/kg·day.

PWS Young Adult (YAP) Study

Patients
Until July 2019, 68 Dutch young adults with PWS who had attained adult height, were included in the PWS Young Adult study (Figure 11).

Inclusion criteria
- Genetically confirmed diagnosis of PWS;
- Age < 35 years
- Adult height attainment, defined as a height velocity less than 0.5 cm per 6 months and complete epiphyseal fusion.
- BMI <38 kg/m^2. Patients with a BMI between 30-38 kg/m^2 have to show that they can comply to a diet and physical exercise program during 6 months resulting in weight loss.

Exclusion criteria
- Non-cooperative behavior;
- Medication to reduce weight or fat;
- Severe sleep related breathing disorders;
- BMI >38 kg/m^2.

Design
Prospective study in young adults with PWS investigating the efficacy and safety of GH treatment after attainment of adult height. Patients who received GH treatment during childhood will restart GH in a dose of 0.33 mg/m^2 per day. Patients who were not treated with GH during childhood will start GH in a dose of 0.15 mg/m^2 per day and after 4 weeks the dose will be increased to 0.33 mg/m^2 per day. Prior to (re)start of GH several medical tests will be performed.

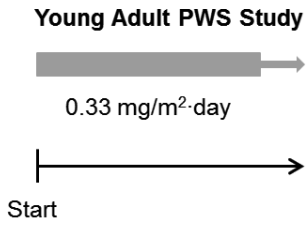

Figure 11. Design of the prospective study in young adults with PWS. GH dose 0.33 mg/m^2·day≈0.012 mg/kg·day.

Please note that the current thesis does not include results about the efficacy and safety of GH treatment in young adults participating in the YAP study. However, all young adults with PWS participating in the YAP study were subjected to a GHRH-Arginine test after attainment of adult height to evaluate adult growth hormone deficiency (results in chapter 4) and 47 of 68 young adults participated in the study investigating leukocyte telomere length (results in chapter 7).

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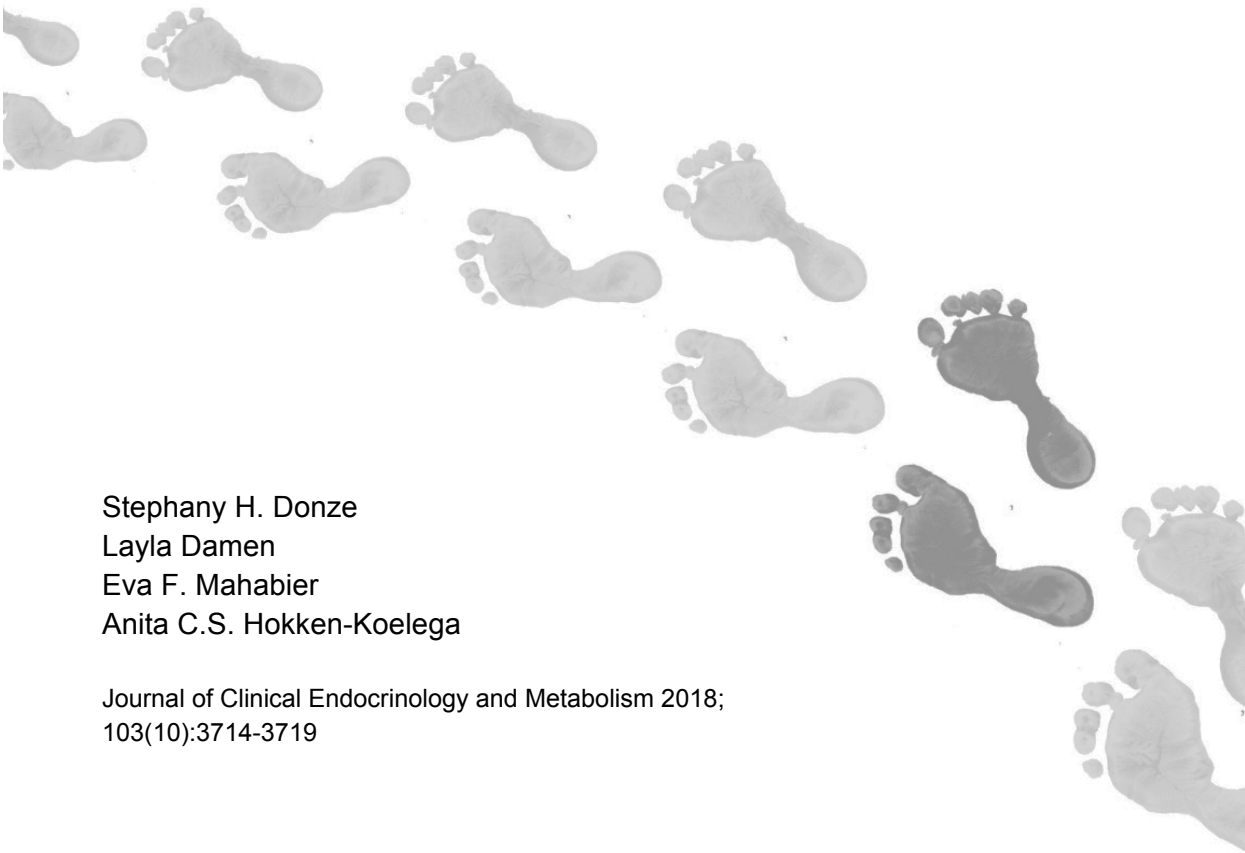
Chapter 2

Improved mental and motor development during 3 years of growth hormone treatment in very young children with Prader-Willi syndrome



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ABSTRACT

Context Infants and toddlers with Prader-Willi Syndrome (PWS) have mental and motor developmental delay. Short-term data suggest a positive effect of growth hormone (GH) on mental and motor development in infants and children with PWS. There are, however, no longer-term results about the effects of GH treatment on mental and motor development.

Objective To investigate the longer-term effects of GH on psychomotor development in infants and toddlers with PWS and the effect of age at start of GH treatment on psychomotor development.

Design Prospective cohort study during 3 years of GH treatment

Intervention All children were treated with growth hormone 1 mg/m^2/day (≈0.035 mg/kg/day).

Main outcome measures Mental and motor developmental age assessed with Bayleys Scales of Infant Development II (BSID-II) and expressed as % of the expected development (100%).

Results During 3 years of GH, mean (SEM) mental development increased from 58.1% (2.8) at baseline to 79.6% (3.7), and motor development from 41.9% (2.9) to 78.2% (3.9; both $p<0.01$). A lower baseline psychomotor development and a younger age at start of GH treatment were associated with a higher increase in mental and motor development ($p<0.01$).

Conclusions Mental and motor development increased significantly during 3 years of GH treatment, reducing the gap between infants with PWS and healthy peers. The younger at start of GH treatment, the greater the improvement in psychomotor development.

INTRODUCTION

Prader-Willi syndrome (PWS) is a neurodevelopmental disorder resulting from the absence of locus q11-q13 (PWS region) on the paternally derived chromosome 15, caused by a paternal deletion, maternal uniparental disomy (mUPD), imprinting center defect or paternal chromosomal translocation. Early features of the syndrome include muscular hypotonia, feeding difficulties with poor weight gain and mental and motor developmental delay. When children get older, they develop short stature, hyperphagia with an increased risk of obesity, neurobehavioral problems and a mild to moderate cognitive impairment with an average IQ between 60 and 70[1-4].

Long-term continuous growth hormone (GH) treatment in children with PWS improves body composition, linear growth, physical strength and adaptive functioning[5,6]. Short-term data suggested a positive effect of GH on mental and motor development in infants and children with PWS[7-10]. In 29 infants with PWS, aged 6 months to 3 years, we found a significant improvement in mental and motor development after 1 year of GH treatment compared to randomized controls. Both mental and motor development declined in infants that were not treated with GH[7]. To our knowledge, there are currently no reports on the longer-term effects of GH on mental and motor development in infants with PWS. In this prospective cohort study, we therefore investigated the effects of 3 years of GH treatment on psychomotor development in a large group of infants with PWS who started GH treatment at a very young age. We hypothesized that psychomotor development would not only improve during the first year of GH treatment but would continue to improve during the second and third year, thereby reducing the disparity between children with PWS and non-PWS peers. As there could be an age dependent effect of GH on psychomotor development, we also investigated if starting GH at a younger age would correlate with greater mental and motor development during 3 years of GH. We hypothesized that starting GH treatment at a younger age would result in a greater improvement in psychomotor development.

METHODS

Patients

The study group consisted of 63 infants and toddlers with PWS. All fulfilled the following inclusion criteria; 1) genetically confirmed diagnosis of PWS, 2) naïve to GH treatment at time of enrolment, 3) uncomplicated pregnancy and delivery, 4) maximum age of 3 years at first evaluation, 5) at least 2 Bayley Scales of Infant Development II (BSID-II) tests during 3 years of GH treatment. Six of 63 infants participated in our previous study investigating psychomotor development in PWS infants and toddlers during 1 year of GH treatment compared to randomized controls[7]. The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center (Rotterdam, The Netherlands) and by the collaborating centers. Written informed consent was obtained from the parents.

Design
All participants were followed at the Dutch PWS Reference Center. To investigate the effect of GH on mental and motor development, BSID-II scores were analysed from the start of GH treatment. Children were treated with Genotropin (Pfizer Inc., New York, NY) administered subcutaneously once daily at bedtime in a dose of 1 mg/m^2/day (≈0.035 mg/kg/day) for at least 3 consecutive years. The GH dose was regularly adjusted based on calculated body surface area and serum insulin-like growth factor (IGF)-I levels. IGF-I levels were expressed as SDS, adjusting for age and gender[11]. GH dose was lowered if serum IGF-I was > 3 SDS.

Anthropometry
All children were examined every 3 months. Standing height was measured using a Harpenden Stadiometer and supine height with a Harpenden Infantometer (Holtain Ltd., Crosswell, UK). Weight was measured on a calibrated electric scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, The Netherlands) and head circumference with a measuring tape. Height, weight, body mass index (BMI) and head circumference were expressed as standard deviation scores (SDS) using Growth Analyser 4.0 (available at www.growthanalyser.org), adjusting for gender and age according to Dutch reference values[12,13].

Psychomotor development
Mental and motor development were measured annually using BSID-II. BSID-II is an individually administered and standardized instrument for developmental diagnostics that can be used to evaluate the cognitive and motor development of young children with a 'developmental age' between 0 and 3.5 years. BSID-II yields two scores: mental developmental age (in months) and motor developmental age (in months). The mental scale consists of items in relation to visual and auditory information processing, language development, memory, eye–hand coordination, imitation and problem solving. The motor scale assesses fine and gross motor skills. All tests were performed by one experienced psychologist (E.M.) in our PWS Reference Center (Erasmus University Medical Center, Rotterdam, the Netherlands), and the results were compared with reference data derived from healthy infants and toddlers of comparable age[14,15]. Mental and motor development were expressed as percentage of the expected mental and motor development for their age (%ed) and calculated as follows: (developmental age / chronological age) * 100.

Body composition
Body composition was annually assessed, using dual-energy x-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare, Chalfont St giles, UK). Total fat mass (FM; kg) and lean body mass (LBM; kg) were measured. LBM was calculated as fat-free mass (FFM) minus bone-mineral content. All scans were made on the same machine, and quality assurance was performed daily. The intra-assay coefficient of variation (CV) for fat tissue was 0.41% to 0.88%; for LBM 1.57% to 4.49%[16]. As reference values

for DXA in this age group are not yet available, FM and LBM were expressed as percentage of total body weight (FM% and LBM%).

Statistical analysis

Statistical analyses were performed with SPSS 24.0 (SPSS Inc., Chicago, IL). Data were expressed as mean (standard deviation, SD). In case of a non-Gaussian distribution data were expressed as median (interquartile range, IQR). Test results of psychomotor development were expressed as developmental age divided by chronological age and multiplied by 100, reflecting the percentage of the expected development (%ed) for that age. To compare psychomotor development with normal development (100%ed), a one-sided t-test was used. Gender and genotypic differences in baseline psychomotor development were calculated by independent samples T-tests. Correlations between baseline psychomotor development and age, head circumference and LBM% were calculated by Pearson's correlation analysis in case of a Gaussian distribution and by Spearman's correlation analysis in case of a non-Gaussian distribution.

Mental and motor development during 3 years of GH were analysed using linear mixed model analysis with years of GH-use (0 = baseline, 1 = 1 year of GH treatment, 2 = 2 years of GH treatment and 3 = 3 years of GH treatment) as a categorical independent variable and a first-order autoregressive (AR(1)) covariance matrix. The effects of genotype, age at start of GH and the change in head circumference and body composition on psychomotor development during GH treatment was determined by using these variables as factors (in case of nominal or ordinal variables) or covariates (in case of scale variables) in the model. Results were adjusted for age and baseline psychomotor development. The effect of age at start of GH on psychomotor development was only corrected for baseline psychomotor development. P-values less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Sixty-three infants and toddlers with PWS (35 boys, 28 girls) participated in the current evaluation of psychomotor development during 3 years of GH treatment. Baseline clinical characteristics are shown in Table 1. Thirty-one children had a deletion (49.2%), 31 children a maternal uniparental disomy (mUPD; 49.2%) and 1 a paternal translocation (1.6%). Birth weight SDS, height SDS, weight for age and weight for height SDS and head circumference for age SDS were all significantly below 0 SDS. GH treatment was started at a median (IQR) age of 1.0 year (0.7-1.6).

Table 1. Baseline characteristics in 63 infants and toddlers with PWS

Boys / girls (n)	35 / 28
Genetic subtype: Deletion / mUPD / translocation	31 / 31 / 1
Gestational age (weeks)	39.8 (38.0 to 41.2)
Birth weight (SDS)	-1.1* (-1.8 to -0.6)
Age at start GH treatment (yrs)	1.0 (0.7 ; 1.6)
Height for age (SDS)	-1.7* (-2.6 to -1.0)
Weight for age (SDS)	-1.8* (-2.5 to -0.7)
Weight for height (SDS)	-0.6* (-1.4 ; 0.5)
FM%	28.6 (26.2 to 34.6)
Head circumference for age (SDS)	-1.3* (-1.7 to -0.5)

*Data expressed as median with (IQR). *P < 0.01, compared to 0 SDS.*

GH: growth hormone. FM%: fat mass percentage.

Baseline percentage of expected mental and motor development (%) are shown in Figure 1. Mean (SEM) baseline mental and motor development were 58.1% (16.1) and 41.9% (13.9), respectively, both being significantly lower than in healthy references (p<0.001).

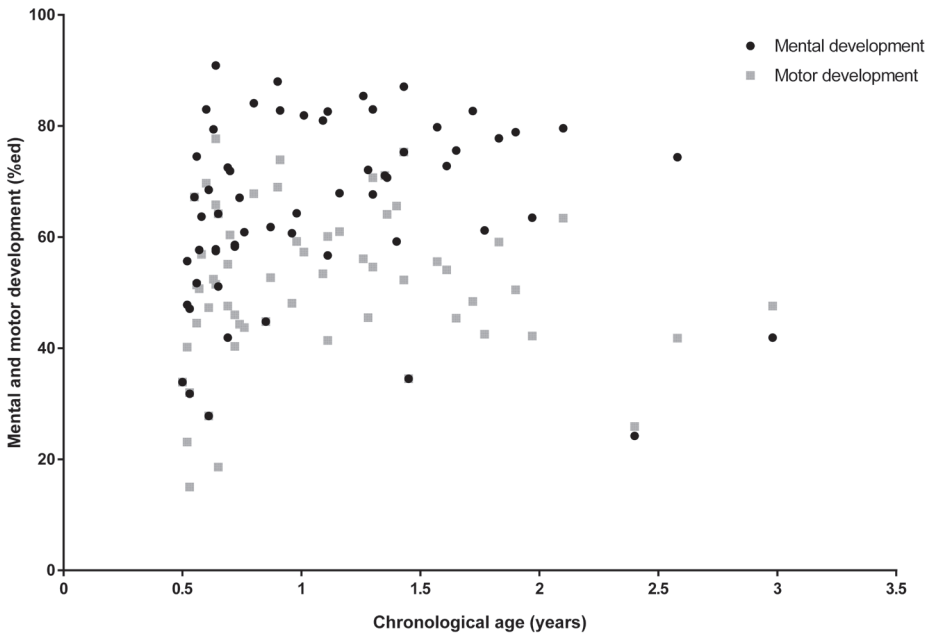

Figure 1. Mental and motor development before start of GH in infants and toddlers with PWS. Black dots represent mental development, grey squares represent motor development.

There was a large variation in psychomotor development with mental development ranging from 24 to 91% of healthy peers and motor development from 15 to 78%. Baseline motor development was significantly lower than mental development ($p<0.001$). Mental and motor development were positively correlated ($r = 0.69$, $p<0.001$), meaning that infants with a higher baseline motor developmental score had a higher baseline mental developmental score and vice versa.

Baseline psychomotor development was not different between boys and girls or between infants with a deletion or an mUPD (all $p>0.07$). We also found no significant association between head circumference and baseline mental and motor development ($p=0.40$ and $p=0.08$, resp.). A younger age was significantly associated with lower baseline mental development, but not motor development ($p=0.01$ and $p=0.29$, resp.). Baseline height SDS was weakly associated with baseline motor development ($r=0.26$, $p=0.047$), but baseline LBM% was not significantly associated with baseline motor development ($p=0.47$).

Psychomotor development during 3 years of GH treatment

During the first year of GH, mean (SEM) mental development increased from 58.1% (2.8) to 65.7% (1.6) and motor development from 41.9% (2.9) to 54.8% (1.7) (both $p<0.01$). After the first year of GH, mean (SEM) mental and motor development increased further to respectively 79.6 (3.7) and 78.2% (3.9) after 3 years of GH (both $p<0.01$; Figure 2), thus further reducing the disparity between infants with PWS and healthy peers of the same age. In spite of this improvement, the average mental and motor development after 3 years of GH were still significantly lower compared to healthy references (both $p<0.001$). The change in mental developmental during 3 years of GH was significantly associated with the change in motor development ($r=0.71$, $p<0.001$).

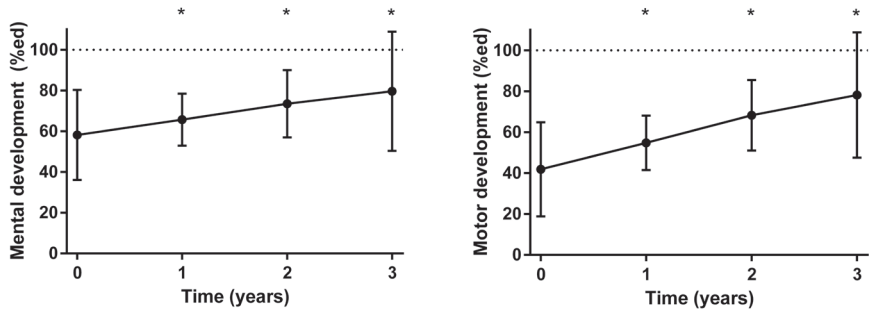

Figure 2. Mental and motor development during 3 years of GH in infants and toddlers with PWS presented as means with 95% CI. Significant changes compared to baseline are indicated with an asterisk. The dashed lines represent the maximum score of 100%.

The course of mental and motor development during 3 years of GH was not significantly different between boys and girls and between infants with a deletion or an mUPD (all p>0.17). A younger age at start of GH treatment was significantly associated with a better improvement in mental development (β=-5.5, SE=1.9, p<0.01) and motor development (β=-7.8, SE=2.1, p<0.001). Head circumference increased significantly from -1.0 SDS at baseline to 0.1 SDS after 3 years (p<0.01), but this change in head circumference was not significantly associated with the course of mental and motor development (p=0.77 and p=0.88, resp.). Neither the change in height SDS nor the change in LBM% significantly influenced motor development during 3 years of GH (p=0.33 and p=0.99, resp.).

DISCUSSION

In this study, we assessed psychomotor development during 3 years of GH in a large group of 63 infants and toddlers with PWS with a median age of 1 year at start of GH treatment. PWS infants and toddlers demonstrated delayed mental and motor development compared to healthy references, but, as psychomotor development in children with PWS significantly increased during 3 years of GH treatment, the disparity between children with PWS and healthy references decreased. Furthermore, the younger the age at start of GH treatment, the greater the increase in psychomotor development.

Mental and motor development did not merely increase during the first year, but also in the second and third year of GH treatment. Hence, psychomotor development kept on improving, raising the mental and motor potential of infants with PWS. We previously found a significant improvement in mental and motor development after 1 year of GH treatment compared to randomized, untreated controls in 29 infants with PWS with a median (IQR) age of 2.0 (1.3 – 3.0) years[7]. In a subsequent study in 20 infants, the combination of GH and physical training resulted in a clinically relevant improvement in motor development[10]. Myers et al. reported a trend towards improved mobility and stability in infants and toddlers with PWS after 1 year of GH (n=14) compared to randomized controls (n=11), and more progression in language and cognitive development (n=7 vs. n=5). The authors noted that it was difficult to quantify differences between groups, because of small groups and large within group variance[8].

Our data show a significant improvement in mental and motor development during 3 years of GH in very young children with PWS. However, we cannot exclude that part of this increase may be explained by spontaneous improvement of hypotonia with age and early start of physical therapy, as has been suggested based on a study comparing the effect of 1 year of GH or Co-enzyme Q10 (CoQ10) in 26 infants and toddlers with PWS[17]. Because all children with PWS in the Netherlands are treated with GH from a young age, we could not compare psychomotor development of GH-treated infants to that of infants without GH treatment.

We acknowledge that an RCT would be the first-choice design to investigate the longer-term effects of GH on mental and motor development in infants with PWS, but it would be unethical to withhold GH for 3 years, knowing the positive effects of GH on numerous outcomes in children with PWS. We do not expect that parents and/or clinicians, anticipating positive results, influenced the results of this study, as the results are based on BSID-II tests carried out by an independent psychologist. Additionally, this study was performed in very young children with PWS and the likelihood of them deliberately influencing the results of the BSID-II is negligible.

We have previously shown that mental and motor development decreased in infants who were not treated with GH, while psychomotor development significantly increased in those who were treated with GH. Thus, it is likely that psychomotor development would have remained similar or would have decreased if children were not treated with GH[7]. This shows that GH treatment plays an important role in psychomotor development in infants with PWS, aside from the spontaneous improvement with age. Six patients also participated in our previous study, but compared to the 63 patients included in this study, this low number did not unduly influence the results presented in this paper. Moreover, the present data of a larger group confirm the preliminary findings of the first study in 29 infants with PWS[7].

Baseline psychomotor development varied widely among infants and toddlers with PWS with developmental scores ranging from 15 to 91% of healthy references. This variability was especially large in infants younger than 1 year of age. Infants with a higher motor developmental score had a higher mental developmental score, which could be related to the fact that some mental developmental milestones cannot be reached without reaching certain motor developmental milestones first. For example, infants with poor head stability will not be able to visually follow (the voice of) their parent(s) and/or caregiver(s). It could be that once infants and toddlers have reached certain motor milestones (e.g. sitting or walking independently), they are capable to better explore their environment, which enhances their mental development. This is supported by the significant correlation between the change in motor and mental development during 3 years of GH. Infants with an improvement in motor development would thus be more likely to improve their mental abilities[7,17].

Brain development during infancy and early childhood is very important for cognitive functioning on the long term[18]. We previously found that children with PWS had a trend towards smaller white matter volume, indicating reduced structural connectivity or aberrant myelinisation in children with PWS, which may reflect a delay in brain maturation and underlie cognitive deficits in these children[19]. GH receptors are expressed throughout the brain and GH and IGF-I are expected to be involved in brain growth, development and myelinisation. As serum IGF-I levels are low in children with PWS prior to starting GH treatment, GH might improve brain development by increasing IGF-I levels[20,21].

We found that the younger the age at start of GH treatment, the greater the improvement in psychomotor development, suggesting that starting GH treatment early, in a critical period of neurodevelopment, could enhance psychomotor and cognitive development on the longer term[21,22]. One of our previous studies also reported a significant effect of age at start of GH on cognitive development in 50 pre-pubertal children[2]. Furthermore, another study in 42 children with PWS stated that if GH was started during infancy, GH had a larger effect on adaptive functioning[6]. More studies are required to confirm these findings in subjects with PWS.

In contrast to our expectations, but in line with previous data[7], we did not find a significant association between psychomotor development and head circumference, neither at baseline, nor during 3 years of GH treatment. Head circumference increased from low-normal to normal during GH, but the change in head circumference relative to the change in height was below one, which means that height SDS increased more than head circumference SDS (data not shown). Thus, it seems that the neuroregulatory role of GH and IGF-I in the central nervous system is more important for psychomotor development than the increase in head circumference[20,23].

Our study is the first to investigate the effects of 3 years of GH treatment on psychomotor development. BSID-II is suitable for children with a developmental age between 0 and 3.5 years and can be compared to references using mental and motor index scores. As infants and toddlers with PWS have delayed psychomotor development, their BSID-II results may only be described in terms of developmental age. Expressing this developmental age as a percentage of the expected mental and motor development for their age allowed us to evaluate psychomotor development during 3 years of GH, despite their delay in psychomotor development[14]. It is not possible to perform a study about the effects of more than 3 years of GH on mental and motor development with the BSID-II, as most children will exceed the maximum developmental age during the first 3 years of GH treatment.

In conclusion, our study shows that, in a large group of infants and toddlers with PWS, mental and motor development increased significantly during 3 years of GH treatment, reducing the gap between infants with PWS and healthy peers. Infants with lower baseline psychomotor development advanced more than infants with higher baseline psychomotor development. Currently, the increased awareness of PWS and the improved genetic tests have made it possible to diagnose PWS during early infancy. As starting GH treatment at a younger age seems to result in better psychomotor development, we nowadays start GH treatment in very young infants with PWS.

