Prader-Willi Syndrome

Clinical Aspects and Effects of Growth Hormone Treatment

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klinische aspecten en effecten van groeihormoon behandeling

Proefschrift

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Aan Jan Pieter, Otto en mijn familie

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Chapter 1

General Introduction

This chapter describes the clinical picture of the Prader-Willi Syndrome (PWS), and the different genetic defects that cause the syndrome. Additionally, the endocrine and metabolic aspects, psychological issues and sleep-related breathing abnormalities are described. It provides previously reported results on growth hormone (GH) treatment in PWS children. Finally, the objectives, study design, and in- and exclusion criteria of the Dutch multicenter GH trial are summarized.

1.1.1 Background

The characteristic features of Prader-Willi syndrome were first documented by Prader, Labhart and Willi in 1956, as "Ein Syndrom von Adipositas, Kleinwuchs, Kryptorchidismus und Oligophrenie nach myotonieartigem Zustand im Neugeborenenalter" [1]. PWS is a neurogenetic disorder characterised by a number of signs and symptoms, including muscular hypotonia, psychomotor delay, obesity, hypogonadism, and short stature. The estimated birth incidence varies from 1:10 000 to 1:30 000 live births, and the reported overall population prevalence of PWS, based on epidemiological studies, varies between 1: 38 395 to 1: 52 000 [2-4]. The differences between these numbers might be caused by different methodological approaches and by including patients with clinical diagnosis or with genetically confirmed diagnosis of PWS. PWS is not associated with social-economic status and the distribution between both sexes is considered equal. Some studies, however, report a slightly higher prevalence among boys, which might be the result of more difficult recognition of hypogonadism in young girls. PWS is considered the most common syndromal cause of severe obesity.

1.1.1.1 Clinical features of PWS change with age

The clinical characteristics of the PWS are age-dependent. Originally, two clinical phases were distinguished: an infantile hypotonic phase and a childhood obese phase. Whitman et al added a third adolescent phase, with particular behavioural features [5], and Donaldson et al [6] extended this model with a foetal and neonatal phase.

1.1.1.2 Foetal and neonatal phase

Decreased foetal movements are commonly reported and there is an increased incidence of breech delivery and of delivery by caesarean section. These abnormalities have been related to hypotonia in neonates with PWS [6], but also foetal hypothalamic dysfunction has been suggested to play an important role [7]. At birth, PWS neonates have severe hypotonia mainly affecting the neck [8,9] (Figure 1), and have a weak

or absent cry [10]. Due to this severe hypotonia and poor sucking reflex, feeding via a nasogastric sonde is usually required. Hypoplasia of the external genitalia is common in both sexes: Boys often have cryptorchidism, a hypoplastic scrotum and a decreased penile length. Girls may have hypoplastic labia, but this is less obvious and easily missed. Sticky saliva may also be a helpful pointer to the clinical diagnosis [9], and neonates may have temperature instability [11]. With the availability of rapid genetic testing and recommendations to exclude the diagnosis of PWS in any neonate with hypotonia, feeding difficulties and low reflexes [12], the diagnosis is now usually confirmed in the neonatal period.

1.1.1.3 Infant phase

After the neonatal stage, hypotonia becomes gradually less marked, although infants with PWS remain to have feeding difficulties, and failure to thrive. Psychomotor development is delayed, in particular gross motor development and speech development [13]. Body composition is abnormal, with a low lean body mass (LBM) and a high body fat percentage, even in infants who are underweight and have failure to thrive [14,15]. Although also present in the neonatal phase, typical dysmorphic features become more pronounced in the infant and childhood phase: a narrow bifrontal diameter, almond shaped eyes, a thin downturned upper lip, and a narrow nose. Many childeren with PWS have hypopigmentation, fair hair and blue eyes [16].