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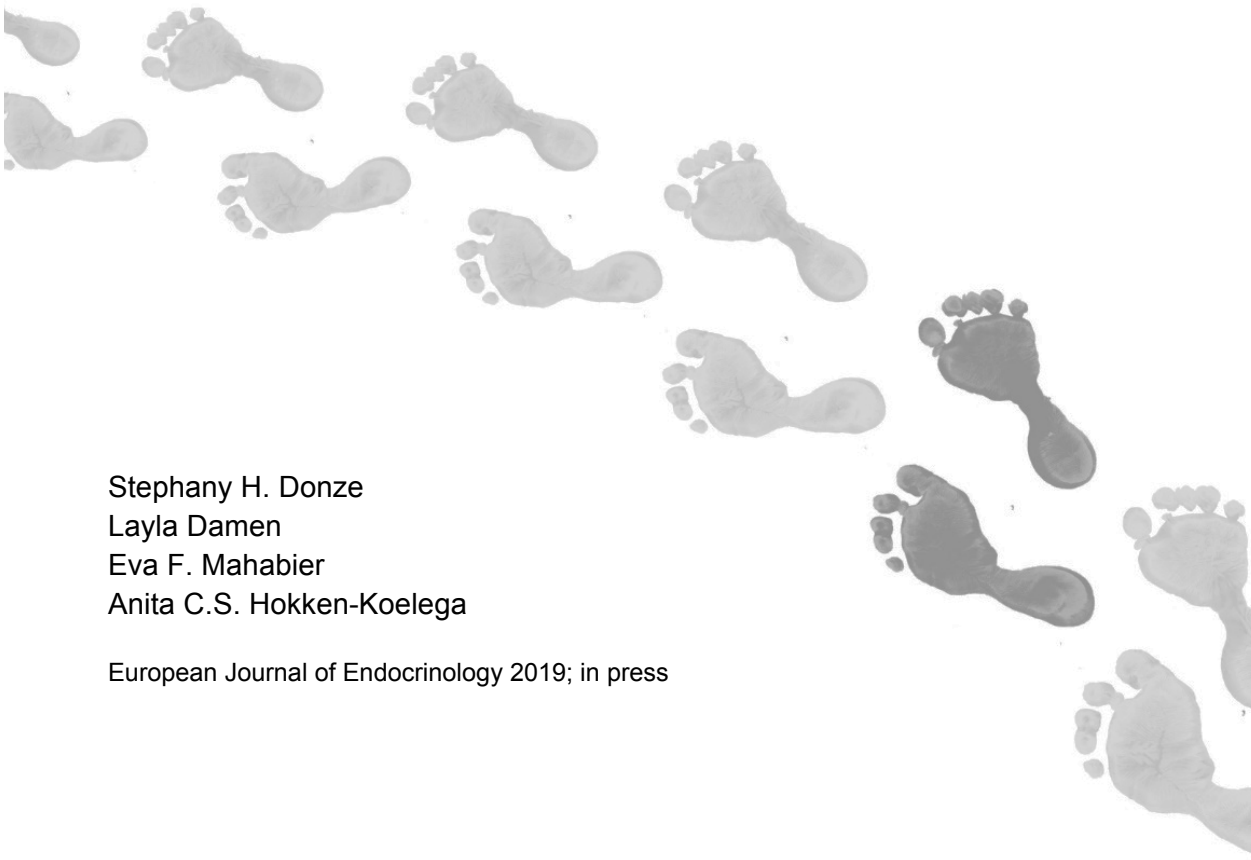
Chapter 3

Cognitive functioning in children with Prader-Willi syndrome during 8 years of growth hormone treatment



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ABSTRACT

Objective: Children with Prader-Willi syndrome (PWS) have mild to moderate cognitive impairment. Short-term studies showed positive effects of growth hormone (GH) on cognitive development. This study investigated the effects of 8 years of GH on cognitive development in children with PWS. We also investigated whether starting GH during infancy results in higher cognitive functioning after 8 years of GH.

Design: Longitudinal study in 43 children with PWS during 8 years of GH (median age at GH start 8.1 years). Cognitive functioning after 8 years was compared to another group of 22 children with PWS (median age at GH start 1.4 years).

Methods: Cognitive functioning measured by Wechsler Intelligence Scale for Children. Vocabulary, Similarities and Block Design subtests were expressed as standard deviation scores (SDS) and total IQ (TIQ) calculated.

Results: Estimated mean (95%CI) Block Design SDS changed from -2.2 (-2.6;-1.8) at GH start to -1.8 (-2.2;-1.4) after 8 years of GH (p=0.18), Similarities SDS from -1.5 (-2.1;-0.9) to -1.3 (-1.9;-0.7, p=0.66), TIQ from 66 (60;72) to 69 (63;75, p=0.57). Vocabulary SDS remained similar, being -1.9 (-2.3;-1.4) at GH start and -1.9 (-2.4;-1.5) after 8 years (p=0.85). After 8 years of GH Vocabulary SDS and TIQ were higher in the children who started GH during infancy, compared to those who started GH later in childhood (p<0.01, p=0.04, resp.).

Conclusions: Cognitive functioning in children with PWS remains similar during long-term GH and develops at the same pace as healthy peers.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare genetic disorder resulting from the loss of expression of locus q11-q13 (PWS region) on the paternally derived chromosome 15. This loss of expression is mostly due to a paternal deletion or maternal uniparental disomy (mUPD) and leads to a characteristic phenotype including muscular hypotonia, short stature, hyperphagia with an increased risk of obesity, psychomotor delay, neurobehavioral problems and cognitive impairment[1]. Patients with PWS typically have mild to moderate cognitive impairment with an average IQ between 60 and 70[2,3].

Growth hormone (GH) is a registered treatment for children with PWS since 2000. The physical benefits have been thoroughly investigated and include an improvement in body composition, linear growth and physical strength[4-7]. GH treatment is also associated with cognitive benefits, which are attributed to the effects of GH and insulin-like growth factor (IGF)-I on brain growth, development and myelinisation[8,9]. A recent review about the effects of GH treatment on cognition concluded that GH could stimulate GH receptors in brain areas involved in learning and memory, thereby improving cognitive functioning[10].

In PWS, few studies have investigated the effects of GH on cognitive functioning. Findings were not unequivocal, with some studies reporting an improvement in psychomotor development and cognition[2,6,11-13], while others described no cognitive benefits of GH[14,15]. We previously demonstrated in a 2-year randomized controlled trial that cognitive functioning in GH-treated children with PWS developed at the same pace as cognitive functioning in healthy references, while abstract verbal reasoning and vocabulary significantly deteriorated in untreated controls[6]. Furthermore, in 50 children with PWS who started GH at a mean age of 7.8 years, we found a significant improvement in abstract verbal reasoning and visuospatial skills during 4 years of GH treatment[2]. Most of these studies evaluated short-term effects of GH in children with PWS and there are currently no studies investigating the effects of more than 4 years of GH in children with PWS.

In this prospective study, we therefore investigated the longitudinal effects of 8 years of GH treatment on cognitive functioning in children with PWS from our Dutch PWS cohort. We also investigated whether starting GH treatment during infancy, i.e. before the age of 2 years, would result in higher cognitive functioning after 8 years of GH compared to starting GH later in childhood. We hypothesized that cognitive functioning would not deteriorate during long-term GH treatment and would progress at the same pace as healthy references. Additionally, we hypothesized that starting GH during infancy could benefit cognitive functioning more than starting GH later in childhood.

METHODS

Patients

A longitudinal, prospective cohort study was performed in 43 children with PWS to investigate the effects of 8 years of GH on cognitive functioning. All children were selected from our Dutch PWS Cohort based on the following criteria; 1) genetically confirmed diagnosis of PWS, 2) naïve to GH treatment at time of enrolment, 3) at least 3 Wechsler Intelligence Scale for Children (WISC) tests performed during 8 years of GH. Thirty-five of 43 children participated in our previous study investigating cognitive development in children with PWS during 4 years of GH treatment[2].

In addition, a cross-sectional study was performed to investigate whether starting GH treatment during infancy, i.e. before the age of 2 years, would result in improved cognitive functioning after 8 years of GH. We compared WISC results after 8 years of GH of a separate group of 22 children with PWS from our Dutch PWS Cohort who started GH treatment during infancy to the 43 longitudinally studied children who started GH later in childhood. We could not evaluate the longitudinal effects of 8 years of GH on cognitive functioning in the first group, because WISC is not suitable for children younger than 6 years of age. After 8 years of continuous GH treatment, all 22 children who started GH during infancy were older than 6 years and cognitive functioning could be tested by WISC.

The study protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center (Rotterdam, The Netherlands) and the collaborating centers. Written informed consent was obtained from parents and from children older than 12 years; assent was obtained from children younger than 12 years.

Design

In this prospective cohort study all participants were followed at the Dutch PWS Reference Center in collaboration with paediatric endocrinologists and paediatricians in other hospitals in the Netherlands. Children were treated with Genotropin (Pfizer, New York), administered subcutaneously once daily at bedtime in a dose of 1 mg/m2/day (≈0.035 mg/kg/day) for 8 consecutive years. All children were GH naïve at start of the study. The GH dose was regularly adjusted based on calculated body surface area and serum IGF-I levels. During the study, all children were seen every three months for anthropometric measurements by the PWS team of the Dutch PWS Reference Center. Cognitive functioning was measured biennially during 8 years of GH treatment by a psychologist experienced in testing children with PWS, in our PWS Reference Center (Erasmus University Medical Center/Sophia Children's Hospital, Rotterdam, The Netherlands).

Anthropometry
Height was measured using a Harpenden stadiometer (Holtain Limited, Crosswell, United Kingdom); weight was measured on a calibrated electronic digital scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, The Netherlands) and head circumference with a measuring tape. Height, weight, body mass index (BMI) and head circumference were expressed as standard deviation scores (SDS) adjusting for gender and age according to Dutch reference values[16,17] using Growth Analyser 4.0 (available at www.growthanalyser.org).

Cognition
Cognitive functioning was assessed by a shortened version of the Wechsler Intelligence Scale for Children (WISC), which is suitable for children between the age of 6 and 16 years[18]. The WISC is a well-recognized measure of cognitive ability and demonstrates strong reliability for diagnosis of mild to moderate intellectual disability[19]. The subtests Block Design, Vocabulary and Similarities were used. Block design was used to assess performance IQ, Vocabulary and Similarities were used to assess verbal IQ[20]. Short versions of the total tests were performed because of the limited attention span of children with PWS. Results of the short version of the WISC are correlated with the full-scale IQ test[21-23].

All scores were expressed as SDS based on a Dutch population with the same age. Normalized scores ranged from 1 (-3 SDS) to 19 (+3 SDS), with a mean of 10 (0 SDS). A subtest score between -2 and +2 SDS was considered within the normal range. Calculation of total IQ was performed using: Total IQ (TIQ) = 45.3 + 2.91 x Vocabulary SDS + 2.5 x Block design SDS). This formula has been used in other studies and is based on a Dutch reference population[24,25].

Statistical analysis
Statistical analyses were performed with the Statistical Package for the Social Sciences 24.0 (SPSS, Chicago, Illinois). Baseline data were expressed as median (interquartile range, IQR). Gender and genotypic differences in baseline cognitive functioning and cognitive functioning after 8 years of GH were calculated by Mann-Whitney U tests. Correlations between cognitive functioning and age, head circumference and serum IGF-I levels were calculated by Spearman's correlation analysis.

Changes in cognitive functioning during 8 years were analysed using repeated measurements analysis, with years of GH use (0 = baseline, 24 = 2 years of GH treatment, 48 = 4 years of GH treatment, 72 = 6 years of GH treatment, 96 = 8 years of GH treatment) as a categorical independent variable and a compound symmetry covariance matrix. The results of the repeated measurement analyses were expressed as estimated mean (95% confidence interval, CI). The effects of age, gender, genotype, head circumference and serum IGF-I levels on cognitive functioning during GH-treatment were investigated by using these variables as

factors (in case of nominal or ordinal variables) or covariates (in case of scale variables) in the model. All subtest scores and TIQ scores were corrected for age, gender and genotype.

Cognitive functioning after 8 years of GH of the children with PWS who started GH during infancy (before the age of 2 years) were compared to the longitudinally studied children with PWS who started GH later in childhood by using unpaired t-tests. P-values less than 0.05 were considered statistically significant.

RESULTS

Baseline clinical characteristics and cognitive functioning

Forty-three children with PWS (29 girls) who started GH at a median (IQR) age of 8.1 (6.6; 11.5) years were studied during 8 years of continuous GH. Baseline clinical characteristics and cognitive functioning are shown in Table 1. Estimated mean baseline Block Design score was below the lower limit of the reference range of a healthy Dutch population without PWS; while Vocabulary and Similarities subtest scores were within the reference ranges, i.e. between -2 and +2 SDS. Estimated mean (95% CI) baseline TIQ was 66 (60; 72), consistent with a mild mental impairment.

Table 1. Clinical characteristics at start of GH in longitudinally studied children with PWS

Number (girls)	43 (29)	
Genetic subtype: Deletion / mUPD / ICD / translocation / #	18 / 17 / 4 / 1 / 3	
Age at start GH treatment (yrs)	8.1	(6.6; 11.5)
Height (SDS)	-2.4	(-3.5; -1.6)
Weight for height (SDS)	1.9	(1.2; 2.6)
BMI for age (SDS)	1.4	(0.7; 1.9)
Head circumference (SDS)	-0.6	(-0.9; -0.2)
IGF-I (SDS)	-1.8	(-2.2; -1.4)
Block Design (SDS)	-2.2	(-2.6; -1.8)
Vocabulary (SDS)	-1.9	(-2.3; -1.4)
Similarities (SDS)	-1.5	(-2.1; -0.9)
Total IQ	66	(60; 72)

Data expressed as median (IQR).
mUPD = maternal uniparental disomy. ICD = imprinting center defect. # = genotype unknown.
GH = growth hormone. BMI = body mass index. IGF-I = insulin-like growth factor 1.
Cognitive functioning, head circumference and IGF-I are expressed as estimated mean (95% CI).

Baseline cognitive functioning was neither different between boys and girls, nor between children with a deletion and a mUPD / imprinting center defect (ICD) / translocation ($p>0.12$). Older age tended to be associated with a lower score on the

Similarities subtest (r=-0.4, p=0.05). The other subtests and estimated TIQ were not associated with age (p>0.16). Baseline head circumference was positively associated with Block Design SDS (r=0.41, p=0.04) and estimated TIQ (r=0.41, p=0.04), but not with Vocabulary and Similarities SDS (r=0.29, p=0.16 and r=0.05, p=0.82, resp.). Baseline IGF-I tended to be associated with Block Design SDS (r=0.42, p=0.05), but not with the other subtests (p>0.17).

Cognitive functioning during 8 years of GH treatment

Estimated mean (95% CI) Block Design SDS changed from -2.2 (-2.6; -1.8) at start of GH to -1.8 (-2.2; -1.4) after 8 years of GH (p=0.18) and Similarities SDS from -1.5 (-2.1; -0.9) to -1.3 (-1.9; -0.7, p=0.66). Vocabulary SDS remained similar, being -1.9 (-2.3; -1.4) at baseline and -1.9 (-2.4; -1.5) after 8 years (p=0.85). These results demonstrate that visuospatial skills, abstract verbal reasoning and vocabulary skills develop at the same pace as healthy references (Figure 1). Mean estimated (95% CI) TIQ changed from 66 (60; 72) to 69 (63; 75, p=0.57). After 8 years of GH, estimated TIQ ranged from 51 to 94 and 30% of the children had an estimated TIQ above 70, the cut-off for mental disability. Overall, cognitive functioning remained similar during 8 years of GH and did not deteriorate.

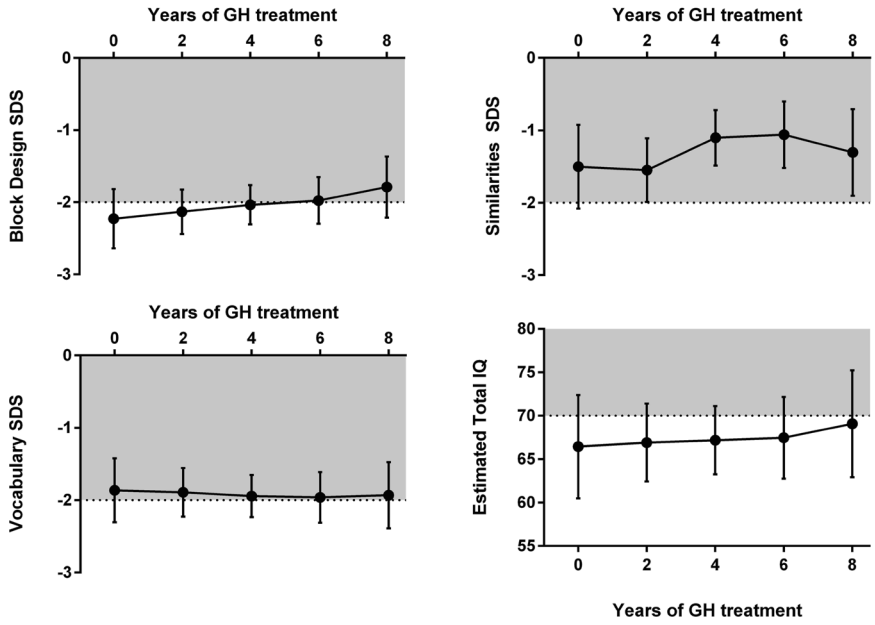

Figure 1. Cognitive functioning during 8 years of GH treatment in children with PWS who started GH during childhood. The grey rectangles define the normal range of cognitive functioning.

Influence of clinical and genetic characteristics on cognitive development

The course of cognitive functioning during 8 years of GH was similar in boys and girls (p>0.26). Genotype significantly influenced the course of Block Design SDS during 8 years of GH (p<0.01): in children with a deletion, estimated mean (95% CI) Block Design SDS changed from -1.8 (-2.3; -1.3) at baseline to -1.5 (-2.0; -1.0) after 8 years (p=0.13), while it remained similar in children with a mUPD / ICD / translocation, being -2.4 SDS (-2.7; -2.1) at baseline and -2.5 (-2.8; -2.2) after 8 years (p=0.76). After 8 years, median Block Design SDS was significantly higher in the children with a deletion (p=0.02). We found no effect of genotype on the course of the other subtests (p>0.30). Estimated mean (95% CI) head circumference SDS increased from -0.6 (-0.9; -0.2) at baseline to -0.1 (-0.5; 0.2) after 8 years (p<0.01) and IGF-I SDS from -1.8 (-2.2; -1.4) to 1.7 (1.3; 2.2, p<0.01), but neither the change in head circumference nor the change in IGF-I SDS was associated with the change in cognitive functioning during 8 years of GH (p>0.25). A higher IGF-I SDS after 8 years of GH was still associated with a higher Block Design SDS after 8 years of GH (r=0.43, p=0.04). The association between IGF-I and the other subtests after 8 years was not statistically significant (p>0.14).

Effect of age at start of GH on cognitive functioning after 8 years of GH

To investigate whether starting GH during infancy, i.e. before the age of 2 years, would result in better cognitive functioning compared to starting GH later in childhood, we compared WISC results after 8 years of GH of a (separate) group of 22 children from our Dutch PWS cohort who started GH at a median (IQR) age of 1.4 (1.0; 1.8) years to those of the 43 longitudinally investigated children who started GH later in childhood (Table 2 and Figure 2). We could not evaluate the longitudinal effects of 8 years of GH on cognitive functioning in the first group, because WISC is not suitable for children younger than 6 years of age. After 8 years of GH, the 22 children who started GH during infancy were significantly younger and taller than the 43 children who started GH later in childhood (p<0.02). Children who started GH during infancy had a significantly higher Vocabulary SDS (p<0.01) and estimated TIQ score (p=0.04; Figure 2) after 8 years of GH. Scores on the Block Design and Similarities subtests were similar (p=0.48 and p=0.16, resp.) between the two groups.

Table 2. Clinical characteristics after 8 years of GH

	Start of GH during childhood	Start of GH during infancy
Number (girls)	43 (29)	22 (9)
Genetic subtype		
Deletion / mUPD / ICD / translocation / #	18 / 17 / 4 / 1 / 3	12 / 9 / 0 / 0 / 1
Age at start GH treatment (yrs)	8.1[1] (6.6; 11.5)	1.4 (1.0; 1.8)
Age after 8 years of GH (yrs)	16.0[1] (14.5; 19.4)	9.5 (9.0; 9.8)
Height (SDS)	-0.9[1] (-1.5; 0.2)	0.2 (-1.0; 1.3)
Weight for height (SDS)	1.5 (0.3; 2.0)	1.3 (0.2; 1.8)
Head circumference (SDS)	0.3 (-1.1; 1.1)	0.2 (0.1; 0.5)

Data expressed as median (IQR). [1]p<0.02 compared to 22 children who started GH before 2 years of age.
mUPD = maternal uniparental disomy. ICD = imprinting center defect. # = genotype unknown. GH = growth hormone.

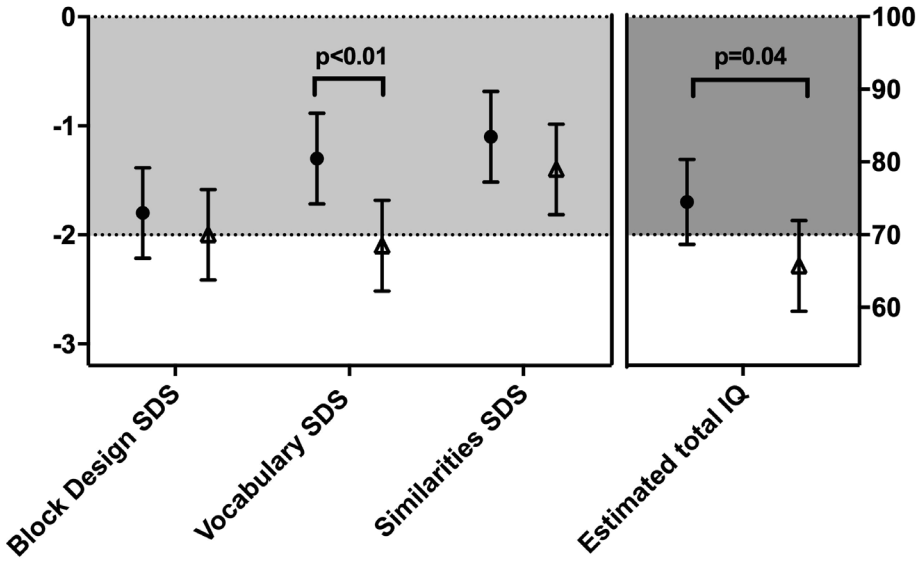

Figure 2. Cognitive functioning after 8 years of GH of the 22 children who started GH treatment during infancy, before the age of 2 years, compared to 43 longitudinally studied children who started GH later in childhood. Data are presented as mean (SEM). The grey rectangles define the normal range of cognitive functioning, the black dots represent the children who started GH treatment during infancy and the white triangles represent the children who started GH later in childhood.

DISCUSSION

This is the first long-term study investigating cognitive functioning during 8 years of continuous GH treatment in children with PWS. Our results demonstrate that the SD scores for visuospatial skills, verbal reasoning skills and vocabulary, as well as estimated TIQ remain similar during 8 years of GH treatment. This shows that cognitive development in GH-treated children with PWS progresses at the same pace as healthy references and does not deteriorate.

We previously demonstrated in a 2-year randomized controlled trial that cognitive functioning in GH-treated children with PWS developed at the same pace as cognitive functioning in healthy references, while there was a significant deterioration in abstract verbal reasoning and vocabulary in untreated controls[2]. In 50 children with PWS who were treated with GH for 4 years, visuospatial skills and abstract verbal reasoning improved significantly[2]. In the current study during 8 years of GH treatment, the improvement in visuospatial skills did not reach statistical significance. Yet, our results are reassuring and they show that GH treatment is able to maintain a similar level of cognitive development compared to healthy references.

We should bear in mind that, although GH seems to benefit cognitive functioning, it does so in the context of a developmental disorder with a lower total IQ than the general population. Longer duration of GH treatment, therefore, does not automatically lead to a continuous increase in IQ scores[12].

Because all children with PWS in the Netherlands are treated with GH from a young age, we could not compare cognitive development of GH-treated children with PWS to children with PWS who were not treated with GH. We acknowledge that an RCT would be the first-choice design to investigate the effects of long-term GH on cognitive functioning in children with PWS, but it would be unethical to withhold GH treatment for 8 years, knowing its positive effects on various outcomes in children with PWS. As our short-term RCT showed a deterioration of cognitive abilities in children with PWS who were not treated with GH during 2 years[2], it is possible that cognitive functioning would have deteriorated if the children in the current study were not treated with GH. The fact that cognitive functioning did not deteriorate and the progress of cognitive development in children with PWS during 8 years of GH treatment was similar to healthy references could therefore be interpreted as a positive result.

How the genetic aberrations underlying PWS lead to cognitive impairment is largely unknown. Studies have shown that it may be related to lower brain volumes and lower cortical complexity, due to alterations in gene networks that are important for early brain development[26,27]. Another question that still needs to be elucidated is the mechanism through which GH could affect cognitive functioning. A recent review about the effects of GH on cognition concluded that GH might stimulate GH receptors in brain areas involved in learning and memory, thereby improving cognitive functioning[10]. It is known that GH receptors are located throughout the brain and that GH and IGF-I affect the genesis of neurons, thereby stimulating brain growth, development and myelinisation[8,9].

A study in untreated adults with PWS reported a correlation between lower IGF-I levels and poorer intellectual skills[28]. We also found a non-significant correlation between lower visuospatial skills and lower serum IGF-I levels ($r=0.42$, $p=0.05$). This shows that GH treatment might improve cognitive functioning by increasing IGF-I levels in patients with PWS. However, it has also been shown that GH has effects on the central nervous system that are independent of IGF-I levels[29]. Baseline head circumference and visuospatial skills at start of GH were significantly associated. However, the increase in head circumference during GH was not associated with the change in cognitive functioning, demonstrating that, even though head circumference has previously been associated with cognitive functioning, other determinants might be more important for cognitive development and head growth[25,30,31].

After 8 years of GH, children with a deletion had significantly better visuospatial skills compared to those with a mUPD / ICD / translocation. Some studies have also described genotypic differences in performance IQ[1-3]. It is however difficult to draw definite conclusions, due to the large variability in cognitive functioning and the small sample-size. After 8 years of GH, estimated TIQ in our study ranged from 51 to 94 and 30% of children had an estimated IQ above 70 points, the cut-off for mental disability. This broad variety in cognitive functioning has been reported in other studies and underlines the importance of individual assessment of cognitive and social functioning in children with PWS, regardless of the genetic defect. By doing so, we can help identify the difficulties that individual children with PWS face and develop strategies to best deal with these difficulties[27].

Some studies show a correlation between age at start of GH and the effect of GH on cognitive functioning. The younger children are at the start of GH treatment, the more they might benefit with regard to psychomotor and cognitive development[11-13]. GH treatment in children with PWS is started in a critical period of neurodevelopment, which might ameliorate cognitive functioning on the long-term. We found a significantly higher vocabulary and estimated TIQ score after 8 years of GH treatment in the 22 children who started GH treatment during infancy compared to the 43 children who started GH later in childhood. Because WISC is not suitable for children younger than 6 years of age, we could not evaluate cognitive functioning at start of GH treatment and the longitudinal effects of 8 years of GH in the first group. Nevertheless, our results suggest that an early start of GH during infancy might be beneficial for cognitive development, as reported in previous studies[11-13].

Combined with an early diagnosis and multidisciplinary support from a very young age, long-term GH treatment during childhood counteracts the clinical course of increasing obesity in PWS and has substantially changed the phenotype of children with PWS. Our study shows that cognitive development during 8 years of GH in children with PWS progresses at the same pace as healthy peers and does not deteriorate. It also shows that vocabulary and estimated TIQ scores after 8 years of GH are higher in children who started GH during infancy, a critical period of neurodevelopment. Further studies are necessary to confirm our findings.

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Chapter 4

Prevalence of Growth Hormone Deficiency in previously GH-treated young adults with Prader-Willi syndrome

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ABSTRACT

Objective Some features of subjects with Prader-Willi syndrome (PWS) resemble those seen in growth hormone deficiency (GHD). Children with PWS are treated with growth hormone (GH), which has substantially changed their phenotype. Currently, young adults with PWS must discontinue GH after attainment of adult height when they do not fulfil the criteria of adult GHD. Limited information is available about the prevalence of GHD in adults with PWS. This study aimed to investigate the GH/IGF-I axis and the prevalence of GHD in previously GH-treated young adults with PWS.

Design Cross-sectional study in 60 young adults with PWS.

Measurements Serum IGF-I and IGFBP-3 levels, GH-peak during combined GHRH-Arginine stimulation test.

Results Serum IGF-I was <-2 SDS in 2 (3%) patients and IGFBP-3 was within the normal range in all but one patient. Median (IQR) GH peak was 17.8 µg/l (12.2; 29.7) [≈53.4 mU/l] and below 9 µg/l in 9 (15%) patients. Not one patient fulfilled the criteria for adult GHD (GH-peak <9 µg/l and IGF-I<-2 SDS), also when BMI-dependent criteria were used. A higher BMI and a higher fat mass percentage were significantly associated with a lower GH peak. There was no significant difference in GH peak between patients with a deletion or a maternal uniparental disomy (mUPD).

Conclusions In a large group of previously GH-treated young adults with PWS, approximately 1 in 7 exhibited a GH peak <9 µg/l during a GHRH-Arginine test. However, none of the patients fulfilled the consensus criteria for adult GHD.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare disorder resulting from the lack of expression of the PWS region (q11-q13) on the paternally derived chromosome 15. This is mostly caused by a paternal deletion or maternal uniparental disomy (mUPD) and in some cases by an imprinting center defect or paternal chromosomal translocation[1,2]. Clinical findings change with age, with infancy being characterized by muscular hypotonia and failure to thrive, while short stature, psychomotor delay, hyperphagia and obesity are more prominent during childhood and adulthood[1,3].

Some features of people with PWS resemble those seen in growth hormone deficiency (GHD), such as short stature and an abnormal body composition with a low lean body mass (LBM) and an increased fat mass (FM). Long-term continuous growth hormone (GH) treatment in children with PWS improves body composition, linear growth, physical strength, cognition and adaptive functioning, substantially changing the phenotype of children with PWS[4-9]. Currently, young adults with PWS have to stop GH treatment after attainment of adult height, when they do not fulfil the criteria of adult GHD. Studies have shown that GH treatment is beneficial for adults with PWS, with a sustained improvement in FM and LBM when GH is continued after attainment of adult height, and a deterioration of body composition when GH treatment is discontinued[10,11].

Reduced serum insulin-like growth factor (IGF)-I levels and a reduced GH response to provocative stimuli were found in a varying percentage of children and adults with PWS[3,12-18]. Most studies in adults with PWS have investigated GHD in adults who were not treated with GH during childhood and the prevalence of GHD varied dependent on the diagnostic test and the chosen cut-off points, which were or were not corrected for BMI. This study aimed to assess the GH response to a GHRH-Arginine stimulation test in a large sample of young adults with PWS who had attained adult height and were previously treated with GH during childhood.

METHODS

Patients

Inclusion criteria for the present study were (1) genetically confirmed diagnosis of PWS by a positive methylation test, (2) growth hormone (GH) treatment during childhood for at least two years, and (3) having attained adult height, defined as a height velocity less than 0.5 cm per six months and epiphyseal closure as demonstrated by a radiograph of the left hand and wrist. Exclusion criteria were (1) medication to reduce weight (fat) or (2) non-cooperative behaviour. Sixty subjects were included, who were all on a diet and exercise program. Written informed consent was obtained from patients and their parents or legal representatives. The study protocol was approved by the Medical Ethics Committee of the Erasmus University Medical Center, Rotterdam.

Design
All participants were followed at the Dutch PWS Reference Center. They were treated with biosynthetic GH (Pfizer Inc., New York, NY) during childhood, administered subcutaneously once daily at bedtime in a dose of 1.0 mg/m^2/day (≈0.035 mg/kg/day). The GH dose was regularly adjusted based on calculated body surface area and serum IGF-I levels. At attainment of adult height, GH treatment was discontinued for at least six weeks prior to performing a standard GH-stimulation test with Growth Hormone Releasing Hormone (GHRH) and Arginine[19].

Anthropometry
Standing height was measured using a Harpenden Stadiometer and weight was measured on a calibrated electric scale (Servo Balance KA-20-150S; Servo Berkel Prior, Katwijk, The Netherlands). Height, weight and body mass index (BMI) were expressed as standard deviation scores (SDS) using Growth Analyser 4.0 (available at www.growthanalyser.org), adjusting for age (18 years) and sex according to Dutch reference values[20,21].

Body composition
Body composition was assessed by dual-energy x-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK), within four months of the GHRH-Arginine stimulation test in 41 participants. Total fat mass (FM; kg) and lean body mass (LBM; kg) were assessed. All scans were made on the same machine, with daily quality assurance. The intra-assay coefficient of variation (CV) for fat tissue was 0.41 to 0.88% and for LBM 1.57 to 4.49%[22]. FM was also expressed as percentage of total body weight (FM%). LBM was calculated as fat-free mass minus bone mineral content. FM% SDS and LBM SDS were calculated according to age- and sex-matched Dutch reference values[23].

GHRH/Arginine test
Growth hormone stimulation tests started at 8.30 am after overnight fasting, with the patients recumbent. After an indwelling catheter had been placed, each participant received GHRH (1 µg /kg as intravenous bolus at 0 minutes, with a maximum dose of 100 µg) and Arginine (0.5 g/kg during 30 minutes, with a maximum dose of 50 g). Blood samples for GH determination were drawn at 0, 15, 30, 45, 60 and 90 minutes after the intravenous bolus of GHRH. Levels of GH, IGF-I and IGFBP-3 were measured using the IDS-iSYS immunoassay system, which is based on chemiluminescence. The intra-assay variations were <6.4%, <7.5% and <5.1%, respectively. Levels of IGF-I and IGFBP-3 were expressed as SDS, adjusting for age and gender[24,25].

Statistical analysis
Statistical analyses were performed with SPSS 24.0 (SPSS Inc., Chicago, IL). Data were expressed as median (IQR). The GH response to GHRH and Arginine was assessed by the evaluation of the highest GH plasma concentration, i.e. the GH

peak. GHD in adults was defined as a GH peak level after GHRH-Arginine test < 9 µg/l in combination with a serum IGF-I SDS level < –2 adjusted for age and gender[3,19]**.** We also applied the BMI-dependent cut-off points for the GHRH-Arginine test: a GH peak of < 11.5 µg/l if BMI is < 25 kg/m^2, a GH peak of < 8.0 µg /l if BMI is 25-30 kg/m^2, and a GH peak of < 4.2 µg/l if BMI > 30 kg/m^2 [26].

Pearson's correlation coefficients were used to assess relationships between IGF-I and GH peak and anthropometric measurements and body composition variables. Student's t-tests were used to calculate differences between groups. *P* values less than 0.05 were considered statistically significant.

RESULTS

Baseline characteristics

Sixty young adults with PWS (27 males, 33 females) with a median (IQR) age of 17.9 (16.3; 19.6) years were included in the current evaluation of GH response to a GHRH-Arginine stimulation test (Table 1). GH treatment during childhood was started at a median (IQR) age of 6.6 (4.0; 8.8) years. Twenty-nine young adults had a deletion (48.3%), 25 a maternal uniparental disomy (mUPD; 41.7%) and five a paternal translocation (8.3%). One patient refused further investigation of the genetic subtype.

Table 1. Clinical characteristics at adult height and during childhood

	Adult height	During childhood
Age (yrs)	17.9 (16.3; 19.6)	6.6 (4.0; 8.8)
Male / Female (n)	27 / 33	27 / 33
Genetic subtype		
Deletion / mUPD / translocation / [a]	29 / 25 / 5 / 1	29 / 25 / 5 / 1
Height for age (SDS)	-1.0 (-1.7; -0.3)	-2.2 (-3.0; -1.8)
BMI	24.2 (21.1; 27.9)	17.9 (16.3; 19.4)
BMI for age (SDS)	1.1 (-0.2; 1.9)	-0.6 (-1.1; 0.1)
FM%	40.5 (35.7; 47.5)	34.2 (28.9; 38.3)
FM% SDS	2.3 (1.8; 2.6)	2.3 (2.1; 2.6)
LBM SDS	-2.3 (-3.1; -1.2)	-2.5 (-2.8; -2.0)

Data expressed as median (IQR). [a]genetic subtype unknown.

BMI: body mass index. FM%: fat mass percentage. LBM: lean body mass.

FM and LBM at adult height was assessed in 41 individuals and in 38 individuals during childhood.

Twenty-nine patients were receiving sex steroid replacement therapy (SSRT) and seven had spontaneous estrogen or testosterone levels within the normal range. The remaining 24 subjects were considered hypogonadal, but were not receiving SSRT at time of evaluation.

GH response to GHRH-Arginine test

Table 2 and Figure 1 show the results of the GHRH-Arginine tests and serum IGF-I and IGFBP-3 levels. Median (IQR) GH peak was 17.8 µg/l (12.2; 29,7) [≈53.4 mU/l]. Median (IQR) serum IGF-I was -0.4 (-1.1; 0.4) SDS and IGFBP-3 1.6 (1.0; 2.2) SDS. Serum IGF-I was < -2 SDS in two (3%) patients and IGFBP-3 was within the normal range in all but one participant. Nine participants had a peak GH level < 9 µg/l (15%) during the GHRH-Arginine test. None of these patients also had an IGF-I level < -2 SDS.

Table 2. GH response to GHRH-Arginine stimulation test

	Adult height		During childhood*	
IGF-I (nmol/l)	32.4	(21.3; 39.6)	7.5	(5.2; 12.7)
IGF-I SDS	-0.4	(-1.1; 0.4)	-1.7	(-2.2; -0.9)
IGFBP-3 (mg/l)	4.7	(4.0; 5.7)	1.2	(0.9; 1.5)
IGFBP-3 SDS	1.6	(1.0; 2.2)	-2.0	(-3.1; -1.4)
GH peak (µg/l)	17.8	(12.2; 29.7)	-	
Time to GH peak (min)	45.0	(30.0; 60.0)	-	

*Data expressed as median (IQR). *GH naïve before start of GH treatment.*
IGF-I: Insulin-like Growth Factor. IGFBP-3: Insulin-like Growth Factor-Binding Protein 3.
GH: growth hormone.

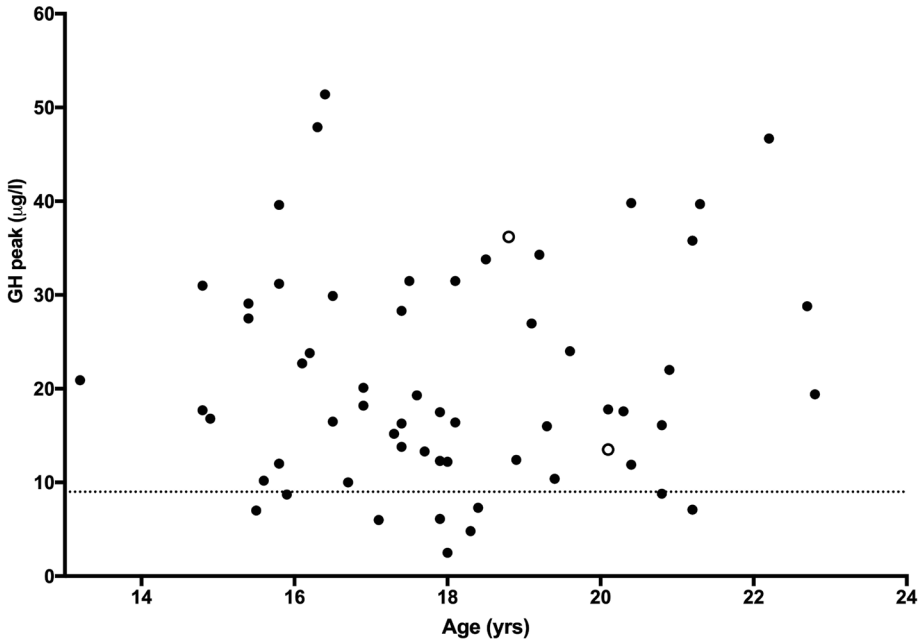

Figure 1. GH Peak and IGF-I SDS according to age in 60 young adults with PWS. The dashed line shows the cut-off value for GHD (9 µg/l). The open circles represent patients with an IGF-I SDS < -2 SDS, the black circles > -2 SDS.

When BMI-dependent criteria were used, nine participants (15%) had a GH peak below the cut-off. Again, none of these patients also had an IGF-I < -2 SDS. Thus, not one of our patients fulfilled the criteria of adult GHD.

GH peak was not significantly different between participants with a deletion and those with a mUPD (p=0.99) or between males and females (p=0.41). There was also no significant difference in GH peak between hypogonadal patients who were not receiving SSRT and patients who were receiving SSRT or had spontaneous estrogen and testosterone levels within the normal range at the time of the GHRH-Arginine stimulation test (p=0.67).

Correlation analyses

A higher BMI SDS and a higher FM% SDS were significantly associated with a lower GH peak (r= -0.46, p<0.01 and r= -0.43, p<0.01, resp.). The GH peak, however, did neither correlate with age or waist-hip ratio (both p>0.13), nor with IGF-I SDS (r=0.01, p=0.93). There was no significant correlation between IGF-I SDS and BMI SDS or FM% SDS (r= -0.14, p=0.94 and r= 0.008, p=0.96, resp.).

IGF-I at start of GH treatment during childhood

Clinical characteristics and median serum IGF-I and IGFBP-3 levels of the young adults at start of GH treatment during childhood are shown in Tables 1 and 2, respectively. Before starting GH treatment at a median age of 6.6 years, 18 children (30%) had an IGF-I < -2 SDS. Serum IGF-I was unknown in eight children (13.3%).

Of the 18 children with an IGF-I < -2 SDS at start of GH, only two had an IGF-I < -2 SDS after cessation of GH. None of them had a GH peak < 9 µg/l during the GHRH-Arginine test. So, none of the young adults with an IGF-I < -2 SDS at start of GH during childhood fulfilled the criteria of adult GHD after cessation of GH treatment at attainment of adult height, also when BMI-dependent cut-off values were used. Before start of GH treatment, there was no significant difference in height, BMI, FM% and LBM SDS between children with an IGF-I < -2 SDS and > -2 SDS (all p>0.34).

DISCUSSION

This study investigated the GH/IGF-I axis and the prevalence of growth hormone deficiency (GHD) in a large group of 60 young adults with PWS, who were treated with GH for more than 10 years during childhood until attainment of adult height. Against expectations, our data show that none of the patients had adult GHD according to the consensus criteria, also when BMI was taken into account. Results were variable, with some patients having a low serum IGF-I and normal GH peak, and others a normal serum IGF-I and low GH peak. In most countries, adults with PWS cannot be treated with GH after attainment of adult height, if they do not fulfil the consensus criteria for adult GHD.

Patients with PWS have several clinical features that resemble those seen in GHD, including short stature and an abnormal body composition with an increased fat mass and decreased muscle mass[1]. Both children and adults with PWS respond favourably to GH treatment, with a complete normalization of stature in children, an increase in serum IGF-I and IGFBP-3 levels and a significant improvement in body composition, without safety concerns[4,10,11]. It was generally believed that young adults with PWS would have GHD and could continue GH treatment after attainment of adult height. However, completely unexpected, none of the 60 young adults fulfilled the current consensus criteria for the diagnosis of adult GHD. Only two of our patients (3%) had an IGF-I level < -2 SDS and none of them had a GH peak < 9 µg/l. Thus, a decreased IGF-I level was not accompanied by a low GH peak during provocative testing. On the other hand, nine (15%) patients had a low GH peak, but completely normal serum IGF-I levels.

The reported prevalence of GHD in children and adults with PWS varies. According to a review by Burman et al. 40-100% of children with PWS fulfilled the criteria for GH deficiency, depending on the stimulation test used[12]. Three recent studies investigated the prevalence of GHD in mostly GH-naïve adult PWS patients, aged 16 – 43 years, by performing GHRH-Arginine tests[3,16,27]. Two of these studies, in 15 and 41 adult patients, also reported a low prevalence of GHD, with only one (8%) and six (15%) of the participants fulfilling the diagnostic criteria for GHD according to BMI-dependent cut-off points, respectively[3,27]. In contrast, Grugni et al. described severe GHD in 16 out of 37 adults with PWS (43.5%)[16]. However, mean BMI of their study group was 45.2 kg/m^2, which is much higher than the median BMI of 24.2 kg/m^2 in our study and the reported median BMI of approximately 27 kg/m^2 in the other two studies[3,27]. Even though BMI-dependent cut-off points were used, a mean BMI of 45.2 kg/m^2 is so far above the upper limit of 30 kg/m^2 that there is an increased likelihood of a blunted GH response to a GHRH-Arginine test. Conceivably, the current BMI-dependent cut-off points may not be suitable for patients with very severe obesity[28]. Also, in contrast to the adults investigated in the aforementioned studies, all young adults participating in our study were treated with GH from a median age of 6.6 years until attainment of adult height.

The GHRH-Arginine test is reported as reliable as the insulin tolerance test (ITT) for (re)testing patients treated with GH during childhood[19,29]. Given the increased risk of severe side effects during an ITT and the higher burden of this test, we chose to perform GHRH-Arginine instead of insulin tolerance tests. There is discussion about the appropriate cut-off values for GH peak during GHRH-Arginine tests. A cut-off value of 15.9 µg/l for severe adult GHD has been proposed for retesting late adolescents with childhood-onset GHD. However, due to the small number of overweight patients, a separate cut-off for adolescents with a high BMI was not obtained[30]. One third of our patients had a BMI > 25 kg/m^2 and the cut-off values as proposed by Dreismann et al., are therefore not applicable to our patients with PWS. We decided to use the BMI-adjusted adult cut-off values instead[26].

Two studies in young children with PWS described a low GH peak after Clonidine or Arginine in 68 - 85%[17,31]. One of these studies in 27 children with PWS also performed a combined test (GHRH-Arginine or GHRH-pyridostigmine test), which showed a much lower prevalence of GHD (15%) than the Clonidine and Arginine tests[31]. As studies in adults with PWS have described a higher prevalence of GHD after combined stimulation tests, the authors of both studies suggested that GHD in PWS might be due to an evolving process, with pituitary GH reserve decreasing with age[17,31]. Although we did not perform GH stimulation tests during childhood before starting GH treatment, our data show that, of the 18 children with an IGF-I < -2 SDS at start of GH treatment, all but two patients had normal serum IGF-I levels (>-2 SDS) after cessation of GH at adult height attainment. This finding contradicts the hypothesis that GHD might become more prevalent with age.

Our data are in line with those of most non-PWS patients with idiopathic childhood-onset GHD, who have a normal GH response when retested as adults[32-34]. The reasons why are unknown, but may include a variable GH response to stimulation, GHD being a transient condition or GHD being partial, preventing normal growth during childhood, but not causing symptoms in adulthood. We can, however, not exclude that GH treatment stimulates GH secretory cells in the pituitary, and that this GH effect persists, even after GH treatment is discontinued. This hypothesis is supported by a recent RCT, which showed that one year of placebo in previously GH-treated young adults with PWS did not deteriorate cognitive functioning and might suggest that the neurotropic effects of long-term GH during childhood last into adulthood[35].

During the transition period, the body undergoes physical and psychological changes that conclude the shift from childhood to adulthood. GH seems to play an important role in this transition, as it favourably influences body composition, lipid profile and peak bone density in adolescents with GHD[36]. In PWS, studies have shown a deterioration of body composition after cessation of GH treatment, while continuing GH after attainment of adult height maintained the improved FM and LBM obtained during childhood, supporting the presence of clinical GHD[10,11]. However, as most adults with PWS do not fulfil the consensus criteria for adult GHD, they currently cannot be treated with GH after attainment of adult height.

In conclusion, in a large group of previously GH-treated patients with PWS, GH peak levels during a GHRH-Arginine test were low in 15% of patients, but not one patient fulfilled the criteria for adult GHD. These results are against expectations, as several studies have shown that GH treatment has positive effects on body composition and health profile in adults with PWS.

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Chapter 5

Bone mineral density in young adults with Prader-Willi syndrome: a randomized, placebo-controlled, cross-over GH trial

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ABSTRACT

Context The prevalence of osteoporosis is increased in adults with Prader-Willi syndrome (PWS). In children with PWS, growth hormone (GH) treatment has beneficial effects on bone mineral density (BMD). BMD might deteriorate after cessation of GH at adult height (AH), while continuing GH might maintain BMD.

Objective To investigate the effects of GH versus placebo, and furthermore the effects of sex steroid replacement therapy (SSRT), on BMD in GH-treated young adults with PWS who had attained AH.

Design Two-year, randomized, double-blind, placebo-controlled, cross-over GH study.

Patients 27 young adults with PWS, stratified for gender and BMI. All patients were randomly and blindly assigned to receive GH (0.67 mg/m^2/day) and placebo, both during one year.

Measurements Bone mineral density of the total body (BMD_{TB}) and lumbar spine (BMD_{LS}) SDS, measured by dual-energy x-ray absorptiometry.

Results At AH, BMD_{TB}SDS was significantly lower compared to healthy peers ($p<0.01$), while $BMAD_{LS}$SDS was similar. Both BMD_{TB}SDS and $BMAD_{LS}$SDS were similar during one year of GH versus one year of placebo. In hypogonadal young adults without SSRT, BMD_{TB}SDS and $BMAD_{LS}$SDS decreased during the two-year study ($p=0.11$ and $p=0.01$), regardless of GH or placebo, while BMD_{TB}SDS increased in those with SSRT ($p<0.01$).

Conclusions: Compared to GH treatment, one year of placebo after attainment of AH does not deteriorate BMD SDS in young adults with PWS. In addition, our data suggest that GH is not able to prevent the decline in BMD SDS in hypogonadal young adults with PWS, unless it is combined with SSRT.

INTRODUCTION

Prader-Willi syndrome (PWS) results from the lack of expression of the PWS region on the paternally inherited chromosome 15, caused by a deletion, maternal uniparental disomy (mUPD), imprinting center defect (ICD) or translocation[1]. Hypothalamic dysfunction is an underlying cause for most symptoms of PWS[2]. Treatment with growth hormone (GH) during childhood improves the abnormal body composition and enhances mental and motor development, cognition, adaptive functioning, linear growth, and bone mineral density (BMD)[3-8].

BMD is influenced by endocrine factors such as GH, insulin-like growth factor (IGF)-I and sex steroids, but also by body composition and body mass index (BMI)[9,10]. Both sex steroids and GH are known to play an important role in the accrual of peak bone mass, which is attained between the age of 18 to 20 years in girls, and 20 to 22 years in boys[11,12]. Recent studies have shown that GH treatment optimizes BMD in prepubertal children with PWS[4,13], leading to a normal BMD compared to peers[4,14]. During puberty, however, we found a decline in BMD in parallel to incomplete pubertal development and low sex hormone levels[4,15,16]. This could explain the reported increased prevalence of osteopenia and osteoporosis resulting in a high fracture risk in adults with PWS[17-19]. The adults in the latter studies were, however, not treated with GH or sex steroid replacement therapy (SSRT).

When young adults with PWS without adult GH deficiency have attained adult height (AH), they have to stop GH treatment. Recently, we found that young adults with PWS who were treated with GH during childhood and have attained AH benefit from continuation of GH, by maintaining the improved body composition obtained during childhood[20]. GH treatment is, however, currently not approved for adults with PWS, unless they are GH-deficient. In GH-deficient young adults without PWS, BMD deteriorates after cessation of GH[21-23]. Given the beneficial effects of GH on BMD during childhood, we hypothesized that BMD would deteriorate during placebo in young adults with PWS, while it would be maintained by GH treatment. We, therefore, investigated the effects of GH versus placebo on BMD, measured by dual-energy x-ray absorptiometry (DXA), in young adults with PWS who had attained adult height (AH) and were treated with GH during childhood, in a two-year, randomized, double-blind, placebo-controlled cross-over GH study. Secondarily, we investigated the effects of no SSRT versus SSRT on BMD in hypogonadal young adults with PWS.