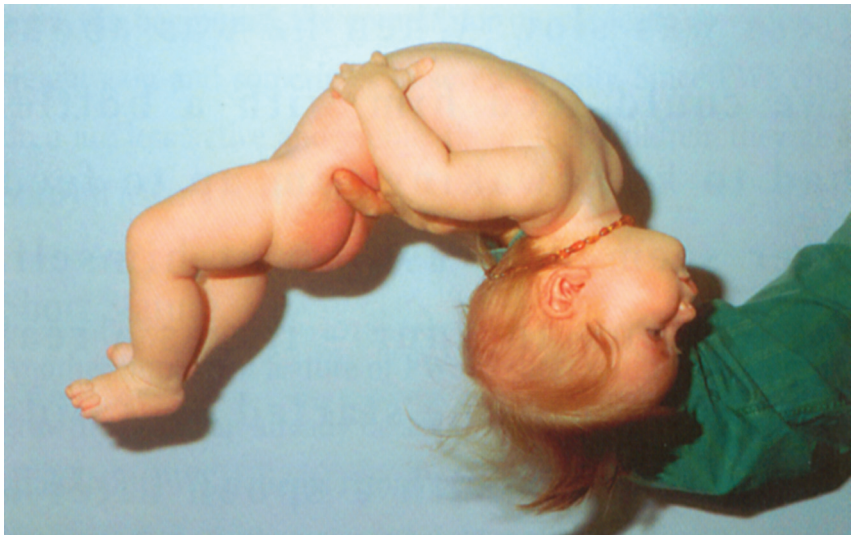


Figure 1. Hypotonia in an infant with Prader-Willi Syndrome Adapted from U. Eiholzer, Prader-Willi Syndrome: Coping with the disease – living with those involved, Karger, Edt, Dr. E. Zangger *(with permission)*.

1.1.1.4 PWS in childhood

In childhood, the hypotonia improves, although difficulties relating to physical activity remain present. The original feeding difficulties improve and excessive appetite with hyperphagia may develop, which may result in extreme obesity, if not controlled by dietary measures. Children with PWS have an abnormal body composition, with a relatively high body fat percentage and a low LBM, which contributes to exercise intolerance. Due to muscle imbalance, children with PWS may have genu valgum and often develop scoliosis [8]. Scoliosis may be present in childhood, but becomes more common and pronounced in adolescence. Their hands and feet are small. They have poor growth and behavioural abnormalities may occur, such as temper tantrums and obsessive compulsive behaviour, often in relation to food. Skin picking may be present, which is related with their decreased pain sensitivity. Other cutaneous symptoms consist of easy bruising and marked erythema after a hot bath. Autism-like symptoms are common in PWS children. Many have mild to moderate learning disability, but some children are able to attend normal schools [17].

1.1.1.5 PWS in adolescence

Puberty may be delayed, incomplete or even absent in adolescents with PWS, although some have precocious puberty [18]. Pubertal delay may be caused by a combination of both hypothalamic dysfunction and primary hypogonadism. Spontaneous growth velocity is impaired, and the pubertal growth spurt might be lacking, which contribute to a decreased adult height. In adolescence, behavioural abnormalities may become worse, in particular in relation to daily life routines [5]. Most patients with PWS have mental disability, although there is a wide variation. Psychosis may occur, in particular in children with uniparental maternal disomy (UPD).

1.1.1.6 PWS in adulthood

The combination of mental disability, excessive appetite with associated food-seeking behaviour, often resulting in extreme obesity, and psychiatric and behavioural abnormalities makes it usually impossible for an adult with PWS to live independently [13,17]. Median adult height is 145-150 cm for women and 155-162 cm for men [13,19, 20]. Secondary sex characteristics are often absent or incomplete [13,17] although pregnancy, resulting in the birth of a healthy baby girl has been reported, in 1 PWS woman [21], whereas another one gave birth to a child with Angelman syndrome (AS) [22]. The incidence of scoliosis in adult PWS patients is high [17]. The adult population of today was usually diagnosed relatively late, when obesity was already present. So far no studies are available on the clinical picture of PWS patients who had been diagnosed early in life by genetic testing and who were treated from infancy onwards, with diet, exercise and hormonal substitution. Complications of severe obesity, such as diabetes mellitus type II or respiratory insufficiency do frequently occur and may lead to an early death [17]. However, if severe obesity can be avoided, patients with PWS may have a reasonable life expectancy [23].