METHODS

Patients

Inclusion criteria of the present study were (1) genetically confirmed diagnosis of PWS; (2) GH treatment during childhood for at least two years and being on GH at time of inclusion (AH); and (3) AH attainment, defined as a height velocity less than

0.5 cm per six months and complete epiphyseal fusion. Exclusion criteria were (1) medication to reduce weight or fat or (2) non-cooperative behavior. From June 2008 to January 2014, 33 young adults with PWS fulfilled the inclusion criteria. Two did not want to continue daily injections and three refused participation due to too large burden of hospital visits. Of the 28 included patients, one 16.7-year old participant (BMI 25.0 kg/m^2) died of gastric rupture and necrosis, three months after inclusion while receiving placebo. Her data were excluded from the present study.

During childhood, patients were treated with the standard GH dose of 1.0 mg/m^2/day. GH dose was lowered in eight children, due to high serum IGF-I levels. In the present study, GH dose was lowered to 0.67 mg/m^2/day (≈0.023 mg/kg/day) in all patients. Eight patients were using thyroid hormone supplementation (29.6%), two modafinil (7.4%), and one patient was using risperidone and citalopram. During the two-year study, 8 out of 16 hypogonadal girls, and 3 out of 7 hypogonadal boys received an adult dose of sex steroid replacement therapy. All patients were on a diet and exercise program.

Design

Two-year, randomized, double-blind, placebo-controlled, cross-over study investigating the effects of one year GH treatment versus one year placebo on bone mineral density. Young adults were stratified according to gender and BMI (below/above 25 kg/m^2) and then randomly and blindly assigned to receive one year of subcutaneous injections once daily at bedtime of either 0.67 mg/m^2/day GH (Genotropin®, 5 mg/ml, Pfizer) or one year of identical appearing placebo (Pfizer), after which they crossed-over to the alternative treatment for another year. An independent statistician generated the random allocation sequence. Investigators, patients and parents were blinded for the allocation. An independent physician monitored the safety during the study. Unblinding was not necessary.

Measurements

Patients were examined every three months by the PWS-team of the Dutch Growth Research Foundation in collaboration with pediatric endocrinologists and pediatricians in the Netherlands. At each visit, the injection dose was adjusted to the calculated body surface area. In addition, patients visited the Erasmus University Medical Center at baseline, 6, 12, 18 and 24 months, to obtain bone mineral density (BMD), body composition, height and weight.

Bone mineral content (BMC; in grams), BMD (in grams per square centimeter) of the total body and lumbar spine (BMD_{TB} and BMD_{LS}), fat mass (FM) and lean body mass (LBM) were measured by DXA (Lunar Prodigy; GE Healthcare). All scans were made on the same machine, with daily quality assurance. The intra-assay coefficients of variation were 1.04% for BMD_{LS}, 0.64% for BMD_{TB} and BMC, 0.41-0.88% for fat tissue and 1.57-4.49% for LBM. In case of short stature, BMD_{LS} is underestimated by the areal presentation and should therefore be corrected for bone size by calculating

the $BMAD_{LS}$. The model: $BMAD_{LS} = BMD_{LS} * [4/(\pi*width)]$ was used, with width as the mean width of the second to fourth lumbar vertebral body[24]. BMD_{TB}SDS, BMD_{LS}SDS and $BMAD_{LS}$SDS were calculated to age- and sex-matched reference values from the Dutch population[25,26]. Low BMD was defined as a BMD_{TB}SDS or $BMAD_{LS}$SDS score of less than -2.0[27]. FM was expressed as percentage of total body weight (FM%). FM% SDS and LBM SDS were calculated according to age- and sex-matched Dutch reference values[26].

Standing height was measured with a calibrated Harpenden stadiometer, weight was determined on a calibrated scale (ServoBalance KA-20-150S) and BMI was calculated. Height, weight and BMI were expressed as SDS[28,29]. SDS values were calculated with GrowthAnalyser 4.0 (available at www.growthanalyser.org).

Assays

Blood samples were collected after an overnight fast and IGF-I, testosterone and estradiol levels measured in one laboratory. IGF-I was measured using an immunometric technique on Immulite 1000 (LKGF1, Siemens Medical Solutions Diagnostics) with an interassay variation <7.3%. IGF-I levels were expressed as SDS, adjusting for age and gender[30]. Total early morning serum testosterone and estradiol levels were determined by coated tube RIA (Immulite, DPC, Siemens). The intra- and interassays CVs of testosterone were below 6 and 9% and for estradiol below 5 and 7%, respectively.

Statistical analysis

Statistical analysis was performed with SPSS 24.0. A power calculation was performed, indicating that 24 patients were required to detect a treatment difference if the true difference between treatments was 0.6, with a power of 80%. This was based on the assumption that the standard deviation of the difference in the response variables was 1. To account for attrition, 4 more patients were added. As data were normally distributed, parametric tests were used and data expressed as mean (standard deviation (SD)). Effects of GH versus placebo were calculated using linear mixed model analysis with BMD_{TB}SDS and $BMAD_{LS}$SDS as dependent variables and a first-order autoregressive (AR(1)) covariance matrix. Possible carry-over effects were analyzed by adding the interaction between period and treatment, but not found.

The effect of no SSRT versus SSRT was evaluated in hypogonadal young adults with PWS using mixed model analysis. In girls, hypogonadism was defined as serum estradiol levels <100 pmol/l and/or Tanner stage 3 or less from the age of 14 years, and/or no menarche from the age of 16 years. In boys, hypogonadism was defined as serum testosterone levels <5 nmol/l and/or Tanner stage 3 or less from the age of 16 years, and/or serum testosterone levels <10 nmol/l from the age of 18 years. P-values less than 0.05 were considered statistically significant.

Study approval

Written informed consent was obtained from patients and parents. The study protocol was approved by the Medical Ethics Committee of Erasmus University Medical Center, Rotterdam, and registered at the Dutch Trial Register (www.trialregister.nl NTR1038).

RESULTS

Baseline characteristics

Table 1 shows the baseline characteristics of 27 young adults with PWS (19 girls, 8 boys). All participants completed all visits. At baseline, mean (SD) age and BMI SDS were 17.2 (1.8) years and 0.9 (1.3), respectively. During childhood, GH treatment was started at a mean (SD) age of 8.5 (3.5) years and patients received GH treatment for 8.7 (3.2) years until attainment of AH. There were significantly more patients with an mUPD, ICD or translocation in the group that received GH in the second phase of the RCT, compared to those who received GH in the first phase (p=0.04). Baseline BMD_{TB}SDS, $BMAD_{LS}$SDS and other baseline characteristics were not significantly different between the treatment groups.

Table 1. Baseline characteristics of total group and per treatment schedule

	PWS (n=27)	Placebo / GH (n=14)	GH / Placebo (n=13)
Boys / girls (n)	8 / 19	4 / 10	4 / 9
Genetic subtype			
Deletion / mUPD / ICD / translocation	9 / 15 / 2 / 1	2 / 10 / 1 / 1	7 / 5 / 1 / 0
Age (yrs)	17.2 (1.8)	17.2 (2.2)	17.3 (1.2)
Height for age (SDS)	-1.3 (0.9)	-1.3 (0.9)	-1.2 (0.9)
BMI for age (SDS)	0.9 (1.3)	1.0 (1.2)	0.7 (1.3)
Age at start GH treatment (yrs)	8.5 (3.5)	8.2 (3.8)	8.9 (3.2)
Duration of GH treatment (yrs)	8.7 (3.2)	8.9 (3.8)	8.4 (2.5)
IGF-I (SDS)	2.2 (1.0)	1.8 (1.2)	2.5 (0.8)
SSRT (n)	11	5	6
Tanner stage (M or G; 2 / 3 / 4 / 5)	5 / 3 / 10 / 9	2 / 3 / 4 / 5	3 / 0 / 6 / 4
FM%	38.0 (10.9)	39.4 (10.9)	36.4 (11.0)
FM (kg)	25.2 (10.0)	26.3 (10.3)	24.0 (9.9)
Lean mass (kg)	37.5 (7.8)	37.1 (8.3)	37.9 (7.6)
BMD_{TB} (SDS)	-0.7 (1.1)	-0.5 (0.9)	-1.0 (1.3)
BMD_{LS} (SDS)	-0.5 (1.1)	-0.2 (1.0)	-0.7 (1.2)
$BMAD_{LS}$ (SDS)	-0.1 (1.1)	0.2 (0.9)	-0.4 (1.2)
BMC (SDS)	-1.0 (1.0)	-0.8 (0.8)	-1.3 (1.1)

Data expressed as mean with (SD). BMI: body mass index. GH: growth hormone. IGF-I: insulin-like growth factor. SSRT: sex steroid replacement therapy. FM%: fat mass percentage. FM: fat mass. BMD: bone mineral density. TB: total body. LS: lower spine. BMAD: bone mineral apparent density. BMC: bone mineral content.

Bone mineral density at adult height

At AH, mean (SD) BMD_{TB}SDS and $BMAD_{LS}$SDS were -0.7 (1.1) and -0.1 (1.1), respectively (Table 1). Compared to healthy peers, mean BMD_{TB}SDS was significantly lower (p<0.01), while $BMAD_{LS}$SDS was similar (p=0.75). Four out of 27 patients had a BMD_{TB} lower than -2.0 SDS (14.8%), while none of the patients had a $BMAD_{LS}$SDS below -2.0 SDS.

Bone mineral density during GH versus placebo

Figure 1 shows BMD during one year GH and one year placebo. Compared to GH treatment, placebo did not significantly deteriorate BMD_{TB}SDS and $BMAD_{LS}$SDS (p=0.51 and p=0.37, resp.). During the two years, BMD_{TB}SDS did not significantly change (p=0.20), but $BMAD_{LS}$SDS declined significantly, independent of GH or placebo (p<0.01). Similarly, there was no significant difference in the change in BMD_{TB} and $BMAD_{LS}$ in g/cm^3 between GH and placebo, also after correcting for age and sex (data not shown). There were no bone fractures, neither during one year of GH nor during one year of placebo.

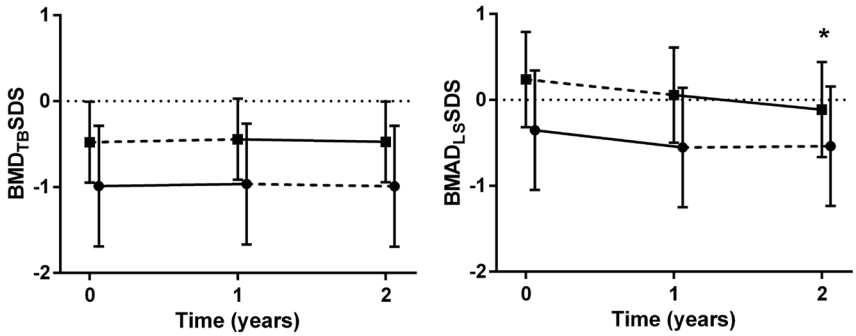

Figure 1. BMD_{TB}SDS and $BMAD_{LS}$SDS during 1 year of GH and 1 year of placebo, presented as means with 95% CI. The dotted lines represent the period with placebo. Significant changes in the total group compared to baseline are indicated with an asterisk.

Bone mineral density and the effect of SSRT in hypogonadal young adults

We evaluated the effect of no SSRT versus SSRT in hypogonadal young adults with PWS during the two-year study. Sixteen out of 19 girls (84.2%) and 7 out of 8 boys (87.5%) were hypogonadal of which 8 girls (50%) and 3 boys (42.9%) received SSRT. At AH, BMD_{TB}SDS and $BMAD_{LS}$SDS were similar in hypogonadal young adults with and without SSRT (p=0.49 and p=0.39, resp.), but the course of BMD_{TB}SDS and $BMAD_{LS}$SDS during the two-year study was significantly different between the two groups (Figure 2). In hypogonadal patients without SSRT, BMD_{TB}SDS slightly decreased from -0.8 to -0.9 SDS (p=0.11), while $BMAD_{LS}$SDS decreased from -0.2 at AH to -0.6 SDS during the two-year study (p=0.01), independent of GH or placebo. In hypogonadal patients receiving SSRT, BMD_{TB}SDS increased significantly from -1.1 at AH to -0.7 SDS during the two-year study (p<0.01), while $BMAD_{LS}$SDS remained similar (-0.5 SDS at AH and after two years, p=0.79). We also evaluated the effect of no SSRT versus SSRT only in hypogonadal women with PWS, but the results were similar to the results in the total group (data not shown). Results remained similar after adjusting for age, gender, genotype and body composition.

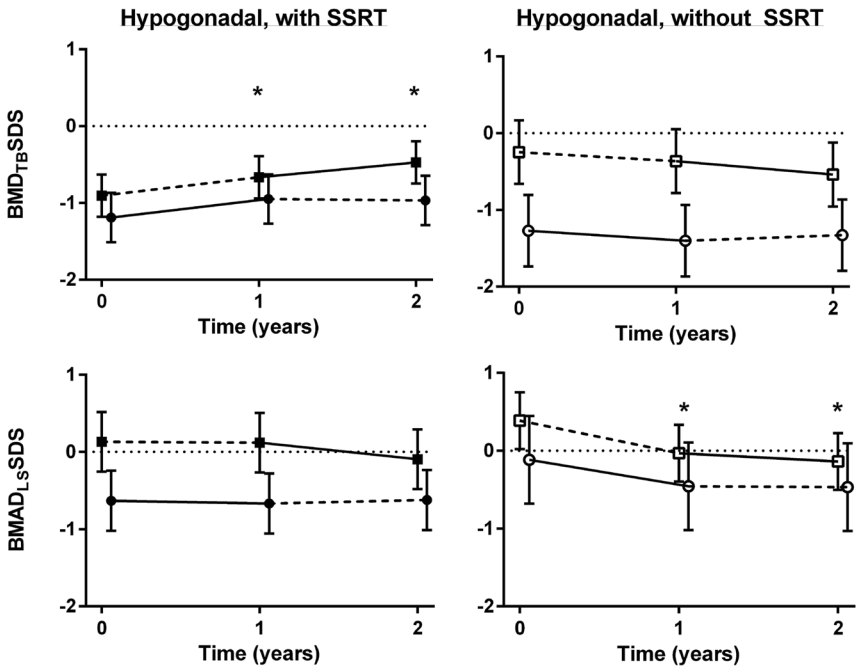

Figure 2. $BMD_{TB}SDS$ and $BMAD_{LS}SDS$ during 1 year of GH and 1 year of placebo, presented as means with 95% CI, in hypogonadal young adults with SSRT (figures on the left; black symbols) and hypogonadal young adults with PWS without SSRT (figures on the right; white symbols). The dotted lines represent the period with placebo. Significant changes in the total group compared to baseline are indicated with an asterisk.

DISCUSSION

This is the first, randomized, double-blind, placebo-controlled cross-over GH study in young adults with PWS who were treated with GH during childhood until attainment of AH, investigating the effects of cessation versus continuation of GH on BMD. Our study demonstrates that, $BMD_{TB}SDS$ and $BMAD_{LS}SDS$ were similar during one year of GH versus one year of placebo. Independent of GH or placebo, sex steroids had a significant role in the course of $BMD_{TB}SDS$ and $BMAD_{LS}SDS$ during the two years, as both $BMD_{TB}SDS$ and $BMAD_{LS}SDS$ decreased in hypogonadal young adults without SSRT, while $BMD_{TB}SDS$ improved in hypogonadal young adults who did receive SSRT.

Our study shows that, compared to GH treatment, one year of placebo does not cause a significant decrease in $BMD_{TB}SDS$ and $BMAD_{LS}SDS$ in young adults with PWS, who were treated with GH during childhood. This is in line with a study in GH-naïve adults with PWS (average age 29 years) who were randomized to GH or placebo for one year, followed by GH for two additional years. Despite an increase in

markers of bone formation, BMD_{TB}SDS did not significantly change, while $BMAD_{LS}$SDS significantly decreased during one year of GH compared to placebo. There were no changes in BMD_{TB}SDS or $BMAD_{LS}$SDS during the two-year open-label GH period[31]. In patients with adult-onset GH deficiency, GH replacement results in an initial increase in bone resorption, with a temporary decrease in BMD, and only after one year of GH treatment, a gradual increase in BMD[32]. It might be that one year is too short to show an effect of GH versus placebo on BMD. Another explanation could be that GH does not predominantly regulate BMD in young adults with PWS.

Our data show that, independent of GH or placebo, both BMD_{TB}SDS and $BMAD_{LS}$SDS decreased in hypogonadal young adults without SSRT, while BMD_{TB}SDS improved in hypogonadal young adults who did receive SSRT. This is in line with Jørgensen et al., who found a higher BMD_{TB}SDS in women with PWS on SSRT or with normal cyclic estrogen levels compared to hypogonadal women with PWS[31]. There is an important relation between estradiol and testosterone and BMD[33]. In women, adequate levels of estradiol prevent excessive bone resorption[34], while androgen stimulates the proliferation of preosteoblasts and differentiation of osteoblasts[33]. Previously, we observed a decrease in both BMD_{TB}SDS and $BMAD_{LS}$SDS in 64 pubertal children in parallel to a lack of pubertal progression and low sex hormone levels[4]. This occurred opposite to the required increase in BMD towards the peak bone mass, which is normally achieved in the early twenties. In this study, almost 15% of adolescents with PWS had a baseline BMD_{TB} lower than -2.0 SDS, which was partially caused by untreated hypogonadism. Not reaching an adequate peak bone mass, will lead to an increased fracture risk in later life[19,35].

Both a decreased BMD and an increased fracture risk have been described in non-GH treated adults with PWS[18,36]. Our data show that BMD SDS decreases in hypogonadal young adults with PWS, increasing the gap between hypogonadal young adults with PWS and healthy references. It seems that GH is not able to prevent this decline in BMD SDS unless it is combined with SSRT, indicating that sex steroids play a major role in the development and conservation of BMD in adolescents and (young) adults with PWS. Although the administration of SSRT was not randomized and our study might have had a selection bias, this suggests that SSRT should be considered in non-eugonadal adolescents with PWS to improve their bone health in adulthood. There is no consensus on when to start SSRT in adolescents with PWS and at which dose. Parents and physicians are reluctant to start SSRT, especially in male patients, because of worries about possible side effects (e.g. increase in behavioral problems). Very preliminary data show that side effects are rare if the dose of SSRT is increased slowly. Further studies are needed to confirm our results and to evaluate the optimal dose and starting age of SSRT and the long-term effects of combining GH with SSRT on BMD in people with PWS.

In conclusion, our study shows that, compared to GH, one year of placebo after attainment of AH does not deteriorate BMD_{TB}SDS and $BMAD_{LS}$SDS in young adults with PWS. As BMD SDS in hypogonadal young adults with PWS without SSRT decreased compared to those who did receive SSRT, our results suggest that GH is not able to prevent the decline in BMD SDS in hypogonadal young adults with PWS, unless it is combined with SSRT. Close monitoring of BMD in adolescents and young adults with PWS is recommended and SSRT should be considered in case of hypogonadism and a declining BMD SDS.

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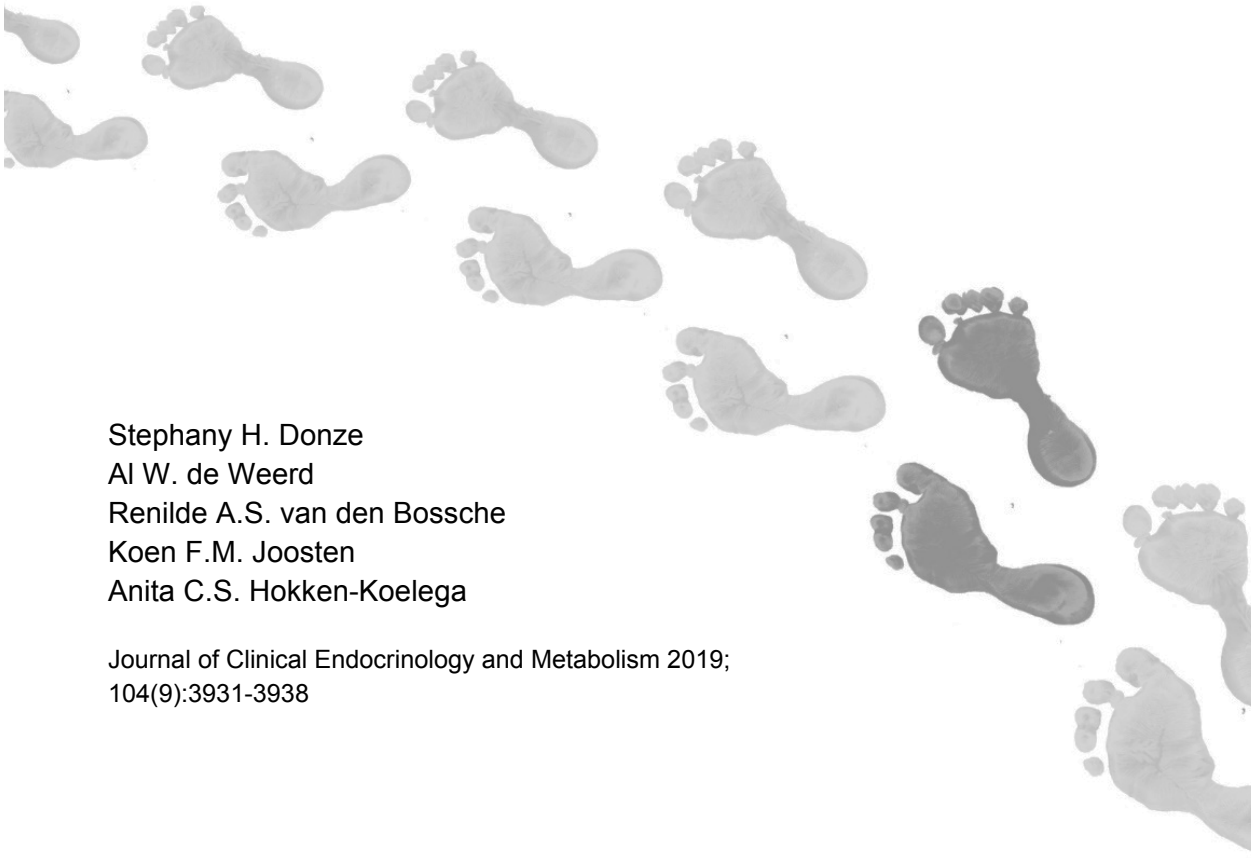
Chapter 6

Sleep-related breathing disorders in young adults with Prader-Willi syndrome: a placebo-controlled, cross-over GH trial



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ABSTRACT

Context Sleep-related breathing disorders (SRBD) are common in people with Prader-Willi syndrome (PWS). Young adults with PWS benefit from GH continuation after adult height by maintaining the improved body composition obtained during childhood. There are, however, no studies about the effects of GH on SRBD in young adults with PWS who were treated with GH during childhood.

Objective To investigate the effects of GH versus placebo on SRBD in young adults with PWS who were treated with GH during childhood and had attained adult height.

Design 2-year, randomized, double-blind, placebo-controlled, cross-over GH-study in 27 young adults with PWS, stratified for gender and BMI.

Intervention Cross-over intervention with GH (0.67 mg/m^2/day) and placebo, both during one year.

Main outcome measures Apnea hypopnea index (AHI), obstructive apnea index (OAI), central apnea index (CAI), measured by polysomnography.

Results Compared to placebo, GH treatment did not increase AHI, CAI or OAI ($p>0.35$). The effect of GH versus placebo was neither different between men and women, nor between patients with a deletion or mUPD/ICD. After 2 years, there was no difference in AHI, CAI or OAI compared to baseline ($p>0.18$). Two patients (7%) fulfilled the criteria of obstructive sleep apnea (OSA), regardless of GH or placebo.

Conclusions GH compared to placebo does not cause a significant increase in AHI, CAI or OAI in adults with PWS who were treated with GH during childhood and have attained adult height. Our findings are reassuring and prove that GH can be safely administered.

INTRODUCTION

Prader-Willi syndrome (PWS) is a genetic disorder, which results from the lack of expression of the PWS region on the paternally inherited chromosome 15, caused by a deletion, maternal uniparental disomy (mUPD), imprinting center defect (ICD) or translocation[1,2]. Hypothalamic dysfunction is an underlying cause for many symptoms related to PWS, which include muscular hypotonia and failure to thrive during infancy, and short stature, hyperphagia and obesity during childhood and adulthood. Both children and adults with PWS have an abnormal body composition with an increased fat mass (FM) and low lean body mass (LBM), even if there is no obesity[1,3].

Sleep-related breathing disorders (SRBD) are common in patients with Prader-Willi syndrome, causing poor sleep quality and excessive daytime sleepiness[4-6]. PWS children have a high Apnea Hypopnea Index (AHI), which is mainly due to central apneas and hypopneas[7]. Obstructive apneas occur more in children and adults with a higher BMI[7,8]. There are several other factors that are thought to influence SRBD in PWS, such as facial dysmorphia, lack of response to hypoxia and hypercapnia, and airway collapse caused by pharyngeal wall hypotonia and adenoid/tonsil hypertrophy[4,9]. We have also demonstrated that there might be an alteration in central ventilatory regulation during stress[10].

Currently, children with PWS are treated with long-term growth hormone (GH) improving the abnormal body composition, mental and motor development, cognition, adaptive functioning and linear growth[11-16]. There have been some reports on SRBD in PWS discussing the safety of GH treatment in children with PWS. We have described a non-significant decline in AHI after 6 months of GH treatment in 35 prepubertal children with PWS and a recent review has concluded that GH can be safely administered, provided that SRBD is monitored and treated appropriately[7,17].

When young adults with PWS have attained adult height, they have to stop GH treatment, unless they are GH deficient. Recently, we found that young adults with PWS benefit from continuation of GH compared to placebo by maintaining the improved body composition obtained during childhood[18]. To our knowledge, there are no studies about the effects of GH versus placebo on SRBD in young adults with PWS who were treated with GH during childhood. We, therefore, investigated the effects of one year of GH versus one year of placebo on SRBD, measured by polysomnography (PSG), in young adults with PWS who had attained adult height and were treated with GH during childhood, in a two-year, randomized, double-blind, placebo-controlled cross-over study. We hypothesized that GH would not negatively influence SRBD. Secondly, we investigated the prevalence of obstructive sleep apnea (OSA) in our cohort of young adults with PWS.

METHODS

Patients

Inclusion criteria were (1) genetically confirmed diagnosis of PWS; (2) GH treatment during childhood for at least two years until adult height and on GH at time of inclusion; and (3) adult height attainment, defined as complete epiphyseal fusion and a height velocity less than 0.5 cm per six months. Exclusion criteria were (1) medication to reduce weight or fat or (2) non-cooperative behavior. From June 2008 to January 2014, 33 young adults with PWS fulfilled the inclusion criteria. Three refused participation due to too large burden of hospital visits and two did not want to continue daily injections. Of the 28 included patients, one 16.7-year old participant (BMI 25.0 kg/m^2) died of gastric rupture and necrosis three months after inclusion while receiving placebo. Her data were excluded from the present analyses.

During childhood, patients were treated with the standard GH dose of 1.0 mg/m^2/day. High serum insulin-like growth factor (IGF)-I levels caused lowering of the GH dose in eight children. Eleven patients received an adult dose of sex steroid replacement therapy (40.7%), 8 patients were using thyroid hormone supplementation (29.6%), two modafinil (7.4%), and one patient was using risperidone and citalopram.

Design

Two-year, randomized, double-blind, placebo-controlled, cross-over study investigating the effects of one year of GH treatment versus one year of placebo on sleep-related breathing disorders (SRBD). Young adults were stratified according to gender and BMI (below/above 25 kg/m^2) and then randomly and blindly assigned to receive one year of subcutaneous injections once daily at bedtime of either 0.67 mg/m^2/day GH (Genotropin®, 5 mg/ml, Pfizer) or one year of identical appearing placebo (Pfizer), after which they crossed-over to the alternative treatment for another year. An independent statistician generated the random allocation sequence. Investigators, patients and parents were blinded for the allocation. An independent physician monitored the safety during the study. Unblinding was not necessary.

Measurements

Patients were examined every three months by the PWS-team of the Dutch Growth Research Foundation, in collaboration with pediatric endocrinologists and pediatricians in the Netherlands. At each visit, the injection dose was adjusted to the calculated body surface area. In addition, patients visited the Erasmus Medical Center at baseline, 6, 12, 18 and 24 months, to obtain body composition, height and weight. FM and LBM were measured by DXA (Lunar Prodigy; GE Healthcare). All scans were made on the same machine, with daily quality assurance. FM was expressed as percentage of total body weight (FM%). FM% SDS and LBM SDS were calculated according to age- and sex-matched Dutch reference values[19]. Standing height was measured with a calibrated Harpenden stadiometer (Holtain Ltd., Crosswell, UK), weight was determined on a calibrated scale (ServoBalance KA-20-

150S) and BMI was calculated. Height, weight and BMI were expressed as SDS[20,21]. SDS values were calculated with GrowthAnalyser 4.0 (available at www.growthanalyser.org).

At start of the study, after one year and after 2 years, a polysomnography (PSG) was performed at the Sleep-Wake Center SEIN, Zwolle, The Netherlands. Young adults were admitted to the sleep center at 17.00h, accompanied by one parent. We inquired for typical symptoms of OSA at each visit. Recordings included electroencephalogram, electro-oculogram, one channel derivation of electrocardiogram, and surface electromyography of the submental muscle and both anterior tibial muscles. Nasal-oral airflow was monitored by nasal pressure prongs fixed in the nose, respiratory effort by thoraco-abdominal gauges and oxygen saturation (SaO_2) by pulse oximetry. All polysomnographic records were evaluated independently by 2 persons, both certified in PSG analysis, and scored according to the adult rules of the American Academy of Sleep Medicine (AASM) Manual[22]. Seventeen patients also had a PSG during childhood GH treatment.

An obstructive apnea was defined as a reduction of airflow of 90% or more for at least 10 seconds with continuous breathing effort during the apnea. An apnea was considered central if the respiratory event was associated with absent breathing effort for 10 seconds or more with an arousal or a decrease in SaO_2 of at least 3%. Hypopneas were defined as a reduction of airflow of at least 30% for 10 seconds or more, with a decrease in SaO_2 of at least 3% or an arousal[22]. The number of obstructive, central and mixed apneas and hypopneas were counted during the total sleep time and an Apnea-Hypopnea Index (AHI) was calculated per hour of sleep. The Obstructive Apnea Index (OAI) was defined as the number of obstructive apneas per hour of sleep and the Central Apnea Index (CAI) as the number of central apneas per hour of sleep. OSA was defined as an AHI ≥ 5 events/h associated with typical symptoms of OSA (e.g., unrefreshing sleep, daytime sleepiness, fatigue or insomnia, awakening with gasping or choking sensation, snoring, witnessed apneas) or an AHI ≥ 15 events/h, regardless of symptoms[23].

Assays

Blood samples were collected after an overnight fast and serum IGF-I levels measured in one laboratory. IGF-I was measured using an immunometric technique on Immulite 1000 (LKGF1, Siemens Medical Solutions Diagnostics) with an interassay variation <7.3%. IGF-I levels were expressed as SDS, adjusting for age and gender[24].

Statistical analysis

Statistical analyses were performed with SPSS 24.0. Data were expressed as mean (SD) in case of a Gaussian distribution and as median (interquartile range (IQR)) in case of a non-Gaussian distribution. Gender and genotypic differences in AHI, CAI

and OAI were calculated by independent samples T-tests or Mann-Whitney U tests depending on the distribution.

Effects of GH versus placebo were calculated using the following formulas for 1) AHI: (ΔAHI during GH - ΔAHI during Placebo) / 2; 2) (ΔCAI during GH - ΔCAI during Placebo) / 2 and 3) (ΔOAI during GH - ΔOAI during Placebo) / 2. The mean outcome of these calculations was compared to 0 using a one-sample t-test. We did not find carry-over or period effects, which were analyzed by comparing the change in AHI, CAI and OAI during GH and placebo between the two treatment sequences (GH followed by placebo and placebo followed by GH) and during the first and second year of the study, respectively. AHI, CAI and OAI at start and after 2 years in the current study were compared to AHI, CAI and OAI during the childhood PSG using Wilcoxon Signed Ranks tests. Correlations between AHI, CAI or OAI and height SDS, BMI SDS, FM% SDS or IGF-I SDS were calculated by Spearman's correlation analysis. P-values less than 0.05 were considered statistically significant.

Study approval

Written informed consent was obtained from patients and parent(s) / caregiver(s). The study protocol was approved by the Medical Ethics Committee of Erasmus University Medical Center, Rotterdam, and registered at the Dutch Trial Register (www.trialregister.nl NTR1038).

RESULTS

Baseline characteristics at adult height

Clinical characteristics

Baseline clinical characteristics of the 27 young adults with PWS who participated in this study after attainment of adult height are shown in Table 1. Mean (SD) age and BMI were 17.2 (1.8) years and 0.9 (1.3) SD-score (SDS), respectively. The group that received GH in the second year of the study consisted of more patients with an mUPD or ICD (p=0.046). There were no other significant differences between the treatment groups (all p>0.24).

Sleep-related breathing

Baseline median (IQR) total sleep time was 8.1 (7.0; 8.7) hours and AHI 3.6 (1.4; 4.7) events/hour (Table 2). Total sleep time was longer in the patients who received GH in the second phase of the RCT (p=0.02). The other polysomnographic characteristics were similar in both treatment arms (all p>0.06). Four participants had an abnormal AHI of ≥ 5 events/hour at baseline (14.8%), but none of them had typical symptoms of OSA and therefore did not fulfil the current criteria of OSA in adults [23].

Table 1. Baseline characteristics of the total group and per treatment schedule

	PWS (n=27)	Placebo / GH (n=14)	GH / Placebo (n=13)
Boys / girls (n)	8/19	4/10	4/9
Genetic subtype			
Deletion / mUPD / ICD / translocation	9 / 15 / 2 / 1	2 / 10 / 1 / 1*	7 / 5 / 1 / 0
Age (yrs)	17.2 (1.8)	17.2 (2.2)	17.3 (1.2)
Duration of childhood GH treatment (yrs)	8.7 (3.2)	8.9 (3.8)	8.4 (2.5)
Adult height (SDS)	-1.3 (0.9)	-1.3 (0.9)	-1.2 (0.9)
BMI (kg/m^2)	24.1 (4.0)	24.6 (4.1)	23.6 (4.1)
BMI (SDS)	0.9 (1.3)	1.0 (1.2)	0.7 (1.3)
IGF-I (SDS)	2.2 (1.0)	1.8 (1.2)	2.5 (0.8)
FM%	38.0 (10.9)	39.4 (10.9)	36.4 (11.0)
FM% (SDS)	1.7 (0.7)	1.8 (0.7)	1.6 (0.7)
Lean mass (kg)	37.5 (7.8)	37.1 (8.3)	37.9 (7.6)
Lean mass (SDS)	-1.6 (1.0)	-1.6 (1.0)	-1.6 (0.9)

Data expressed as mean with (SD). GH: growth hormone. FM%: fat mass percentage.

**The group that received GH in the second year of the study consisted of more patients with an mUPD or ICD (p=0.046).*

Table 2. Baseline sleep-related breathing in the total group and per treatment schedule

	PWS (n=27)	Placebo / GH (n=14)	GH / Placebo (n=13)
Total Sleep Time (hours)	8.1 (7.0; 8.7)	8.4 (7.7; 8.9)*	7.4 (6.5; 8.1)
Apnea hypopnea index	3.6 (1.4; 4.7)	3.4 (1.0; 4.7)	3.8 (1.7; 4.8)
Central apnea index	1.1 (0.6; 2.0)	1.0 (0.5; 2.3)	1.1 (0.6; 1.9)
Obstructive apnea index	0.0 (0.0; 0.3)	0.0 (0.0; 0.2)	0.0 (0.0; 0.6)
Duration longest apnea (seconds)	22.2 (18.6; 27.9)	23.3 (20.1; 28.1)	20.4 (18.1; 25.1)
Median sleep SaO_2 (%)	96.0 (95.5; 96.7)	96.1 (94.6; 96.7)	96.0 (95.6; 96.8)
Minimum SaO2 (%)	90.0 (86.0; 92.0)	89.0 (83.0; 92.0)	91.0 (88.5; 92.5)

Data expressed as median (IQR). SaO_2: oxygen saturation.

**Total Sleep Time was significantly longer in the patients who received GH in the second phase of the RCT (p=0.02).*

Male PWS patients had a higher median AHI and CAI than female PWS patients (4.7 vs. 2.1 and 1.9 vs. 0.8, resp., both p=0.01). Median OAI was 0 in both male and female patients (p=0.67). There was no difference in AHI and CAI between young adults with a deletion and an mUPD or ICD (all p>0.24). OAI was slightly higher in patients with an mUPD or ICD than in patients with a deletion (0.2 vs. 0.0, p=0.04). Neither height SDS nor BMI SDS, FM% SDS or IGF-I SDS at adult height were associated with AHI, CAI or OAI at adult height.

Sleep-related breathing during GH versus placebo

Compared to placebo, one year of GH did not increase AHI, CAI or OAI (p>0.35) (Figure 1). The effect of GH versus placebo on AHI, CAI or OAI was neither different between boys and girls (p>0.23), nor between young adults with a deletion or an mUPD or ICD (p>0.14). After 2 years, there was no significant difference in AHI, CAI or OAI compared to baseline, regardless of GH or placebo (p>0.18).

Table 3 shows the 11 patients with an AHI of ≥ 5 events/hour during at least one of the PSG's performed during the 2-year study. At start of the study, 4 patients had an AHI of ≥ 5 events/hour. Seven patients developed an AHI of ≥ 5 events/hour during the study, 5 during placebo and 2 during GH. Two of them fulfilled the current criteria of OSA (7% of the total group), because they also developed mild complaints of

snoring and daytime sleepiness, one during GH, the other during placebo [23]. AHI in these 2 patients consisted mainly of hypopneas, OAI being 0 and 1.2 events/hour. Both were obese and had tonsillar hypertrophy, for which they were referred to an otolaryngologist. One patient with an AHI ≥ 5 events/hour and moderate complaints of daytime sleepiness did not fulfil the criteria of OSA, because he was also diagnosed with narcolepsy and on modafinil during the study.

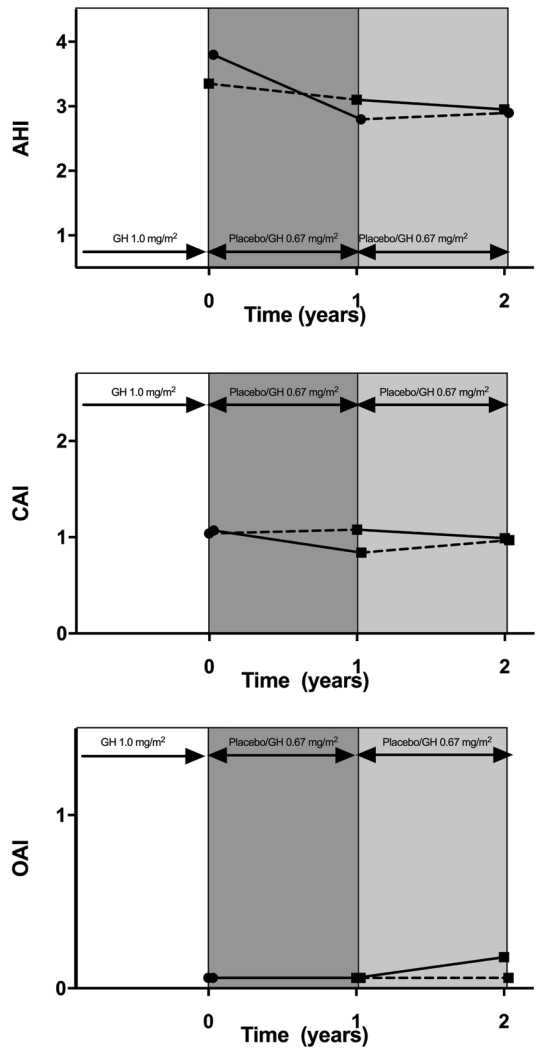

Figure 1. Apnea Hypopnea Index (AHI), Central Apnea Index (CAI) and Obstructive AI (OAI) during one year of GH and one year of placebo, presented as median. The dotted lines represent the period with placebo. There were no significant changes during GH versus placebo.

Table 3. Patients with an Apnea Hypopnea Index > 5 events/hour during the study

Patient no. (genotype)	Age at baseline	Gender	Complaints	Tonsillar hypertrophy		AHI			
						GH phase	At baseline	After 1 year	After 2 years
1 (DEL)	18.9	M	no	no	Apnea Hypopnea Index	1	**5.2**	**5.5**	**7.4**
					BMI (kg/m^2)		18.4	18.4	19.6
2 (DEL)	18.3	F	no	no	Apnea Hypopnea Index	1	**6.6**	**11.3**	**7.0**
					BMI (kg/m^2)		29.9	31.4	36.3
3 (DEL)	17.4	F	mild	mild	Apnea Hypopnea Index	1	1.7	4.3	**5.4**
					BMI (kg/m^2)		28.6	32.2	31.6
4 (UPD)	17.9	M	no	no	Apnea Hypopnea Index	1	4.5	5.0	**9.9**
					BMI (kg/m^2)		19.2	18.8	20.3
5 (UPD)	15.9	F	no	no	Apnea Hypopnea Index	2	**5.0**	3.1	4.6
					BMI (kg/m^2)		24.1	24.9	25.9
6 (DEL)	20.2	M	no	no	Apnea Hypopnea Index	2	**11.1**	3.1	3.0
					BMI (kg/m^2)		24.3	24.2	24.6
7 (UPD)	14.5	F	mild	mild	Apnea Hypopnea Index	2	1.9	1.6	**5.8**
					BMI (kg/m^2)		30.8	36.4	36.5
8 (ICD)	18.8	M	moderate	no	Apnea Hypopnea Index	2	4.7	4.7	**5.8**
					BMI (kg/m^2)		24.9	26.4	24.4
9 (UPD)	18.4	M	no	no	Apnea Hypopnea Index	2	4.7	**5.9**	-
					BMI (kg/m^2)		32.3	32.2	32.3
10 (UPD)	16.1	F	no	no	Apnea Hypopnea Index	2	4.2	**6.6**	3.7
					BMI (kg/m^2)		24.6	26.2	26.0
11 (UPD)	18.7	M	no	no	Apnea Hypopnea Index	2	0.6	**8.1**	2.9
					BMI (kg/m^2)		18.5	19.6	19.5

The grey areas represent the year in which participants received GH treatment. Patient 3 and 7 fulfilled the diagnostic criteria of OSA, because they also developed mild complaints of snoring anddaytime sleepiness. Patient 8 was also diagnosed with narcolepsia and therefore did not fulfil the diagnostic criteria of OSA.

Sleep-related breathing during childhood and adulthood

Seventeen participants (62.9%) had a PSG during childhood GH treatment (Table 4). AHI ranged from 1.0 to 14.7 events/hour and 6 children had an AHI of ≥ 5 events/hour (35%). Only two of these children were reported to have obstructive apneas, OAI being 1.9 and 2.0 events/hour.

Table 4. Sleep-related breathing during childhood, at adult height and after 2 years

	Childhood (n=17)		Adult height (n=17)		After 2 years (n=14)	
Age (years)	10.0	(8.0; 11.5)	17.4	(15.2; 18.3)*	19.3	(16.7; 20.4)*
Total Sleep Time (hours)	8.4	(7.7; 8.7)	8.2	(7.2; 8.9)	7.9	(7.4; 9.0)
Apnea hypopnea index	4.1	(2.6; 6.2)	3.8	(1.4; 4.8)	3.0	(1.2; 5.5)
Central apnea index	1.2	(0.6; 2.8)	1.0	(0.5; 2.1)	1.0	(0.3; 1.5)
Obstructive apnea index	0.0	(0.0; 0.4)	0.0	(0.0; 0.9)	0.0	(0.0; 0.5)
Duration longest apnea (seconds)	18.0	(16.0; 24.5)	22.3	(19.3; 27.2)	21.9	(17.8; 33.8)
Median sleep SaO_2 (%)	92.0	(87.0; 98.0)	96.0	(95.5; 96.5)*	96.1	(95.0; 97.0)*
Number of awakenings	13	(7; 18)	14	(11; 21)	16	(11; 26)

Data expressed as median (IQR). SaO_2: oxygen saturation.

*Wilcoxon Signed Rank Tests were performed. * = p<0.05 compared to childhood PSG.*

Compared to the childhood PSG, AHI, CAI and OAI were neither significantly higher at adult height nor after 2 years in the study (all p>0.21). The change in AHI, CAI or OAI from childhood to adulthood was neither different between boys and girls (p>0.09), nor between patients with a deletion or an mUPD or ICD (p>0.13).

DISCUSSION

This randomized, double-blind, placebo-controlled cross-over GH study in young adults with PWS who were treated with GH during childhood until attainment of adult height, investigated the effects of GH versus placebo on sleep-related breathing. We have previously shown that young adults with PWS benefit from continuation of GH by maintaining the improved body composition obtained during childhood, without safety concerns regarding their metabolic health profile[18,25]. Our study demonstrates that, compared to placebo, GH does not cause an increase in AHI, CAI or OAI in young adults with PWS. These findings are reassuring and prove that GH can be safely administered with regard to SRBD.

Only one other study examined SRBD during GH treatment in 10 adults with PWS[26]. The authors performed a PSG at baseline and 6 weeks after the initiation of GH treatment. Nine of 10 adults showed an improvement in AHI. One adult with worsening of the AHI had a concurrent respiratory infection and tonsillar hypertrophy[26]. In children with PWS, several studies investigated the effects of GH on SRBD[7,27-29]. We previously found a non-significant decline in AHI after 6 months of GH treatment in 35 prepubertal children with PWS with a median age of 6.0 years at start of GH[7]. Al-Saleh et al. reported no significant change in obstructive apnea hypopnea index (OAHI) and CAI during 2 years of GH treatment in 15 children with PWS with a median age of 3.7 years at start of GH[27]. The same research group

investigated OAHI and CAI in 28 young children with PWS and found a significant improvement of the CAI and no significant difference in OAHI between the baseline and follow-up PSG, regardless of GH treatment[28]. Overall, studies about the effects of GH on SRBD in children and adults with PWS conclude that GH treatment does not negatively influence AHI. However, medical professionals should be aware that (mild) upper respiratory tract infections and/or adenotonsillar hypertrophy can cause increased obstructive apneas[7,29].

The reported prevalence of OSA in people with PWS is variable, due to wide age ranges, different diagnostic criteria and a potential referral bias[5,6,30]. Recent guidelines define OSA in adults as an AHI of ≥ 5 events/hour combined with typical complaints for OSA (e.g. daytime sleepiness, snoring, or witnessed apneas), or an AHI of ≥ 15 events/hour regardless of complaints[23]. According to these guidelines, two participants in our study (7%) fulfilled the diagnostic criteria of OSA, regardless of GH or placebo. However, only one of them had obstructive apneas during sleep with an OAI of merely 1.2 events/hour. Based on these patients we would suggest, besides evaluating AHI, to specifically assess the number of obstructive apneas. OAI might be a more accurate parameter than AHI for diagnosing OSA.

We could compare a PSG performed during childhood GH treatment with a PSG performed at adult height and after 2 years in the current study in 17 and 14 participants, respectively. There was no significant difference in AHI, OAI or CAI between the childhood PSG and the PSG at adult height or the PSG after 2 years in the study, regardless of GH treatment or placebo. During the childhood PSG, 35% of children had an AHI of ≥ 5 events/hour. At start and finish of the current study, respectively 15% and 22% of young adults, had an AHI of ≥ 5 events/hour. These data demonstrate that long-term GH treatment during childhood has no negative consequences with regard to the prevalence of SRBD in young adults with PWS.

We have previously shown that long-term GH treatment in combination with a healthy lifestyle can counteract the clinical course of PWS during childhood and reduce the prevalence of morbid obesity[12]. Median baseline BMI of the participants in the current study was 24.1, which is much lower than (young) adults with PWS who were never treated with GH. Also, unlike previous reports, we did not find a correlation between AHI and BMI or FM% SDS. It is likely that, due to the reduced prevalence of obesity in our cohort of young adults with PWS, SRBD occurs less common.

The current consensus guideline for GH treatment in PWS recommends performing a PSG before and 3-6 months after starting GH treatment in children with PWS[31]. Based on our data in adults with PWS, we would not recommend a standard PSG at attainment of adult height in non-obese patients with PWS who were treated with GH during childhood and will continue GH treatment in adulthood. However, clinical signs of SRBD need to be monitored and, when indicated, a PSG should be performed.

In conclusion, our study shows that, compared to placebo, AHI does not significantly increase during GH treatment in young adults with PWS who were previously treated with GH during childhood. GH treatment has positive effects on body composition and health profile in adults with PWS. The results of the current study are reassuring and prove that GH can be safely administered. However, clinical signs of SRBD need to be monitored in children and adults with PWS, as obstructive apneas occur in patients with adenotonsillar hypertrophy and/or (mild) upper respiratory tract infections.

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Chapter 7

Evidence for accelerated biological ageing in young adults with Prader-Willi syndrome

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ABSTRACT

Objective Adults with Prader-Willi syndrome (PWS) are at increased risk of developing age-associated diseases early in life and, like in premature ageing syndromes, ageing might be accelerated. We investigated leukocyte telomere length (LTL), a marker of biological age, in young adults with PWS and compared LTL to healthy young adults of similar age. As all young adults with PWS were treated with growth hormone (GH), we also compared LTL in PWS subjects to GH-treated young adults born short for gestational age (SGA).

Design Cross-sectional study in age-matched young adults; 47 with PWS, 135 healthy and 75 born SGA.

Measurements LTL measured by quantitative PCR, expressed as T/S ratio.

Results Median (IQR) LTL was 2.6 (2.4; 2.8) at a median (IQR) age of 19.2 (17.7; 21.3) years in PWS, 3.1 (2.9; 3.5) in healthy young adults and 3.1 (2.8; 3.4) in the SGA group. Median LTL in PWS was significantly lower compared to both control groups ($p<0.01$). In PWS, a lower LTL tended to be associated with a lower total IQ ($r=0.35$, $p=0.08$). There was no association between LTL and duration of GH treatment, cumulative GH dose or several risk factors for cardiovascular disease or type 2 diabetes mellitus.

Conclusions Young adults with PWS have significantly shorter median LTL compared to age-matched healthy young adults and GH-treated young adults born SGA. The shorter telomeres might play a role in the premature ageing in PWS, independent of GH. Longitudinal research is needed to determine the influence of LTL on ageing in PWS.

INTRODUCTION

Prader-Willi syndrome (PWS) is a rare disorder resulting in a variable phenotype with muscular hypotonia and failure to thrive during infancy, and short stature, mental retardation, hyperphagia and obesity in childhood and adulthood[1,2]. PWS is caused by a lack of expression of the PWS region (q11-q13) on the paternally derived chromosome 15, mostly caused by a paternal deletion or maternal uniparental disomy (mUPD) and in some cases by an imprinting center defect (ICD) or paternal chromosomal translocation[1,3]. Growth hormone (GH) is an approved treatment for children with PWS improving body composition, linear growth, physical strength, cognition and adaptive functioning[4-9]. Studies have shown that GH treatment also benefits adults with PWS, with a sustained improvement in body composition when GH is continued after attainment of adult height[10,11]. However, up to now GH treatment is not registered for adults with PWS. Studies in adults with PWS who were not treated with GH describe an increased risk of age-associated diseases early in life, e.g. diabetes mellitus type 2 (T2DM), cardiovascular disease (CVD), and cognitive decline[12,13]. The mortality rate of people with PWS was estimated to be 3% per year across all ages, rising to 7% in those aged over 30[14]. One explanation for the increased mortality rate and risk of age-associated diseases could be that, like in premature ageing syndromes, the ageing process is accelerated in PWS[15-18].