1.1.2 Pathophysiology: the role of the hypothalamus

Most symptoms of the PWS are considered to result from hypothalamic dysfunction [7]. The hypothalamus is important in the regulation of many processes, including body temperature, activity level, appetite, body weight, and breathing. In addition, the hypothalamus regulates the release of hormones from the anterior lobe of the pituitary gland, which is often dysregulated in PWS patients (Endocrine and metabolic aspects). The higher incidence of breech delivery and caesarian section in PWS may be the result of foetal hypothalamic dysfunction. In addition, in PWS a decreased cell number and a lower volume of oxytocin-expressing neurons in the hypothalamic

paraventricular nucleus, the putative satiety neurons, have been reported and might be the basis of the insatiable hunger and obesity [245]. Abnormal temperature control, excessive daytime sleepiness and rapid eye movement (REM) sleep abnormalities as have been described in PWS, have been related to hypothalamic dysfunction [25] as well as abnormal chemoreceptor function in PWS [26]. In a subgroup of patients, Magnetic Resonance Imaging (MRI) studies showed an absence of the posterior pituitary bright spot. The posterior pituitary bright spot represents the neuropituitary and its presence confirms the integrity of the hypothalamic-pituitary axis [27].

1.1.3 Genetic background

In 1981, PWS was reported to be related to a deletion in chromosome 15 in a subgroup of patients [28]. Subsequently, it was discovered that the deletion in PWS affected only the paternally inherited chromosome 15 [29]. In 1989, Nicholls et al found that in most of the remaining patients maternal disomy was the underlying genetic defect, suggesting genetic imprinting of the PWS region of chromosome 15 [30] (Figure 2). Gene imprinting is a mechanism by which part of a chromosome is "imprinted" or silenced during gametogenesis, which leads to a different expression according to the parent of origin. In some rare cases, PWS is considered to result from a mutation in the imprinting control region (imprinting center mutation, ICM), which causes a defect of paternal imprinting of chromosome 15 genes [31,32].

A deletion, a disomy or an ICM in the same region on the maternal chromosome, results in a completely different syndrome (AS).

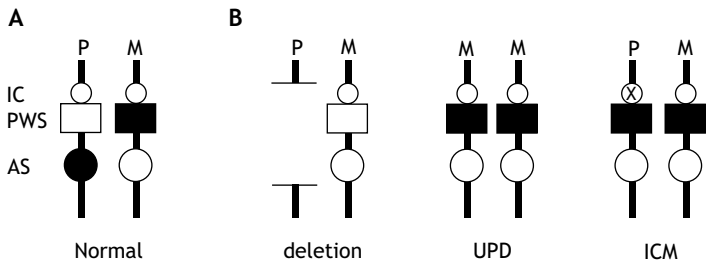


Figure 2. Schematic presentation of the different genetic defects in PWS. (**A**) represents a normal imprinting of the paternal (P) and maternal (M) regions of 15q11-13. In paternal chromosome 15, PWS genes are active (open square) and AS genes are imprinted (silenced, solid circle), whereas in maternal chromosome 15, PWS genes are imprinted (solid square) and AS genes are active (open circle). (**B**) represents the possible genetic defects, resulting in lack of expression of paternal genes in PWS (a paternal deletion, a uniparental maternal disomy (UPD), or an imprinting defect, due to a imprinting center (IC) mutation (ICM), respectively.
Adapted from H. Kokkonen; Genetic changes of chromosome region 15q11-13 in Prader-Willi syndrome and Angelman syndrome in Finland ISBN951-42-7027-4 URL: http//herkules.oulu.fi/isbn9514270274

1.1.4 The link between genotype and phenotype

Several mouse models of PWS have been developed, with maternal disomy or paternal deletions of the PW region, or mutations in the imprinting center. These mice have a consistent phenotype of failure-to-thrive, hypotonia, neonatal lethality, and growth retardation [33], but not of hyperphagia and obesity. For most genes, however, the link between genotype (Figure 3) and phenotype has not yet been established.