Ageing is characterized by a progressive time-dependent decline of normal tissue and organ function and recent studies have shown that telomere shortening is involved in this process[19-22]. Telomeres are highly conserved TTAGGG tandem repeat DNA sequences at the end of each chromosome arm. Their main function is to protect the end of the chromosomes from inappropriate DNA repair mechanisms, preventing the loss of crucial DNA. Telomeres shorten during proliferation and telomere length declines as a function of chronological age. When telomere length becomes critically short, the cell enters either senescence (i.e. irreversible cessation of division) or apoptosis (i.e. programmed cell death). The accumulation of senescent cells might be driving the process of tissue and organismal ageing[21-23].

We hypothesized that accelerated biological ageing could partly explain the increased mortality rate and increased risk of developing age-associated diseases early in life in adults with PWS. Since telomeres are suggested to play a role in biological ageing and telomere length had not yet been studied in people with PWS, we investigated leukocyte telomere length (LTL) in young adults with PWS and compared LTL to healthy young adults of similar age. As all young adults who participated in this study were treated with GH, we also investigated LTL in young adults born short for gestational age (SGA) who were also treated with GH. We hypothesized that LTL would be shorter in young adults with PWS compared to both groups, independent of GH treatment. Finally, we assessed whether cognitive functioning and putative risk factors for T2DM and CVD correlated with LTL.

METHODS

Patients

We included 47 young adults participating in the Dutch Young Adult PWS (YAP) study, whose primary objective was to evaluate the effects and safety of GH treatment in young adults with PWS who were treated with GH during childhood. Inclusion criteria were 1) genetically confirmed diagnosis of PWS by a positive methylation test, 2) at least two years of GH treatment during childhood, and (3) having attained adult height, defined as a height velocity less than 0.5 cm per six months and epiphyseal closure as demonstrated by a radiograph of the left hand and wrist. Exclusion criteria were (1) medication to reduce weight (fat) or (2) non-cooperative behaviour.

We compared LTL in GH-treated young adults with PWS to healthy participants from the PROGRAM cohort[24] and to young adults born SGA of similar age treated with ≥ 7 years of GH (1 mg/m^2/day) because of their short stature[25]. The healthy participants from the PROGRAM cohort were all 1) 17 - 24 years, 2) born singleton, 3) Caucasian and 4) had an uncomplicated neonatal period without severe asphyxia, sepsis, or long-term complications of respiratory ventilation and/or oxygen supply. The Medical Ethics Committee of the Erasmus University Medical Center approved the study protocols. Written informed consent was obtained from patients and/or their parents or legal representatives.

Anthropometric measurements

All patients with PWS were followed at the Dutch PWS Reference Center and treated with biosynthetic GH (Pfizer Inc., New York, NY) during childhood in a dose of 1.0 mg/m^2/day (≈0.035 mg/kg/day) and, at the time of LTL measurement, in a dose of 0.33 mg/m^2/day (≈0.012 mg/kg/day). The GH dose was regularly adjusted based on calculated body surface area and serum insulin-like growth factor (IGF)-I levels. Standing height was measured using a Harpenden Stadiometer and weight on a calibrated electric scale (ServoBalance KA-20-150S). Height, weight and body mass index (BMI) were expressed as standard deviation score (SDS) using GrowthAnalyser 4.0 (available at www.growthanalyser.org), adjusting for age and sex according to Dutch reference values[26,27]. Systolic and diastolic blood pressure was measured using an appropriately sized cuff while sitting and expressed as SDS, adjusting for height and sex[28].

Body composition and cognitive functioning

Body composition was assessed by dual-energy x-ray absorptiometry (DXA, Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Total fat mass (FM; kg) and lean body mass (LBM; kg) were assessed. All scans were made on the same machine, with daily quality assurance. The intra-assay coefficient of variation (CV) for fat tissue was 0.41 to 0.88% and for LBM 1.57 to 4.49%[29]. FM was also expressed as percentage of total body weight (FM%). LBM was calculated as fat-free mass minus

bone mineral content. FM% SDS was calculated according to age- and sex-matched Dutch reference values and LBM SDS according to height- and sex-matched Dutch reference values[30]. The Wechsler Adult Intelligence Scale (WAIS) was used to assess total IQ (TIQ) in patients over 16 years of age[31] and the Wechsler Intelligence Scale (WISC) in patients below 16 years of age[32].

Laboratory measurements

Blood samples were collected after overnight fasting. Genomic DNA was isolated from peripheral leukocytes using standard procedures. DNA samples were kept frozen at -20°C until assayed. All LTL measurements were performed in the same laboratory at the University of Leicester, UK. LTL was measured by the quantitative PCR-based technique as previously described[33-35]. Telomere sequence copy number (T) and single copy gene (36B4,S) were measured in separate reactions and calculated relative to a calibrator sample (genomic DNA from K562 cell line) included on each run. Leukocyte telomere length was subsequently expressed as T/S ratio. For quality control, all samples were checked for concordance between duplicate values and samples with >0.2 cycle difference in take-off value were excluded and re-run. Samples which amplified outside of the linear range of the assay were also excluded and re-run. Reproducibility of the assay was tested by re-running 47 samples of the age-matched healthy participants and the SGA group together with the PWS samples[36]. The correlation between the original and new LTL results was 0.87 and the mean CV 6.4%.

Blood levels of glucose, insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDLc) and high-density lipoprotein cholesterol (HDLc), triglyceride (TG) were determined in the Biochemical and Endocrine laboratories of the Erasmus Medical Center, Rotterdam.

Statistical analysis

Statistical analyses were performed with SPSS 24.0 (SPSS Inc., Chicago, IL). LTL was expressed as T/S ratio and data as median (interquartile range, IQR). Continuous variables of young adults with PWS, healthy young adults and young adults born SGA were compared using Kruskal-Wallis and Mann-Whitney U tests and categorical variables were compared using Chi-Square tests. ANCOVA was used to correct for age, gender, gestational age, birth length and birth weight SDS.
In young adults with PWS, gender and genotypic differences in clinical characteristics were calculated by independent samples T-tests in case of a Gaussian distribution and by Mann-Whitney U tests in case of a non-Gaussian distribution. Correlations between LTL and anthropometric measurements, body composition, cognitive functioning and metabolic health parameters were calculated by Pearson correlation analysis in case of a normal distribution and by Spearman correlation analysis in case of a non-Gaussian distribution. *P* values less than 0.05 were considered statistically significant.

RESULTS

Clinical characteristics

Forty-seven young adults with PWS (24 females) with a median age of 19.2 (17.7; 21.3) years participated in the current evaluation of LTL (Table 1). We compared LTL to 135 age-matched healthy participants (71 females, median age 20 years) and to 75 young adults born SGA (33 females, median age 20 years) who were treated with GH during childhood because of their short stature. The distribution of males and females was similar between the 3 groups. Gestational age, BMI and FM% in the PWS group were significantly higher and LBM significantly lower compared to the healthy participants and the SGA group (p<0.02). Age, birth weight SDS, birth length SDS and adult height SDS were lower in young adults with PWS compared to healthy participants (p<0.04). Compared to the SGA group, birth weight, birth length, adult height SDS and duration of GH treatment were higher in young adults with PWS (p<0.02), and age was similar (p=0.47).

Table 1. Clinical characteristics of young adults with PWS, healthy young adults and young adults born SG**A**

	PWS	Healthy	SGA
Men / women (n)	23 / 24	64 / 71	42 / 33
Genetic subtype			
Deletion/mUPD/ICD/#	21 / 22 / 3 / 1	NA	NA
Gestational age (weeks)	40.6[1] (38.9; 41.7)	37.0 (33.0; 40.0)	37.4 (33.0; 40.0)
Birth weight (SDS)	-1.2[1] (-2.1; -0.1)	0.4 (-0.4; 1.0)	-2.7 (-3.3; -1.6)
Birth length (SDS)	-1.1[1] (-2.2; -0.2)	0.1 (-0.4; 0.7)	-3.1 (-4.5; -2.3)
Age (yrs)	19.2[2] (17.7; 21.3)	20.0 (19.0; 22.0)	20.0 (18.0; 21.7)
Height (SDS)	-1.0[2,3] (-1.8; -0.3)	0 (-0.4; 0.7)	-1.5 (-2.0; -0.8)
BMI (kg/m^2)	25.0[1] (21.8; 27.5)	21.8 (20.5; 23.4)	20.2 (18.8; 22.1)
BMI for age (SDS)	1.2[1] (0.0; 1.8)	-0.1 (-0.6; 0.6)	-0.9 (-1.4; 0.1)
Duration of GH treatment (years)	13.0[3] (11.5; 14.0)	NA	9.2 (7.1; 11.0)
Fat percentage (%)	39.8[1] (36.0; 43.6)	22.4 (14.3; 31.4)	18.9 (11.6; 27.8)
Fat percentage (SDS)	2.2[1] (1.8; 2.5)	0.6 (-0.1; 1.1)	0.5 (-0.1; 1.1)
Lean mass (SDS)	-1.3[2,3] (-2.1; -0.5)	-0.6 (-1.3; 0.1)	-0.7 (-1.6; 0.1)
Telomere length (T/S ratio)	2.6[1] (2.4; 2.8)	3.1 (2.9; 3.5)	3.1 (2.8; 3.4)

Data expressed as median (IQR).

mUPD = maternal uniparental disomy. ICD = imprinting center defect. # = genotype unknown. BMI = Body mass index.

1) p<0.01 compared to healthy young adults and SGA group.

2) p<0.04 compared to healthy young adults.

3) p<0.02 compared to SGA group.

Telomere length in PWS and control groups

Median LTL was 2.6 (2.4; 2.8) in the PWS group (Figure 1). Forty-four young adults with PWS (94%) had a LTL below the 50th percentile of healthy young adults and 20 (43%) below the 10th percentile. The healthy age-matched young adults and the young adults born SGA had a similar median (IQR) LTL, being 3.1 (2.9; 3.5) and 3.1 (2.8; 3.4), respectively (Figure 1). Median LTL was significantly lower in young adults with PWS compared to both control groups (p<0.01), also after correction for age, gender, gestational age, birth weight and birth length SDS.

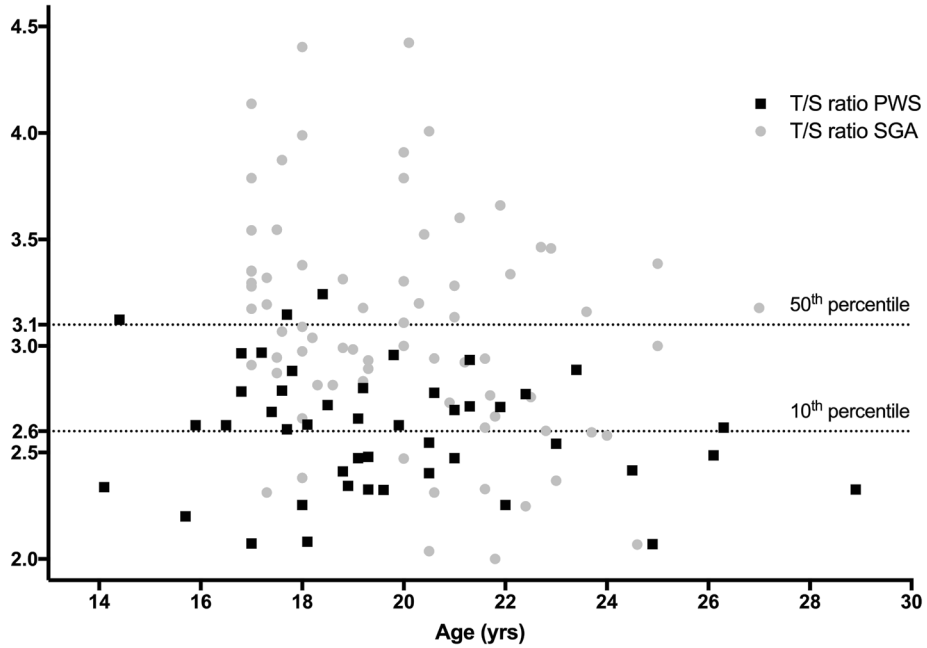

Figure 1. Leukocyte telomere length for age in 47 GH-treated young adults with PWS and 75 young adults born SGA who were also treated with GH. The dotted lines represent the 10th and 50th percentile of the healthy young adults. Forty-four young adults with PWS (94%) have a LTL below the 50th percentile and 20 (43%) below the 10th percentile of the healthy young adults.

Correlation analyses in PWS

Age was not significantly associated with LTL (r=-0.18, p=0.22), which could be related to the fact that subjects in our study population had approximately the same age. There was no significant difference in LTL between males and females, median (IQR) LTL being 2.5 (2.3; 2.8) in males and 2.7 (2.4; 2.8) in females with PWS (p=0.35). Median (IQR) LTL was similar in young adults with a deletion and a mUPD/ICD, being 2.6 (2.4; 2.8) in both groups (p=0.97). There was no significant association between LTL and gestational age, birth weight, birth height, adult height, BMI or FM% (p>0.28). A lower LTL tended to be associated with a lower LBM SDS and a lower total IQ (r=0.29, p=0.06 and r=0.36, p=0.08, resp.), showing that LBM SDS and cognitive functioning in young adults with PWS tend to be lower in those with a shorter LTL. Since GH treatment could potentially cause increased replicative stress by inducing catch-up growth, we analysed whether LTL was associated with the duration of GH treatment or the cumulative GH dose, but we found no significant association between either. Table 2 shows metabolic health parameters in young adults with PWS. Neither blood pressure SDS and serum cholesterol levels, nor HOMA-IR were associated with LTL (p>0.21).

Table 2. Metabolic health parameters in 47 young adults with PWS

Systolic blood pressure (SDS)	0.8 (0; 1.6)
Diastolic blood pressure (SDS)	0.6 (0.3; 1.2)
Total Cholesterol (mmol/L)	4.3 (3.9; 4.8)
HDL (mmol/L)	1.3 (1.1; 1.5)
LDL (mmol/L)	2.8 (2.3; 3.2)
Triglycerides (mmol/L)	0.8 (0.7; 1.2)
HOMA-IR	1.7 (1.2; 2.9)

Data expressed as median (IQR).
HDL = high-density lipoprotein cholesterol. LDL = low-density lipoprotein cholesterol.
HOMA-IR = Homeostatic Model Assessment for Insulin Resistance.

DISCUSSION

This cross-sectional study in 47 GH-treated young adults with PWS shows that median leukocyte telomere length (LTL) is shorter in young adults with PWS compared to age-matched healthy young adults and young adults born SGA who were also treated with GH. We found no significant association between LTL and the duration of GH treatment or the cumulative GH dose. This is the first study to show that a shorter LTL might play a role in the reported accelerated ageing process in adults with PWS, independent of GH treatment[15-17]. Sinnema et al described excess functional impairment, morbidity and mortality and evidence of premature ageing in 12 adults with PWS above the age of 50 years[17]. Ageing is characterized by a progressive time-dependent functional decline of tissues and telomere shortening is considered to be involved in this process[20,21]. Individuals with shorter telomere length show increased mortality risk, providing support for an association between telomere length and lifespan[37].

Besides the finding that LTL is shorter in young adults with PWS, we found a tendency towards an association between a shorter LTL and a lower total IQ, which might imply a role of LTL in cognitive functioning in PWS. A cross-sectional MRI-study in 20 young adults with PWS showed that predicted brain age was on average 8.7 years higher than chronological age[38]. Together with a brain structure resembling healthy older brains, indicative of premature neuronal loss and atrophy, this suggests premature brain ageing in young adults with PWS[38]. The same research group found a 2.5-year higher predicted brain age than chronological age in 46 adults with Down syndrome. A higher brain-predicted age was significantly associated with lower cognitive functioning[39] and shorter telomeres have been associated with cognitive decline and dementia status in patients with Down syndrome[40,41]. These studies support that people with PWS and Down syndrome age prematurely. Our study suggests a possible role of shorter LTL in this accelerated ageing process in adults with PWS. However, longitudinal studies on LTL, cognitive functioning and brain development in a larger cohort of children and (older) adults with PWS are needed to

elucidate the natural course of brain development in relation to LTL and cognitive functioning.

There is a strong correlation between telomere length in different tissues in humans and in other mammals, which shows that telomere length in leukocytes reflects systemic telomere length in other tissues[42]. Besides chronological ageing, a wealth of genetic and environmental factors are reported to affect telomere length[22]. Even though none of the genes in the PWS region on chromosome 15 are associated with telomere homeostasis, several clinical features of people with PWS are associated with an increased risk of shorter telomeres, including obesity, a reduced level of physical activity and increased psychosocial stress. Furthermore, adults with PWS are prone to develop T2DM and CVD in early life[12,13] and several studies have shown that shortened telomeres are associated with an increased risk of T2DM and CVD[20,22,37]. As none of the investigated young adults with PWS were diagnosed with T2DM and CVD, we investigated the interaction between LTL and putative risk factors for T2DM and CVD. We found no significant correlation between LTL and age, BMI, FM%, blood pressure, serum cholesterol levels and HOMA-IR. This is in accordance with an earlier study demonstrating that the association between LTL and CVD is independent of risk factors for CVD[35]. Probably our study group of young adults with PWS was too young to already have T2DM or CVD. Also, with the more recent trends of early diagnosis, GH treatment from a young age, and the enhanced prevention of potentially impairing health conditions, T2DM and CVD might occur later in life. It would be interesting to analyse the association between (risk factors for) T2DM and CVD and LTL at a later age, when age-associated diseases become more apparent.

All young adults with PWS who participated in the current study were treated with long-term GH improving body composition, linear growth, physical strength, cognition and adaptive functioning[4-9]. To evaluate whether GH treatment would cause increased replicative stress and shorter LTL, we compared young adults with PWS to young adults born SGA who were also treated with GH. The fact that LTL in young adults with PWS was shorter compared to both groups and similar in healthy young adults and GH-treated young adults born SGA, make adverse effects of long-term GH treatment on LTL very unlikely[36]. Besides, the lack of an association between LTL and the duration of GH or the cumulative GH dose in young adults with PWS is reassuring with regard to possible negative effects of GH on LTL.

One could even argue that GH treatment might positively influence LTL by improving body composition. We know that long-term GH treatment during childhood counteracts the clinical course of increasing obesity in children with PWS and has substantially changed their phenotype[4]. Combined with an early diagnosis and multidisciplinary support from a very young age, GH treatment has resulted in a new generation of children and young adults with PWS without severe obesity. Thus, it could be that if our group of young adults had not been treated with GH, they would

have had a higher BMI and FM% and their LTL could have been even shorter. The limited age range of our PWS cohort is both a strength and a constraint of our study, since it eliminates the confounding effect of age, but restricts generalizations of the results to other age groups. Also, we were not able to compare LTL in GH-treated young adults with PWS to (young) adults with PWS who were not treated with GH and more research is needed on LTL across the life course to be able to determine its role in ageing in both GH-treated and untreated people with PWS.

In contrast to previous studies, we found no significant difference in median LTL between males and females. Females have been reported to have longer LTL, which is thought to be due to higher levels of oestrogen, conferring anti-inflammatory and antioxidant properties and promoting telomerase expression[22]. Since serum oestrogen levels are generally low in females with PWS, this could explain the lack of a difference between males and females in our study[43]. We also found similar LTL in young adults with a deletion and those with a mUPD or ICD. A recent study by Whittington et al reported on 26 adults with PWS over the age of 40 years. In 3 participants (age 41, 48 and 55) there was a significant deterioration in executive functioning with possible dementia. All were women, had UPD or a disomic region and all had had psychotic episodes[18]. Psychiatric disorders are reported more commonly in people with a mUPD and a higher level of psychosocial stress is associated with shorter telomeres[22]. Based on these studies, a difference in LTL between the deletion and mUPD subtypes was expected. However, since the study group of Whittington et al. is considerably older than our group and none of the affected individuals were treated with GH, it is difficult to compare results. We suggest investigating possible differences in LTL between genetic subtypes in future studies.

In conclusion, we found a shorter LTL in 47 GH-treated young adults with PWS compared to untreated healthy young adults and young adults born SGA who were also treated with GH, which might suggest that a shorter LTL is involved in the reported accelerated ageing process in adults with PWS. More research on LTL across the life course is needed to be able to determine its exact role in ageing in people with PWS.

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Chapter 8

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

GENERAL DISCUSSION

In the last few decades, considerable progress has been made in the knowledge and care of children and (young) adults with Prader-Willi syndrome (PWS). The Dutch Growth Research Foundation started investigating children with PWS and the effects and safety of growth hormone (GH) treatment in 2002. Thereafter, the care for Dutch children and adolescents markedly improved. These efforts eventually led to the development of the Dutch PWS Reference Center, which currently provides high-quality care to children and adults with PWS in close collaboration with paediatricians, paediatric-endocrinolgists and internists throughout the Netherlands. Nowadays, children with PWS are treated with GH from a very young age and, combined with an early diagnosis and multidisciplinary support, this counteracts the clinical course of increasing obesity and has resulted in a new generation of children and young adults with PWS.

This thesis describes six new studies about children and young adults with PWS. In this chapter our results are discussed, also in view of recent literature. Furthermore, clinical implications of our results and recommendations for future research are presented.

8.1 MENTAL AND MOTOR DEVELOPMENT - INFANTS

Children with PWS are generally delayed in reaching major mental and motor developmental milestones. Mental development entails acquirement of mental capabilities, such as language, memory and problem solving, while motor development refers to the acquirement of fine and gross motor developmental milestones. Our group previously showed that delayed brain maturation may underlie the delayed psychomotor development in children with PWS, as we found a smaller white matter volume, indicating reduced structural connectivity and aberrant myelinisation[1].

The second chapter of this thesis describes the effects of 3 years of GH treatment on psychomotor development in 63 children with PWS with a median age of 1.0 year at start of GH. Mental and motor development were measured using the Bayley Scales of Infant Development, an individually administered and standardized instrument for developmental diagnostics that can be used to evaluate the cognitive and motor development of young children with a ‘developmental age’ between 0 and 3.5 years[2,3]. Mental and motor development were expressed as percentage of the expected mental and motor development for their age and calculated as (developmental age / chronological age) * 100%.

Our results demonstrate that both mental and motor development increase significantly during 3 years of GH: mental development increases from 58.1% of expected at start of GH to 79.6% of expected after 3 years of GH and motor

development from 41.9% to 78.2%. One explanation for this improvement could be that GH and insulin-like growth factor (IGF)-I are involved in brain growth, development and myelinization. GH receptors are expressed throughout the brain and serum IGF-I levels are low in children with PWS prior to starting GH treatment. Therefore, GH might improve brain development by increasing IGF-I levels[4,5]. Another explanation may be that mental development improved because GH treatment improves motor development by increasing lean body mass (LBM). Once infants and toddlers reach certain motor milestones, they would be able to better explore their environment, enhancing mental development[6,7]. Although there was no association between the change in LBM and the change in motor development, the significant correlation between the change in motor and mental development during 3 years of GH supports this hypothesis.

We cannot exclude that part of the increase in psychomotor development may be explained by the increased awareness of PWS and the spontaneous improvement of hypotonia with age and early start of physical therapy, as has been suggested in a study comparing the effect of 1 year of GH or Co-enzyme Q10 in 26 infants and toddlers with PWS[7]. We acknowledge that an RCT would have been the first-choice design to investigate the effects of 3 years of GH on mental and motor development in infants with PWS, but it would be unethical to withhold GH for 3 years, knowing the positive effects of GH on numerous outcomes in children with PWS. Also, we previously performed an RCT during 1 year in 29 infants with PWS, which showed that mental and motor development deteriorated in those who were not treated with GH[6]. This shows that GH treatment plays an important role in psychomotor development in infants with PWS, aside from the spontaneous improvement of hypotonia with age and early start of physical therapy.

Our study also shows that the younger the age at start of GH treatment, the greater the improvement in psychomotor development. It suggests that starting GH treatment early, in a critical period of neurodevelopment, could enhance psychomotor and cognitive development on the longer term[5,8]. This is in line with 2 of our previous studies[9,10]. The first study also reported a significant positive effect of age at start of GH on cognitive development in 50 pre-pubertal children[9]. The second study in 42 children with PWS found that if GH was started during infancy, GH had a greater effect on adaptive functioning[10]. In spite of the significant improvement in psychomotor development, the average mental and motor development after 3 years of GH was still significantly lower compared to healthy references, indicating that young children with PWS keep needing extra support with regard to their development.

We conclude that mental and motor development increase during 3 years of GH, reducing the gap between infants with PWS and healthy peers. Secondly, we conclude that, the younger at start of GH treatment, the greater the improvement in psychomotor development.

8.2 COGNITIVE DEVELOPMENT - CHILDREN

In chapter 3 we describe the benefits of long-term GH treatment in children with PWS on cognitive functioning. People with PWS typically have mild to moderate cognitive impairment with an average IQ between 60 and 70[9,11]. Previous studies have only investigated the effects of GH on cognitive functioning in children with PWS who received GH treatment for a maximum duration of 4 years. We, therefore, conducted a prospective cohort study during 8 years of GH in 43 children with PWS, who started GH at a median age of 8.1 years. We longitudinally investigated cognitive functioning during 8 years of GH and, secondly, cross-sectionally investigated whether starting GH during infancy, i.e. before the age of 2 years, results in higher cognitive functioning after 8 years of GH. Cognitive functioning was assessed by a shortened version of the Wechsler Intelligence Scale for Children (WISC), which is suitable for children between the age of 6 and 16 years[14]. The subtests Vocabulary, Similarities and Block Design were used to assess vocabulary, abstract verbal reasoning and visuospatial skills, respectively[15].

The results of our study show that visuospatial skills slightly improve during 8 years of GH, while abstract verbal reasoning and vocabulary remain similar. After 8 years of GH, estimated total IQ ranged from 51 to 94 and 30% of the children had an estimated TIQ above 70, the cut-off for mental disability. We previously demonstrated in a 2-year randomized controlled trial that cognitive functioning in GH-treated children with PWS developed at a similar rate as in healthy references, while there was a significant deterioration in abstract verbal reasoning and vocabulary in untreated controls[9]. Furthermore, we have previously shown that visuospatial skills and abstract verbal reasoning improved significantly during 4 years of GH treatment in 50 children with PWS. In the current study, the change in visuospatial skills and abstract verbal reasoning during 8 years of GH was not significant. Nonetheless, our results are reassuring as they show that cognitive functioning does not deteriorate during long-term GH treatment and progresses at a similar rate compared to healthy peers.