SNURF-SNRPN (imprinting center)

The promoter and first exon of the SNURF-SNRPN gene appears to be the imprinting center in the PWS region [34]. The imprinting center has a role in erasing the grandmaternal imprint and establishing the paternal imprint during spermatogenesis, and in postzygotic maintenance of the paternal imprint [33].

Maternally imprinted genes

Maternally imprinted genes are normally only expressed in the paternally inherited chromosome, and are therefore lacking in PWS.

Necdin

Necdin (neurally differentiated embryonal carcinoma-cell derived factor) is involved in the neural differentiation and survival. Necdin deficient neonatal mice have severe neonatal respiratory distress, with an abnormal rhytm-generating center in the medulla [35]. Most mice don't survive the neonatal period, but the ones that do, have increased skin-scraping activity, improved spatial learning and structural abnormalities in the hypothalamus, which resembles the PWS phenotype [36]. Necdin deficient mice show a reduction in both hypothalamic oxytocin producing and luteinizing hormone releasing hormone (LHRH) producing neurons [36]. A potential role for the Necdin (and other) genes in the other PWS symptoms outside the brain, such as small hands and feet, growth retardation, hypotonia, articulation defects, dysmorphic mouth, viscous saliva, and genital hypoplasia, are indicated by mouse models [37,38].

Paternally imprinted genes

Paternally imprinted genes are expressed only in the maternally inherited chromosome, and might therefore be over-expressed in PWS patients with UPD.

UBE3A

Patients with UPD are reported to have significantly higher scores on verbal IQ tests, but not performance IQ tests and a higher risk of developing psychotic disorders in adult life than patients with a paternal deletion [39]. This is suggested to result from an overexpressing of the Ubiquitin protein ligase E3A (UBE3A) gene, which is located in the paternally imprinted AS region. Since UBE3A is expressed only on the maternally inherited chromosome 15, UBE3A may be overexpressed in patients with UPD [40].

Non-imprinted genes

Hypopigmentation of skin, hair and eyes is more frequent in patients with a paternal deletion [41]. Hypopigmentation is caused by the loss of expression of the non-imprinted P gene which is also involved in oculocutaneous albinism [42].

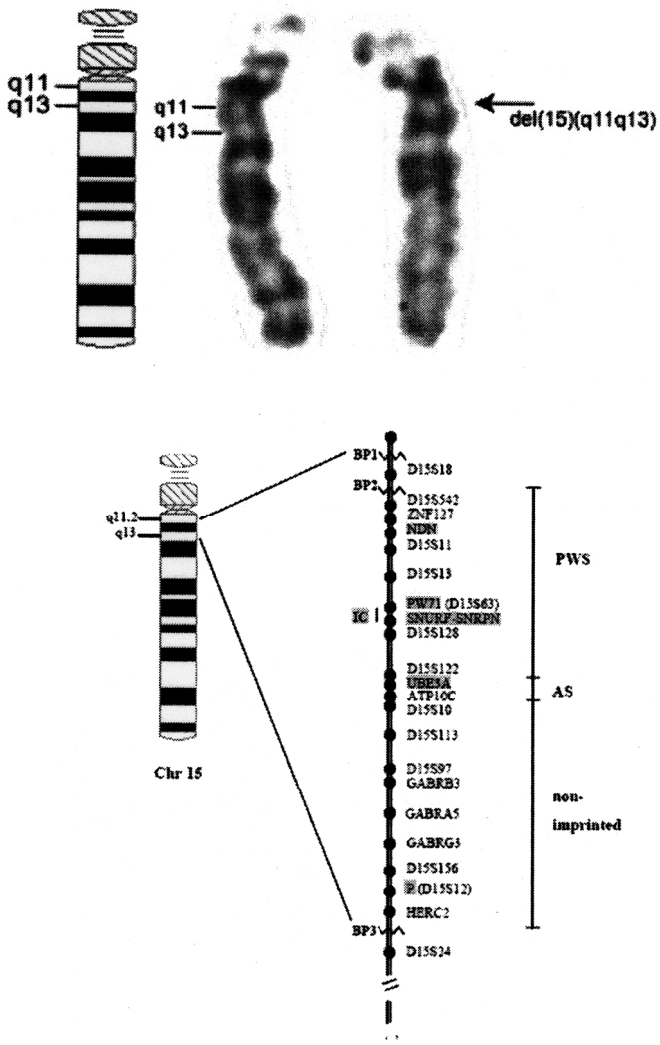


Figure 3. Human chromosome 15q11-13. Common deletion breakpoints (BP1, BP2, BP3), and imprinting center (IC). *Adapted from H. Kokkonen; Genetic changes of chromosome region 15q11-13 in Prader-Willi syndrome and Angelman syndrome in Finland ISBN951-42-7027-4 URL: http//herkules.oulu.fi/isbn9514270274*

1.1.5 Diagnosis

Clinical diagnostic criteria were developed by Holm et al [11] for the diagnosis in children aged 0 to 3 years and from 3 years to adulthood (Table 1). The PW71 (D15S63) methylationtest is presently the method of choice for rapid diagnostic testing of patients suspected of having PWS [43]. The PW71 methylation test, using methylation-

sensitive restriction enzymes, is based on the observation that the D15S63 (PW71) locus in chromosome 15q11-13 is methylated on the maternally derived chromosome, but unmethylated on the paternally derived chromosome [43]. The PW71 methylation test does not differentiate between paternal deletion, maternal disomy or ICMs. Further characterisation of the genetic defect is required to distinguish between those defects. The estimated recurrence risk is lower than 1%. In case of an imprinting center mutation the recurrence risk is higher [44].

Table 1: Consensus diagnostic criteria: *Holm et al (Pediatrics 1993;91:398-340)*

Major criteria
1. Neonatal and infantile central hypotonia with poor suck, gradually improving with age.
2. Feeding problems in infancy with need for special feeding techniques and poor weight gain/ failure to thrive.
3. Excessive or rapid weight gain on weight-for-length chart (excessive defined as crossing 2 centile channels) after 12 months but before six years of age.
4. Characteristic facial features with dolichocephaly in infancy, narrow face or bifrontal diameter, almond shaped eyes, small appearing mouth, with thin upper lip, down-turned corners of the mouth (3 or more required).
5. Hypogonadism – with any of the following, depending on age:
(a) genital hypoplasia, male: scrotal hypoplasia, cryptorchidism, small penis and/or testes for age (<5th percentile); female: absence or severe hypoplasia of labia minora and/or clitoris.
(b) delayed or incomplete gonadal maturation, with delayed pubertal signs in the absence of intervention after 16 years of age (male: small gonads, decreased facial and body hair, lack of voice change; female: amenorrhoea, oligomenorrhoea after age 16).
6. Global developmental delay in a child younger than 6 years of age: mild to moderate mental retardation or learning problems in older children.
7. Hyperphagia/food foraging/obsession with food.
8. Deletion 15q11-13 on high resolution (>650 bands) or other cytogenetic/molecular abnormality of the PWS chromosome region, including maternal disomy.
Minor criteria
1. Decreased foetal movement or infantile lethargy or weak cry, improving with age.
2. Characteristic behaviour problems: temper tantrums, violent outbursts and obsessive-compulsive behaviour; tendency to be argumentative, oppositional, rigid, manipulative, possessive, and stubborn; perseverating, stealing and lying (5 or more of these symptoms required).
3. Sleep disturbance or sleep apnoea.
4. Short stature for genetic background by age 15 (in the absence of growth hormone intervention)
5. Hypopigmentation: fair skin and hair compared to family.
6. Small hands (<25th centile) and/or feet (<10th centile) for height age.

7. Narrow hands with straight ulnar border.
8. Eye abnormalities (esotropia, myopia).
9. Thick, viscous saliva with crusting of corners of mouth.
10. Speech articulation defects.
11. Skin picking.

Supportive findings

12. High pain treshold.
13. Decreased vomiting.
14. Temperature instability in infancy or altered temperature sensitivity in older children and adults.
15. Scoliosis and/or kyphosis.
16. Early adrenarche.
17. Osteoporosis.
18. Unusual skill with jigsaw puzzles.
19. Normal neuromuscular studies.