Previous studies have shown that cognitive impairment in PWS may be related to lower brain volumes and lower cortical complexity, due to alterations in gene networks that are important for early brain development[1,16]. It is, however, still unknown how the genetic aberrations underlying PWS lead to cognitive impairment. Another question that remains to be elucidated is how GH could affect cognitive functioning. A recent review concluded that GH could stimulate GH receptors in brain areas involved in learning and memory, thereby improving cognitive functioning[5]. It is known that GH receptors are located throughout the brain and that GH and IGF-I affect the genesis of neurons, thereby stimulating brain growth, development and myelinisation[4,13]. In our study we found an association between visuospatial skills and serum IGF-I levels, with a tendency towards significantly better visuospatial skills with higher serum IGF-I levels. A study in untreated adults with PWS also reported a

correlation between lower IGF-I levels and poorer intellectual skills[17]. This indicates that GH treatment might improve cognitive functioning by increasing IGF-I levels in patients with PWS. We should, however, also keep in mind that GH has effects on the central nervous system that are independent of IGF-I levels[18]. Therefore, further investigation is still warranted.

After 8 years of GH, 22 children who started GH before the age of 2 years had significantly higher vocabulary skills and a higher estimated total IQ, compared to the children who started GH during childhood, suggesting that early start of GH could be beneficial for cognitive functioning on the long term. Even though we could not evaluate the longitudinal effects of 8 years of GH in the 22 children who started GH during infancy, because WISC is not suitable for children younger than 6 years, these results support previous studies showing beneficial effects of early start of GH on psychomotor and cognitive development[8,19,20].

We conclude that cognitive skills in children with PWS during 8 years of GH treatment do not deteriorate and develop at the same pace as healthy references. We also conclude that starting GH treatment before the age of 2 years, a critical period of neurodevelopment, might benefit cognitive functioning on the long-term.

8.3 GROWTH HORMONE DEFICIENCY - YOUNG ADULTS

Some features of people with PWS resemble those seen in growth hormone deficiency (GHD), such as short stature, and an abnormal body composition with a low LBM and an increased fat mass (FM). The benefits of GH treatment in children with PWS are well established as it improves body composition, bone mineral density (BMD), adaptive functioning and linear growth[10,12,21]. Studies have also shown that continuation of GH treatment after attainment of adult height is beneficial for people with PWS, with a sustained improvement in FM and LBM, and a deterioration of body composition when GH treatment is discontinued[22,23]. However, GH treatment is currently not approved for adults with PWS and can only be reimbursed when they fulfil the criteria of adult GHD.

We, therefore, examined the prevalence of adult GHD in 60 previously GH-treated young adults with PWS with a median age of 17.9 years. As the insulin tolerance test is not feasible in patients with PWS because of the cumbersome procedure and side effects, we performed a GHRH-Arginine test. Consensus guidelines for the diagnosis of adult GHD using a GHRH-Arginine test require a GH peak level < 9 µg/L in combination with a serum IGF-I SDS level < -2 adjusted for age and gender[24,25]. We also applied the BMI-dependent cut-off points for the GHRH-Arginine test: a GH peak < 11.5 µg/L if BMI is < 25 kg/m^2, a GH peak < 8 µg/L if BMI is 25-30 kg/m^2 and a GH peak < 4.2 µg/L If BMI > 30 kg/m^2[2,26].

Our results demonstrate that serum IGF-I was <-2 SDS in only 2 (3%) patients and serum IGFBP-3 was within the normal range in all but one patient. Nine participants had a GH peak < 9 µg/L (15%) during the GHRH-Arginine test. None of these patients also had an IGF-I < -2.0 SDS. Therefore, not one patient fulfilled the criteria for adult GHD, also when BMI-dependent criteria were used. Although GHD cannot be demonstrated by conventional testing, it does not mean that it does not exist. It is generally accepted that people with PWS have a dysfunction of the hypothalamus, which complicates the evaluation of GHD as most GH stimulation tests stimulate secretion of GH from the pituitary. Also, there is discussion about the appropriate cut-off values for GH peak during GHRH-Arginine tests. We chose to use the BMI-adjusted adult cut-off values since the decision of whether GH treatment is reimbursed is made based on these criteria.

The fact that there is hypothalamic dysfunction in PWS and both children and adults with PWS respond very well to GH treatment, with a significant improvement in body composition, health profile, normalization of stature in children, and a significant increase in serum IGF-I and IGFBP-3 levels strongly supports the likelihood of GHD in these patients. As the GHRH-Arginine test fails to demonstrate adult GHD it seems to be an unreliable test in adults with PWS.

Currently, GH treatment can only be reimbursed when adults fulfil the consensus criteria of adult GHD. Our study shows that a GHRH-Arginine test to diagnose adult GHD is unreliable in adults with PWS. As GH treatment has been shown to have very positive effects in both children and adults with PWS, GH treatment is highly recommended for adults with PWS and confirming adult GHD should not be a prerequisite for treating adults with PWS.

8.4 BONE MINERAL DENSITY - YOUNG ADULTS

The prevalence of osteopenia and osteoporosis in adults with PWS is increased[34-36]. Bone mineral density (BMD) and peak bone mass are influenced by endocrine factors such as GH, IGF-I and sex steroids, and by body composition and body mass index[27-29]. Recent studies have shown that long-term GH treatment optimizes body composition and BMD in prepubertal children with PWS[21,30], leading to a normal BMD compared to peers[21,31]. During puberty, however, we found a decline in BMD in parallel to incomplete pubertal development and low sex hormone levels[21,32,33].

When young adults with PWS without adult GHD have attained adult height, they have to stop GH treatment. In GH-deficient young adults without PWS, BMD deteriorates after cessation of GH[37-39]. The fifth chapter of this thesis describes the effects of one year of GH versus one year of placebo on BMD in 27 young adults with PWS who had attained adult height and were treated with GH during childhood. We also studied the effects of no sex steroid replacement therapy (SSRT) versus SSRT on BMD in hypogonadal young adults with PWS. BMD of the total body and lumbar

spine (BMD_{TB} and BMD_{LS}) were measured by DXA and BMD_{LS} was corrected for bone size by calculating bone mineral apparent density of the lower spine ($BMAD_{LS}$) and compared to age- and sex-matched references[40,41].

Our data show that compared to 1 year of GH, 1 year of placebo did not deteriorate BMD_{TB} or $BMAD_{LS}$. This is in line with a study in GH-naïve adults with PWS who were randomized to GH or placebo for 1 year, followed by GH for 2 additional years[42]. In that study, BMD_{TB} did not significantly change, while $BMAD_{LS}$ significantly decreased during 1 year of GH compared to placebo. There were no changes in BMD_{TB} or $BMAD_{LS}$ during the 2 years of GH treatment[42]. In patients with adult-onset GHD, GH replacement results in an initial increase in bone resorption, and only after 1 year of GH treatment, a gradual increase in BMD[45]. Thus, it could be that 1 year of GH versus 1 year of placebo is too short to show a positive effect of GH on BMD in young adults with PWS who were treated with GH during childhood.

Our study also shows that BMD_{TB} did not significantly change during 2 years, while $BMAD_{LS}$ decreased, independent of GH or placebo. We previously observed a decrease in BMD_{TB} and $BMAD_{LS}$ SDS in 64 pubertal children, in parallel to a lack of pubertal progression and low sex hormone levels[21]. Because there is an important relation between serum oestradiol and testosterone levels and BMD[43,44], we suggest that the increased prevalence of hypogonadism in PWS might cause the decrease in BMD. Our results demonstrate that both BMD_{TB} and $BMAD_{LS}$ decrease in hypogonadal young adults without SSRT, while BMD_{TB} improves in hypogonadal young adults who receive SSRT. Another study also found a higher BMD_{TB} in women with PWS on SSRT or with normal cyclic oestrogen levels compared to hypogonadal women with PWS[42]. It seems that GH is not able to prevent the decline in BMD unless it is combined with SSRT, indicating that sex steroids play a major role in the development and conservation of BMD in adolescents and (young) adults with PWS. Although the administration of SSRT was not randomized, our findings suggest that SSRT should be considered in hypogonadal adolescents with PWS to improve their bone health in adulthood.

We conclude that, compared to GH treatment, 1 year of placebo after attainment of adult height does not deteriorate BMD in young adults with PWS. In addition, our data suggest that GH is not able to prevent the decline in BMD in hypogonadal young adults with PWS, unless it is combined with SSRT.

8.5 SLEEP-RELATED BREATHING DISORDERS - YOUNG ADULTS

Sleep-related breathing disorders (SRBD) are common in patients with PWS, causing poor sleep quality and excessive daytime sleepiness[46-48]. Several factors are thought to influence SRBD in PWS, such as facial dysmorphia, lack of response to hypoxia and hypercapnia, and airway collapse caused by pharyngeal wall hypotonia and adenoid/tonsil hypertrophy[4]. Since 2002, children with PWS are being treated

with long-term GH[12,49,50] and in the early years there were safety concerns regarding the effects of GH on SRBD. These concerns were, however, unfounded, as we have described a non-significant decline in apnea hypopnea index (AHI) after 6 months of GH in 35 prepubertal children with PWS and a recent review has concluded that GH can be safely administered, provided that SRBD is monitored and treated appropriately[54].

Since GH treatment is not registered for adults with PWS, young adults with PWS have to stop GH treatment after attainment of adult height, unless they fulfil the criteria for adult GHD. We have previously shown that young adults with PWS benefit from continuation of GH, without safety concerns regarding their metabolic health profile[22,51]. There were, however, no studies about the safety of GH with regard to SRBD. The sixth chapter of this thesis, therefore, investigated the effects of one year of GH versus one year of placebo on SRBD in a randomized, double-blind, placebo-controlled cross-over GH study. A polysomnography (PSG) was performed at start of the study, after one year and after two years in 27 young adults with PWS who had attained adult height and were treated with GH during childhood. The number of obstructive, central and mixed apneas and hypopneas were counted during the total sleep time and an AHI, Obstructive Apnea index (OAI) and Central Apnea Index (CAI) were calculated per hour of sleep.

Our results show that, compared to placebo, one year of GH did not increase AHI, OAI or CAI. Furthermore, after two years in the study, there was no difference in AHI, CAI or OAI compared to baseline, regardless of GH or placebo. These findings are reassuring and prove that GH can be safely administered to young adults with PWS with regard to SRBD. Our study is in line with a previous study that found an improvement in AHI in 9 out of 10 GH-treated adults with PWS[52]. We also evaluated the prevalence of obstructive sleep apnea (OSA). Recent guidelines define OSA in adults as an AHI ≥ 5 events/h combined with typical complaints for OSA (e.g. daytime sleepiness, snoring or witnessed apneas) or an AHI ≥ 15 events/h regardless of complaints[53]. According to these guidelines 2 patients in our study (7%) fulfilled the criteria of OSA, one after 1 year of GH, the other after 1 year of placebo. However, only one of them had obstructive apneas during sleep with an OAI of merely 1.2 events/hour. Based on these patients we would suggest, besides evaluating AHI, to specifically assess the number of obstructive apneas. OAI might be a more accurate parameter than AHI for diagnosing OSA in PWS.

The reported prevalence of OSA in people with PWS is variable, but generally higher than the 7% we found in our cohort. One reason could be that the median baseline BMI of the participants in the current study was 24.1 kg/m^2, which is much lower than (young) adults with PWS who were never treated with GH. We also did not find a correlation between AHI and BMI or FM% SDS, unlike in previous reports. It is likely that, due to the reduced prevalence of obesity in our cohort, SRBD occur less common. Based on our data in adults with PWS, we would not recommend standard

PSG at attainment of adult height in non-obese patients with PWS who were treated with GH during childhood and will continue GH in adulthood. However, medical professionals should be aware that (mild) upper respiratory tract infections and/or adenotonsillar hypertrophy can cause increased obstructive apneas[54,55].

We conclude that, compared to placebo, GH treatment does not increase AHI in young adults with PWS who were previously treated with GH during childhood. These results are reassuring and prove that GH can be safely administered. However, clinical signs of SRBD need to be monitored in children and adults with PWS, as obstructive apneas can occur in patients with adenotonsillar hypertrophy and/or (mild) upper respiratory tract infections.

8.6 LEUKOCYTE TELOMERE LENGTH - YOUNG ADULTS

Studies in GH-untreated adults with PWS describe an increased risk of developing age-associated diseases in early adulthood, such as diabetes mellitus type 2 (T2DM), cardiovascular disease (CVD) and cognitive decline[10,35]. An approximate death rate of 3% per year was calculated across all ages, rising to 7% in those aged over 30 years[56]. As described in premature ageing syndromes, the ageing process might be accelerated in PWS[57-60] and may partly explain the increased mortality rate and the increased risk of developing age-associated diseases early in life. Ageing is characterized by a progressive time-dependent decline of normal tissue and organ function and recent studies have shown that telomere shortening is involved in this process[61-64]. Telomeres are highly conserved TTAGGG tandem repeat DNA sequences at the end of each chromosome arm. Their main function is to protect the end of the chromosomes from inappropriate DNA repair mechanisms, preventing the loss of crucial DNA. Telomeres shorten during proliferation and telomere length declines as a function of chronological age[63-65]. Individuals with shorter telomere length show an increased mortality risk, providing support for an association between telomere length and lifespan[66].

The seventh chapter of this thesis investigated leukocyte telomere length (LTL) in 47 GH-treated young adults with PWS with a median age of 19.2 years. LTL was compared to 135 age-matched healthy young adults. Because all young adults with PWS were treated with GH, we also compared LTL to 75 young adults born short for gestational age (SGA) who were also treated with GH. LTL was measured using a quantitative PCR-based technique, telomere sequence copy number (T) and single copy gene (36B4,S) were measured in separate reactions and calculated relative to a calibrator sample (genomic DNA from K562 cell line) included on each run. LTL was subsequently expressed as T/S ratio[67].

Our results demonstrate that LTL in young adults with PWS is shorter compared to age-matched healthy young adults and GH-treated young adults born SGA. There are no other studies on LTL in PWS, but previous reports described excess

functional impairment, morbidity and mortality and evidence of premature ageing in 12 adults with PWS above the age of 50 years[35]. Shorter LTL might play a role. Another interesting finding in our group was the tendency of a lower IQ in subjects with a shorter LTL, which might imply a role of LTL in cognitive functioning in PWS. A cross-sectional MRI-study in 20 young adults with PWS showed that predicted brain age was 8.7 years higher than chronological age and their brain pattern resembled those of older brains[68]. Even though LTL was not measured in the latter study, there could be a possible role of LTL in accelerated brain ageing in adults with PWS. Longitudinal studies on LTL, cognitive functioning and brain development in a larger cohort of children and (older) adults with PWS are needed to elucidate the natural course of brain development in relation to LTL and cognitive functioning.

In addition to chronological ageing, many genetic and environmental factors, which are often present in people with PWS, have been reported to affect telomere length, including obesity, a reduced level of physical activity and increased psychosocial stress[64]. Also, several studies have shown that shortened telomeres are associated with an increased risk of T2DM and CVD[62,64,66] and adults with PWS are prone to develop T2DM and CVD in early life. We found no significant association between LTL and age, BMI, FM%, blood pressure, serum cholesterol levels or insulin sensitivity, which could be explained by the young age of our study group and positive effects of GH treatment on (determinants) of T2DM and CVD. It would be interesting to analyze the association between (risk factors for) T2DM and CVD and LTL in subjects with PWS at a later age, when age-associated diseases become more apparent.

To evaluate whether GH treatment would cause increased replicative stress and shorter LTL, we compared young adults with PWS to young adults born SGA who were also treated with GH. Despite the fact that subjects with PWS were treated with GH for a significantly longer time than those born SGA, we found no association between LTL and the duration of GH treatment or the cumulative GH dose in young adults with PWS. This is reassuring with regard to possible negative effects of GH on LTL. Furthermore, LTL was similar in healthy young adults and GH-treated young adults born SGA, which makes adverse effects of long-term GH treatment on LTL very unlikely[69]. We could even argue that GH treatment might positively influence LTL, because long-term GH treatment during childhood counteracts the clinical course of increasing obesity in children with PWS and has resulted in a new generation of children and young adults with PWS without severe obesity. It could be that LTL would have been even shorter in our group of young adults with PWS if they had not been treated with GH and had a higher BMI and FM%. More research is needed on LTL across the life course to be able to determine its role in ageing in both GH-treated and untreated people with PWS.

We conclude that LTL was shorter in GH-treated young adults with PWS compared to untreated healthy young adults and young adults born SGA who were also treated with GH during childhood. This suggests that a shorter LTL might be involved in the reported accelerated ageing process in adults with PWS. More research on LTL across the life course is needed to be able to determine its role in ageing in people with PWS.

8.7 GENERAL CONCLUSIONS AND CLINICAL IMPLICATIONS

Since 2002, our longitudinal PWS studies investigated the short and long-term effects of GH treatment in a large group of children and young adults with PWS. In this thesis we describe 6 new studies regarding GH treatment in children and young adults with PWS. Chapter 2 shows that mental and motor development improve during 3 years of GH, reducing the gap between infants with PWS and healthy peers. It also shows that the younger the age at start of GH treatment, the greater the improvement in psychomotor development. This suggests that starting GH treatment early, in a critical period of neurodevelopment, could enhance psychomotor and cognitive development on the longer term. The mean mental and motor development after 3 years of GH was, however, still lower than in healthy references, indicating that young children with PWS need extra support with regard to their development.

We also demonstrate that visuospatial skills slightly improve during 8 years of GH in children with PWS, while abstract verbal reasoning and vocabulary remain similar. These results are reassuring as cognitive functioning did not deteriorate and developed at a similar rate compared to healthy references. After 8 years of GH, children who started GH before the age of 2 years had significantly better vocabulary skills and a higher estimated total IQ, compared to children who started GH during childhood, suggesting that early start of GH could be beneficial for cognitive functioning on the long term. Combined with an early diagnosis and multidisciplinary support from a very young age, long-term GH treatment during childhood counteracts the clinical course of increasing obesity in PWS and substantially changes the phenotype of children with PWS. This study provides further support for the importance of GH treatment in children with PWS with regard to cognitive functioning.

In the fourth chapter of this thesis we show that not one patient fulfills the criteria for adult growth hormone deficiency (GHD), when tested with a GHRH-Arginine stimulation test. However, children and adults with PWS have hypothalamic dysfunction and several features that resemble those seen in GHD. Furthermore, the fact that they respond very well to GH treatment, with a significant improvement in body composition, health profile, normalization of stature in children, and a significant increase in serum IGF-I and IGFBP-3 levels strongly supports the likelihood of GHD in these patients. In other words, although adult GHD cannot be demonstrated by conventional testing using the current consensus criteria, one cannot conclude that it does not exist. It seems that conventional testing for adult GHD is not reliable in

adults with PWS and adult GHD should not be a prerequisite for treating adults with PWS with GH. GH treatment is highly recommended for adults with PWS, regardless of whether they fulfil the consensus criteria of adult GHD.

Our randomized, double-blind, placebo-controlled, cross-over study in young adults with PWS who were treated with GH during childhood demonstrates that compared to one year of GH treatment, one year of placebo after attainment of adult height, does not deteriorate BMD. However, we did find that BMD decreased during two years, regardless of GH or placebo. This decline was present in hypogonadal young adults with PWS without SSRT, but not in hypogonadal young adults with PWS with SSRT. It seems that GH is not able to prevent the decline in BMD unless it is combined with SSRT, indicating that sex steroids play a major role in the development and conservation of BMD in adolescents and (young) adults with PWS. This suggests that SSRT should be considered in hypogonadal adolescents with PWS to improve their bone health in adulthood. Our two-year study also shows that compared to one year of placebo, one year of GH treatment did not increase sleep-related breathing disorders (SRBD) in young adults with PWS who were treated with GH during childhood and had attained adult height. This is reassuring and proves that GH can be safely administered with regard to SRBD. However, clinicians should keep track of clinical signs of SRBD, e.g. snoring or daytime sleepiness in children and adults with PWS, as obstructive apneas can occur or increase in patients with adenotonsillar hypertrophy and/or (mild) upper respiratory tract infections.

We found a shorter Leukocyte Telomere Length (LTL) in young adults with PWS compared to age-matched healthy young adults and young adults born SGA who were also treated with GH during childhood. There was a tendency towards a lower IQ in subjects with shorter LTL, which might imply a role of LTL in cognitive functioning in PWS. Adults with PWS are at increased risk of developing age-associated diseases in early adulthood and the ageing process might be accelerated. The shorter LTL might be involved in the reported accelerated ageing process in adults with PWS. However, more research is needed on LTL across the life course to determine its role in ageing in both GH-treated and untreated people with PWS.

8.8 DIRECTIONS FOR FUTURE RESEARCH

GH in adults with PWS

Long-term GH treatment during childhood has well-known beneficial effects on body composition, psychomotor development, bone mineral density, linear growth and quality of life[6,12,21,77]. Up to now GH treatment is only approved for children with PWS, and adolescents with PWS who reach adult height currently have to stop GH treatment if they do not fulfil the criteria of adult growth hormone deficiency. However, multiple studies have shown beneficial effects of short-term GH treatment in young adults with PWS[22,78-80].

There are, however, no long-term studies on the efficacy and safety of GH treatment in adults with PWS. Future research should focus on the long-term effects of GH treatment on body composition, bone mineral density, cognitive functioning, quality of life and safety in both GH-treated and GH-naïve adults with PWS.

Genotype-phenotype

PWS is caused by a lack of expression of the paternally inherited genes located on 15q11-13, i.e. the Prader-Willi region. The loss of the imprinted genes on the PWS region causes the PWS phenotype[72]. Even though some studies have described phenotypic differences between people with a deletion and mUPD[73-76], the exact function of each gene, still needs to be elucidated.

Another subject of interest is the clinical variation between subjects with PWS. Some people with PWS eat everything they can find, including inedible products, while the appetite of other people with PWS is normal. Severe behavioral problems are more of a problem in some persons with PWS than others. It could be that (epi)genetic variation in people with PWS cause this variable phenotype. Future studies should focus on which (epi)genetic deviations cause different features of PWS, to be able to treat and/or prevent particular features accurately. This could improve the life of people with PWS and their families.

Appetite regulating hormones

Various clinical features in subjects with PWS change during their life, the most obvious being the change in appetite and body composition. Most babies with PWS require tube feeding, and infants with PWS show feeding difficulties and a lack of appetite. From the age of 2 years an increased interest in food emerges, which shifts to an unlimited appetite[70]. If parents and caregivers do not control the unlimited appetite, it will result in morbid obesity with severe complications including diabetes mellitus and cardiovascular disease in early adult life.

Appetite regulating hormones play an important role in the regulation of food intake and body composition by signaling satiety and energy reserves through hypothalamic receptors. Future studies focusing on appetite regulating hormones and hypothalamic function are needed to unravel the cause of hyperphagia in patients with PWS. Currently, an effective treatment for hyperphagia does not exist. Potential treatments for hyperphagia in PWS should be addressed in future studies, as this would relieve families of children with PWS from the great responsibility to retain food from their children, and would reduce the risk for obesity and comorbidities.

Prader-Willi like

In the last few decades, considerable progress has been made in the knowledge about children and (young) adults with PWS. Children with PWS are treated with GH from a very young age and, combined with an early diagnosis and multidisciplinary support, this has resulted in a new generation of children with PWS. Children with PWS have a healthier weight and body composition, reach a normal adult height and are able to take part in society.

In the past years we have been frequently contacted by parents and caregivers of children with similar symptoms as children with PWS, but without the typical genetic defect of children with PWS. These Prader-Willi like (PWL) children have the same severe medical and behavioural problems as children with PWS, but did not receive multidisciplinary care and treatment. Since the beginning of 2019 the Dutch PWS Reference Center started providing multidisciplinary care to children and young adults with PWL and aims to investigate how multidisciplinary care influences quality of life of children with PWL and their families and which (epi)genetic defects underlie PWL.

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Chapter 9

Summary
Samenvatting

SUMMARY

This thesis reports several studies we have performed in the last few years in children and young adults with Prader-Willi syndrome (PWS). Their main aim was to improve the knowledge about PWS and to optimize the care for patients with PWS. This chapter summarizes the studies and their most important outcomes.

Chapter 1

The introduction discusses the clinical manifestations of children and young adults with PWS in different stages of life, the genetic cause, hypothalamic dysfunction and describes the current knowledge on the effects of GH treatment in children and young adults with PWS. Furthermore, the objectives of the studies in this thesis are introduced.

Chapter 2

Infants and toddlers with PWS have a mental and motor developmental delay. Short-term data suggest a positive effect of growth hormone (GH) treatment on psychomotor development. Longer-term studies were, however, lacking. We, therefore, prospectively investigated the effects of 3 years of GH treatment on psychomotor development (by Bayley Scales of Infant Development II) in a large group of 63 infants with PWS who started GH treatment at a median age of 1 year. We also investigated if a younger age at start of GH treatment would lead to greater mental and motor development during 3 years of GH.

We found that both mental and motor development increased during 3 years of GH treatment. Our study also demonstrated that the lower psychomotor development and the younger the age at start of GH, the greater the improvement in psychomotor development.

We conclude that mental and motor development increase during 3 years of GH, reducing the gap between infants with PWS and healthy peers. Secondly, we conclude that, the younger at start of GH treatment, the greater the improvement in psychomotor development.

Chapter 3

Children with PWS generally have mild to moderate cognitive impairment with an IQ between 60 and 70. GH is an approved treatment for children with PWS and has been associated with cognitive benefits, attributed to the effects of GH and insulin-like growth factor on brain growth and development. Short-term data suggest positive effects of GH treatment on cognitive functioning in children with PWS. There were, however, no long-term studies about the effects of GH on cognitive functioning.

We, therefore, conducted a prospective cohort study in 43 children with PWS, who started GH at a median age of 8 years. We evaluated the effects of 8 years of GH treatment on cognitive functioning and whether starting GH before the age of 2 years results in higher cognitive functioning after 8 years of GH.

Our study showed that visuospatial skills slightly improve during 8 years of GH treatment, while abstract verbal reasoning and vocabulary remained similar. After 8 years of GH, 22 children who started GH before the age of 2 years had significantly higher vocabulary skills and a higher estimated total IQ, compared to the children who started GH during childhood.

From these data we conclude that cognitive skills in GH-treated children develop at the same pace as in healthy references. We also conclude that starting GH treatment before the age of 2 years might lead to higher cognitive functioning on the long-term.

Chapter 4

Some features of subjects with PWS resemble those seen in growth hormone deficiency (GHD). Children with PWS are treated with GH, which has substantially changed their phenotype. Studies in adults with PWS have also shown beneficial effects of GH treatment. GH treatment for adults with PWS is currently not approved and can only be reimbursed if they fulfill the consensus criteria of adult GHD. We, therefore, investigated the prevalence of GHD in 60 previously GH-treated young adults with PWS by GHRH-Arginine stimulation tests. GHD in adults was defined as a GH peak level < 9 µg/l in combination with a serum IGF-I SDS level < -2.0.

Our results demonstrate that serum IGF-I was < -2 SDS in 2 (3%) patients and IGFBP-3 was within the normal range in all but one patient. Nine participants had a GH peak < 9 µg/L (15%) during a GHRH-Arginine test. None of these patients also had an IGF-I < -2.0 SDS. Therefore, not one patient fulfilled the criteria for adult GHD, also when BMI-dependent criteria were used.

Our study shows that a GHRH-Arginine test to diagnose adult GHD is unreliable in adults with PWS. As GH treatment has very positive effects in adults with PWS, adult GHD should not be a prerequisite for treating adults with PWS with GH.

Chapter 5

The prevalence of osteoporosis is increased in adults with PWS. In children, GH treatment has beneficial effects on bone mineral density (BMD). BMD might deteriorate after cessation of GH at adult height, while continuing GH might maintain BMD. In our 2-year, randomized, double-blind, placebo-controlled cross-over GH study with stratification for gender and BMI, we investigated the effects of 1 year of GH versus 1 year of placebo on BMD of the total body (BMD_{TB}) and of the lumbar

spine ($BMAD_{LS}$) in 27 GH-treated young adults with PWS who had attained adult height. Furthermore, we investigated the effects of sex steroid replacement therapy (SSRT) on BMD in hypogonadal young adults with PWS.

At adult height, BMD_{TB} was significantly lower compared to healthy peers, while $BMAD_{LS}$ was similar. Compared to 1 year of GH, 1 year of placebo did not deteriorate BMD_{TB} or $BMAD_{LS}$. We also showed that BMD_{TB} did not significantly change during 2 years, while $BMAD_{LS}$ decreased, independent of GH or placebo. This could be due to the higher prevalence of hypogonadism in PWS. We found that BMD_{TB} and $BMAD_{LS}$ decreased during the 2-year study in hypogonadal young adults without SSRT, while BMD_{TB} increased in those with SSRT.

We conclude that, compared to GH treatment, 1 year of placebo after attainment of adult height does not deteriorate BMD in young adults with PWS. Our data suggest that GH is not able to prevent the decline in BMD in hypogonadal young adults with PWS, unless it is combined with SSRT.

Chapter 6

Sleep-related breathing disorders (SRBD) are common in people with PWS causing poor sleep quality and excessive daytime sleepiness. Young adults with PWS benefit from GH continuation after adult height by maintaining the improved body composition obtained during childhood. There were, however, no studies about the effects of GH on SRBD in young adults with PWS who were treated with GH during childhood. We, therefore, investigated SRBD in 27 young adults with PWS who were treated with GH during childhood and had attained adult height in our 2-year, randomized, double-blind, placebo-controlled, cross-over GH-study, stratified for gender and BMI. We also investigated the prevalence of obstructive sleep apnea (OSA).

Compared to placebo, one year of GH did not increase Apnea hypopnea index (AHI), obstructive apnea index (OAI) or central apnea index (CAI). After 2 years, there was no difference in AHI, CAI or OAI compared to baseline, regardless of GH or placebo. Two patients (7%) fulfilled the criteria of obstructive sleep apnea (OSA). AHI in these 2 patients consisted mainly of hypopneas, OAI being 0 and 1.2 events/hour.

We conclude that GH compared to placebo does not cause a significant increase in AHI, CAI or OAI in adults with PWS who were treated with GH during childhood and have attained adult height. Our findings are reassuring and prove that GH can be safely administered with regard to SRBD.

Chapter 7

Adults with PWS are at increased risk of developing age-associated diseases early in life and, like in premature ageing syndromes, ageing might be accelerated. Recently, telomere length has been associated with ageing and individuals with shorter telomere length show an increased mortality risk. We, therefore, investigated leukocyte telomere length (LTL), a marker of biological age, in 47 young adults with PWS and cross-sectionally compared LTL to 135 healthy young adults of similar age. As all young adults with PWS were treated with growth hormone (GH), we also compared LTL in PWS subjects to 75 GH-treated young adults born short for gestational age (SGA).

We demonstrate that LTL in young adults with PWS is shorter compared to age-matched healthy young adults and GH-treated young adults born SGA, suggesting a possible role of LTL in the reported accelerated ageing process in PWS. Despite the fact that subjects with PWS were treated with GH for a significantly longer time, there was no association between LTL and duration of GH treatment or cumulative GH dose.

We conclude that young adults with PWS have a significantly shorter median LTL compared to age-matched healthy young adults and GH-treated young adults born SGA. The shorter telomeres might play a role in premature ageing in PWS, independent of GH treatment.

Chapter 8

In the general discussion, we examine our results in view of the current literature and suggest clinical implications of our findings. Furthermore, we report our overall conclusions and give recommendations for future research.

SAMENVATTING

Dit proefschrift geeft een overzicht van de resultaten van 6 studies die we in de afgelopen jaren hebben verricht bij kinderen en jongvolwassenen met Prader-Willi syndroom (PWS). Het belangrijkste doel was het vergroten van de kennis over PWS en het optimaliseren van de zorg voor kinderen en jongvolwassenen met PWS en hun gezin. In dit hoofdstuk geven we een samenvatting van de resultaten van de verrichte studies.

Hoofdstuk 1

Hoofdstuk 1 geeft achtergrondinformatie over PWS. De kenmerken per levensfase, de genetische achtergrond en groeihormoon (GH) behandeling worden besproken. Ook wordt een korte inleiding gegeven over de studies die worden beschreven in dit proefschrift.

Hoofdstuk 2

Jonge kinderen met PWS hebben een mentale en motorische ontwikkelingsachterstand. Korte termijn studies vonden een positief effect van GH op de mentale en motorische ontwikkeling bij jonge kinderen met PWS. Tot op heden, waren de langere termijn effecten van GH op de mentale en motorische ontwikkeling van jonge kinderen met PWS nooit onderzocht. In deze prospectieve studie onderzochten we daarom bij 63 peuters met PWS, die op een mediane leeftijd van 1 jaar waren gestart met GH, de psychomotorische ontwikkeling tijdens 3 jaar GH middels de Bayley Scales of Infant Development II. Ook onderzochten we of eerder starten met GH leidt tot een betere psychomotorische ontwikkeling.

We vonden dat zowel de mentale als de motorische ontwikkeling verbeterden tijdens 3 jaar GH behandeling. Ook vonden we dat kinderen die een lagere psychomotorische ontwikkeling hadden of jonger waren bij het starten van GH een grotere verbetering toonden in hun psychomotorische ontwikkeling.

Uit deze resultaten concluderen we dat zowel de mentale, als de motorische ontwikkeling verbeteren tijdens 3 jaar GH behandeling. Dit verkleint de psychomotorische verschillen tussen kinderen met PWS en gezonde kinderen. Daarnaast concluderen we dat, hoe jonger het kind is bij de start van GH, hoe groter de verbetering in psychomotorische ontwikkeling.

Hoofdstuk 3

Kinderen met PWS hebben een milde tot matige verstandelijke beperking met een gemiddeld IQ tussen 60 en 70. GH is een geregistreerde behandeling voor kinderen met PWS en verschillende studies beschreven positieve effecten van GH op de

cognitieve ontwikkeling. Echter, tot op heden waren de lange-termijn effecten van GH op de cognitieve ontwikkeling nooit onderzocht. We hebben daarom een prospectieve cohort studie verricht bij 43 kinderen met PWS met een mediane leeftijd van 8 jaar bij start van GH. We onderzochten de cognitieve ontwikkeling tijdens 8 jaar GH behandeling. Ook onderzochten we of de cognitieve ontwikkeling na 8 jaar GH beter is bij kinderen die voor de leeftijd van 2 jaar waren gestart met GH.

We vonden dat het performaal IQ iets verbeterde tijdens 8 jaar GH, terwijl het verbaal IQ gelijk bleef. Na 8 jaar GH vonden we een significant hoger verbaal en totaal IQ bij kinderen die voor de leeftijd van 2 jaar waren gestart met GH, ten opzichte van de kinderen die na de leeftijd van 2 jaar waren gestart met GH.

We concluderen dat het totaal IQ van kinderen met PWS lager is dan bij gezonde kinderen, maar dat de cognitieve ontwikkeling van GH-behandelde kinderen met PWS gelijk is aan die van gezonde kinderen. Dit betekent dat de cognitieve achterstand niet groter wordt. We concluderen ook dat het starten van GH voor de leeftijd van 2 jaar mogelijk tot een betere cognitieve ontwikkeling leidt.

Hoofdstuk 4

Sommige kenmerken van patiënten met PWS komen overeen met het hebben van groeihormoondeficiëntie (GHD). Langdurige behandeling met GH bij kinderen met PWS verbetert onder andere de lichaamssamenstelling en de groei. Op dit moment moeten jongvolwassenen met PWS stoppen met GH bij het bereiken van de eindlengte als ze niet voldoen aan de criteria voor volwassen GHD. Echter, verschillende studies hebben aangetoond dat GH gunstig is voor volwassenen met PWS, met een blijvende verbetering in de lichaamssamenstelling en de metabole gezondheid. Er was tot op heden weinig bekend over de prevalentie van GHD bij volwassenen met PWS. We hebben daarom middels een GHRH-Arginine stimulatietest de prevalentie van GHD onderzocht bij 60 jongvolwassenen met PWS die tot het bereiken van de volwassen lengte waren behandeld met GH. De definitie van volwassen GHD was een IGF-I <-2 standaarddeviaties (SDS) en een groeihormoon piek <9 µg/l. We vonden dat het IGF-I in het bloed lager was dan -2 SDS bij 2 jongvolwassenen (3%). Negen deelnemers (15%) hadden een GH piek <9 µg/L. Geen enkele jongvolwassene had een IGF-I < -2 SDS én een GH piek <9 µg/L. Geen van de jongvolwassenen met PWS voldeed daarom aan de volwassen GHD criteria, ook als er gecorrigeerd werd voor body mass index (BMI).

We concluderen dat de standaard GHRH-Arginine test niet geschikt is om volwassen GHD aan te tonen bij jongvolwassenen met PWS. Omdat de positieve effecten van GH bij volwassenen met PWS in meerdere studies zijn bewezen, zou het aantonen van volwassen GHD geen vereiste moeten zijn voor GH behandeling bij volwassenen met PWS.

Hoofdstuk 5

Kinderen en adolescenten met PWS hebben een hoger risico op een lagere botdichtheid, omdat ze vaak lage groeihormoon (GH) spiegels in het bloed hebben, niet goed in de puberteit komen en een inactieve leefstijl hebben. Het is bekend dat ouderen met PWS meer risico hebben op osteoporose. Uit eerder onderzoek bij kinderen met PWS is gebleken dat GH zorgt voor een verbetering van de botdichtheid (BMD). De gevolgen van het stoppen met GH op het moment dat jongeren met PWS zijn uitgegroeid was onbekend. In onze gerandomiseerde, dubbelblinde, placebo-gecontroleerde cross-over studie onderzochten we de effecten van 1 jaar GH versus 1 jaar placebo op de botdichtheid van het gehele lichaam (BMD_{TB}) en de botdichtheid van de lumbale wervelkolom ($BMAD_{LS}$) bij 27 jongvolwassenen met PWS die tot de volwassen lengte waren behandeld met GH. Ook onderzochten we bij jongvolwassenen met PWS en hypogonadisme de effecten van behandeling met geslachtshormonen (SSRT) op de botdichtheid.

Ten opzichte van 1 jaar GH, zorgde 1 jaar placebo niet voor een afname in BMD_{TB} of $BMAD_{LS}$. Wel zagen we dat $BMAD_{LS}$ gedurende de 2 jaar durende studie afnam, onafhankelijk van groeihormoon of placebo. Omdat we weten dat geslachtshormonen een belangrijke rol spelen bij de botdichtheid werden de jongvolwassenen met hypogonadisme die behandeld werden met SSRT vergeleken met de jongvolwassenen die niet werden behandeld. We vonden dat de jongvolwassenen met hypogonadisme die wel SSRT kregen een verbetering hadden in BMD_{TB}, terwijl BMD_{TB} en $BMAD_{LS}$ daalden bij de jongvolwassenen die geen SSRT kregen.

We concluderen dat een jaar placebo geen achteruitgang geeft van de botdichtheid bij jongvolwassenen met PWS die tijdens de kindertijd langdurig met groeihormoon zijn behandeld. Ook concluderen we dat GH bij jongvolwassenen met PWS en hypogonadisme de afname in de botdichtheid niet lijkt te kunnen voorkomen, behalve als GH gecombineerd wordt met behandeling met SSRT.

Hoofdstuk 6

Een (sterk) verminderde ademhaling (hypopneu) of een ademstilstand (apneu) in de slaap komen regelmatig voor. Bij volwassenen is er sprake van een slaapstoornis als een apneu 's nachts meer dan vijf keer per uur plaatsvindt en dit minimaal tien seconden duurt. Er is sprake van een obstructieve slaapstoornis (OSA) als iemand wel een prikkel heeft om adem te halen, maar de ingeademde lucht niet verder komt dan de keel door een gedeeltelijke of volledige blokkade van de luchtweg. Slaap-gerelateerde ademhalingsproblemen (SRBD) komen vaak voor bij mensen met PWS. Het effect van GH of placebo op SRBD bij jongvolwassenen met PWS die de eindlengte hadden bereikt was tot op heden nooit onderzocht.

In onze gerandomiseerde, dubbelblinde, placebo-gecontroleerde cross-over studie onderzochten we de effecten van 1 jaar GH versus 1 jaar placebo op SRBD bij 27 jongvolwassenen met PWS die tot de volwassen lengte waren behandeld met GH. We onderzochten ook hoe vaak OSA voorkomt in ons cohort van jongvolwassenen met PWS. We vonden dat, in vergelijking met placebo, 1 jaar GH niet zorgde voor een toename in het aantal apneus en hypopneus. Na 2 jaar was er geen significant verschil in het aantal apneus en hypopneus ten opzichte van start van de studie, onafhankelijk van GH of placebo. Twee jongvolwassenen (7%) voldeden aan de OSA criteria. Beiden waren obees en hadden vergrote amandelen.

We concluderen dat er geen toename is in het aantal apneus en hypopneus tijdens 1 jaar GH. Onze bevindingen zijn geruststellend en laten zien dat GH behandeling veilig is met betrekking tot SRBD bij volwassenen met PWS.

Hoofdstuk 7

Veel volwassenen met PWS ontwikkelen op jonge leeftijd ouderdomsziekten. In de literatuur wordt een versnelde veroudering beschreven bij mensen met PWS. Mogelijk spelen telomeren hierin een rol. De telomeerlengte was nooit eerder onderzocht bij mensen met PWS. We hebben daarom bij 47 jongvolwassenen met PWS de telomeerlengte in leukocyten (LTL) gemeten en vergeleken met 135 gezonde jongvolwassenen. Omdat alle jongvolwassenen met PWS behandeld werden met GH, hebben we LTL ook vergeleken met 75 jongvolwassenen die te klein waren bij de geboorte en tot het bereiken van de eindlengte ook behandeld waren met GH.

We vonden dat de LTL korter was bij jongvolwassenen met PWS in vergelijking met gezonde jongvolwassenen en GH-behandelde jongvolwassenen die te klein waren bij de geboorte. Dit suggereert een mogelijke rol van LTL in het versnelde verouderingsproces bij PWS. We vonden geen verband tussen LTL en de duur van de GH-behandeling of de cumulatieve GH-dosis.

We concluderen dat jongvolwassenen met PWS een significant kortere mediane LTL hebben in vergelijking met gezonde jongvolwassenen en GH-behandelde jongvolwassenen die te klein waren bij de geboorte. Mogelijk speelt de kortere telomeerlengte een rol in de versnelde veroudering bij mensen met PWS, onafhankelijk van GH.

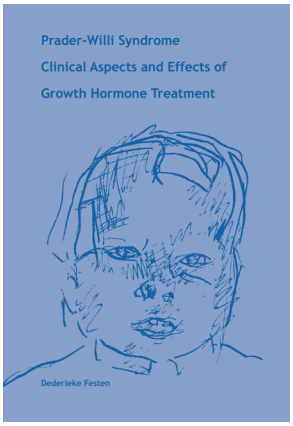
Hoofdstuk 8

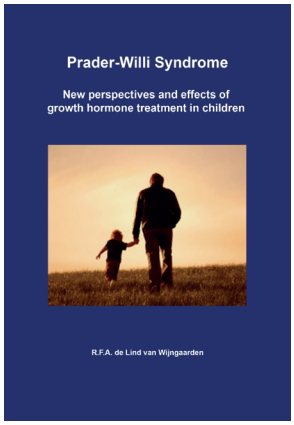
In de algemene discussie bespreken we onze resultaten in het kader van de huidige literatuur, presenteren we de praktische implicaties van onze bevindingen en geven we suggesties voor toekomstig onderzoek.

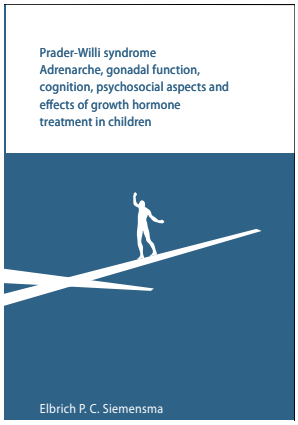
Chapter 10

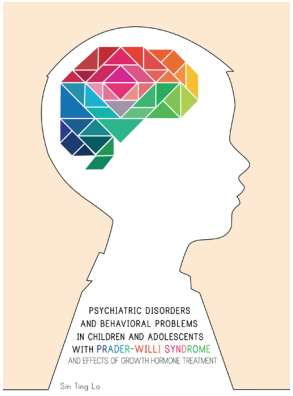
Overview of publications by PWS-team of Dutch Growth Research Foundation

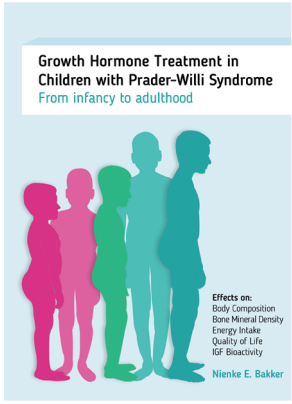
OVERVIEW OF PUBLICATIONS BY PWS-TEAM OF DUTCH GROWTH RESEARCH FOUNDATION











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Chapter 11

List of abbreviations
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LIST OF ABBREVIATIONS

ACTH	corticotropin
AH	adult height
AHI	apnea hypopnea index
$BMAD_{LS}$	bone mineral apparent density of the lumbar spine
BMD	bone mineral density
BMD_{LS}	bone mineral density of the lumbar spine
BMD_{TB}	bone mineral density of the total body
BMI	body mass index
BP	break point
BSID	Bayley Scales of Infant Development
CAI	central apnea index
CI	confidence interval
CoQ10	co-enzyme Q10
CRH	corticotropin-releasing hormone
CV	coefficient of variation
CVD	cardiovascular disease
DNA	deoxyribonucleic acid
DXA	dual-energy x-ray absorptiometry
%ed	percentage of expected development
FFM	fat free mass
FM	fat mass
FM%	fat mass percentage
FSH	follicle stimulating hormone
GH	growth hormone
GHD	growth hormone deficiency
GHRH	growth hormone-releasing hormone
GnRH	gonadotropin-releasing hormone
HDLc	high-density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment of insulin resistance
ICD	imprinting center defect
IGFBP-3	IGF binding protein 3
IGF-I	insulin-like growth factor type I
IQ	intelligence quotient
IQR	interquartile range
LBM	lean body mass
LBM%	lean body mass percentage
LDLc	low-density lipoprotein cholesterol
LH	luteinizing hormone
LTL	leukocyte telomere length
MD	medical doctor
mUPD	maternal uniparental disomy
OAI	obstructive apnea index

OSA	obstructive sleep apnea
PRL	prolactin
PSG	polysomnography
PWS	Prader-Willi syndrome
RCT	randomized controlled trial
SaO_2	oxygen saturation
SE	standard error
SEM	standard error of the mean
SGA	short for gestational age
SDS	standard deviation score
SPSS	Statistical Package for Social Sciences
SRBD	sleep related breathing disorders
T2DM	diabetes mellitus type 2
TC	total cholesterol
TG	triglyceride
TIQ	total IQ
TRH	thyrotropin-releasing hormone
TSH	thyrotropin
WAIS	Wechsler Adult Intelligence Scale
WISC	Wechsler Intelligence Scale for Children

LIST OF PUBLICATIONS

Donze SH, Codd V, Damen L, Goedegebuure WJ, Denniff M, Samani NJ, van der Velden AAEM, Hokken-Koelega ACS. Evidence for accelerated biological ageing in young adults with Prader-Willi syndrome. *Journal of Clinical Endocrinology and Metabolism 2019; in press.*

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Name: Stephany Hermina Donze
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PhD period: January 2015 – August 2019

Summary of PhD training	**Year**	**ECTS**
1. General courses		
Good Clinical Practice (BROK), Erasmus Medical Center	2016	1.5
Integrity in Medical Research, Medical Ethics and Philosophy, Erasmus MC	2017	2.0
Biomedical English Writing and Communication, MolMed, Erasmus MC	2017	4.0
2. Specific courses		
Radiation protection 5A, Zorgacademie, Erasmus MC	2015	1.0
Basic Introduction to SPSS, Erasmus MC	2016	1.0
PubMed and Endnote, Medical Library, Erasmus MC	2016	0.3
3. Seminars and workshops		
Weekly research meeting, Pediatric Endocrinology, Erasmus MC	2015-2019	4.0
Annual PhD day, Erasmus MC	2015-2019	1.2
Annual Sophia Research Day, Sophia Children's Hospital, Erasmus MC	2015-2019	0.8
Behaviour and Sleeping disorders in children with congenital or hereditary disorders, Erasmus MC	2017	0.8
Epigenetic regulation in health and disease, Erasmus MC	2018	0.8
4. International and national conferences		
54th Meeting of the European Society of Pediatric Endocrinology (ESPE), Barcelona, Spain (1 poster presentation)	2015	1.0
Invitational Conference PWS, Utrecht, The Netherlands	2015	1.0
55th Meeting of the European Society of Pediatric Endocrinology (ESPE), Paris, France (1 poster presentation)	2016	1.0
Expert meeting PWS, Utrecht, The Netherlands	2018	1.0
57th Meeting of the European Society of Pediatric Endocrinology (ESPE), Athens, Greece (2 poster presentations and 2 oral presentations)	2018	1.0
5. Lecturing		
PWS Parents information day, Nijmegen (oral presentation)	2016	1.0
Annual IMC Weekendschool 'Growth and Development', Rotterdam	2016	1.0
PWS Parents information day, Rotterdam (oral presentation)	2018	1.0
Grand round meeting section Pediatric, Rotterdam (oral presentation)	2018	1.0

6. Research proposals

Co-author ‘Randomized, double-blind, placebo-controlled oxytocin study in children with Prader-Willi syndrome’	2017	2.0

7. Miscellaneous

Co-author several grant applications	2015-2019	
Clinical advisor ‘Informatie voor de huisarts over Prader-Willi syndroom’	2016	2.0
Clinical expert ‘Digitaal Expertisecentrum Prader-Willi syndroom’	2016-2019	2.0
Co-author Business case Prader-Willi Expert Center	2017	2.0
10th International Meeting Pediatric Endocrinology (ESPE), Washington, USA Travel Award	2018	

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First and foremost, I want to thank the people who this thesis is about: the children and young adults with PWS and their families. They have not only invested large amounts of time and effort in cooperating with the research this work is based on, but they have also enriched and touched my life in numerous other ways. I have immensely enjoyed meeting so many lovely and interesting families. They are what makes working at the Prader-Willi Reference Center truly special.

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As mentioned, this booklet is a product of a collective effort. This is of course true in the most straightforward sense about the people who have co-written it. I could not have asked for a more supportive, intelligent and committed group of collaborators.

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Mariëlle van Eekelen's presence always makes sure that our patients, and their parents, are at ease and happy to come to the hospital. She had the same effect on me. It was always a pleasure to work with her in Rotterdam, as well as during our trips around the countryside. I have particularly good memories of our little trips up north and I'm honoured that she has agreed to be my paranimf.

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CURRICULUM VITAE

Stephany Hermina Donze (Terneuzen, 1988) grew up in Zeeuws-Vlaanderen and moved to Leiden in 2007 to start her medical training at the Leiden University Medical Center. After finishing her senior clinical elective internship in pediatrics at the 'Reinier de Graaff Gasthuis' in Delft, she started a research internship at the pediatric endocrinology department in the Leiden University Medical Center, where she studied the effects of growth hormone treatment in children with anomalies in the *SHOX* gene. Stephany obtained her medical degree in January 2015 and started working as a PhD fellow at the Dutch Growth Research Foundation and the department of Pediatric Endocrinology of the Erasmus Medical Center-Sophia Children's Hospital in Rotterdam.

During her PhD Stephany dedicated her research to children and young adults with Prader-Willi syndrome under supervision of professor Anita Hokken-Koelega. The results of this PhD project are presented in this thesis.