Major criteria are scored as 1 point, minor criteria as ½ point each. In children <3 years, a total of 5 points, of which at least 4 major criteria are required for diagnosis. In children >3 years, a total of 8 points, of which at least 5 major criteria are required for diagnosis

1.1.6 Growth and body composition in PWS

1.1.6.1 Growth

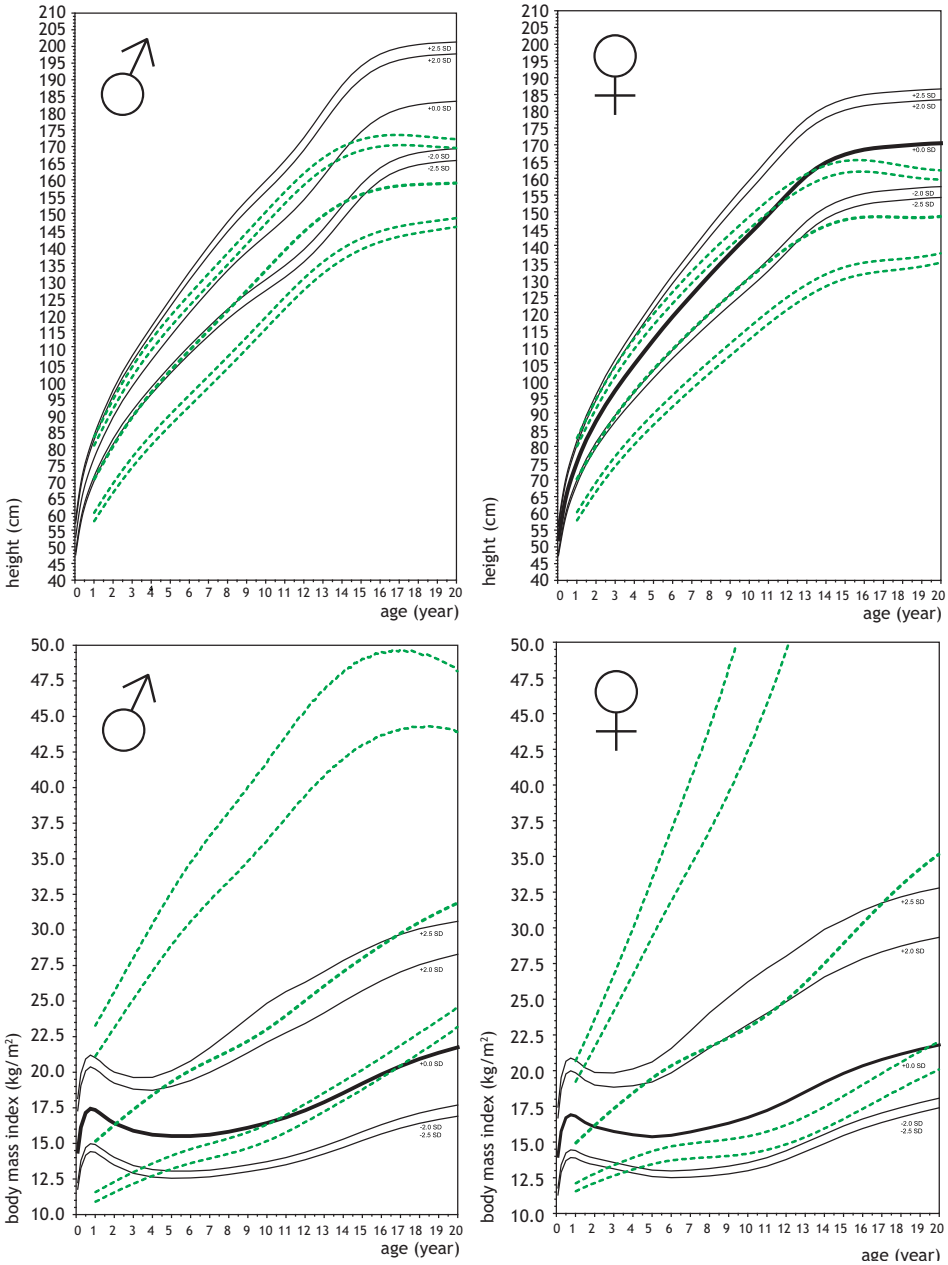


Figure 4. Reference curve indicating height-for-age and BMI-for-age in healthy boys and girls (straight line) and for boys and girls with PWS. *Adapted from (19) and Growthanalyser (www.growthanalyser.org)*

Birth weight, but not birth length is significantly reduced in PWS. Growth during the first months of life may be compromised by feeding difficulties secondary to the muscular hypotonia. Catch-up in length is rare, even when nutrition becomes adequate or even excessive, and some loss of height SDS during the first three years of life has been reported. A close to normal growth rate, however, can be maintained until puberty (with the 50th percentile of the PWS growth curve, corresponding to the 3rd percentile of the reference population), when a gradual decline from the reference curves starts [20,45]. A distinctive pubertal growth spurt is rare, and mean adult height is 155 to 162 cm in males and 145 to 150 cm in females [13,19,20].

Until a height of about 90 cm, nearly all PWS patients have a body weight within the normal range for the general population. After the initial feeding difficulties, obesity is common, generally starting at age 2-5 years and, if uninhibited, proceeding to extremes. When a height of about 120 cm is reached, nearly all patients have surpassed the normal weight-for-height range [20].

1.1.6.2 Body composition

LBM is significantly lower in PWS patients than in healthy normal-weight or obese individuals, which resembles a growth hormone deficient state [46,47]. PWS patients have significantly higher adiposity and lower LBM in the limbs, whereas trunk fat percentage is similar compared to normal weight subjects [46]. Bone mineral content (BMC) is significantly lower in PWS patients than in healthy normal-weight and obese subjects [46]. These abnormalities are more severe in patients over 12 years, indicating a role of hypogonadism in the abnormal body composition [46]. When the diagnosis of PWS is established in the newborn period, parents are prepared for the risk of obesity, and with food restriction weight can often be maintained within normal limits. However, studies of body composition by Dual Energy X-ray absorbtiometry (DXA) have shown that even under these conditions, the ratio between fat and LBM is still elevated in PWS children due to subnormal muscle mass and a relatively high body fat percentage. Even in young, underweight infants, body fat percentage is relatively high [14,15].

Besides GH deficiency, hypogonadism, and increased energy intake, a decreased energy expenditure and reduced physical exercise may play a role in the development and maintenance of obesity in PWS. Total energy expenditure in PWS subjects is low compared to healthy obese controls, and compared to predicted metabolic rate based on height, and weight [47]. The difference between energy expenditure in PWS children and controls could largely be explained by the small fat free mass as found in PWS [47,48].

1.1.7 Endocrine and metabolic aspects

1.1.7.1 Hypothalamic-pituitary axis

The hypothalamus regulates the release of hormones from the anterior pituitary. These hormones are growth hormone (GH), gonadotrophins (LH/FSH), thyrotropin (TSH), prolactin (PRL), and corticotropin (ACTH) (Figure 5). The endocrine status of children with PWS is characterised by a hypothalamic dysfunction. Hypoplasia of the pituitary gland is reported in a subgroup of PWS patients [18], which might reflect the hypothalamic insufficiency. Also a size reduction or even a complete absence of the posterior pituitary bright spot was found in 4 out of 15 patients [27]. The posterior pituitary bright spot is synonymous with the neuropituitary and its presence confirms the integrity of the hypothalamic-pituitary axis.

1.1.7.1.1 Growth hormone (GH) and Insulin-like growth factor I (IGF-I)

Most attention has been paid to the GH-IGF-I axis. Many studies reported that PWS individuals have a lower spontaneous 24-hour GH secretion [49] and a lower response to GH provocation testing with arginine, clonidine, or insulin-induced hypoglycaemia, compared to lean controls, but not compared to obese controls [45,49-52]. In non-syndromal obese children, stimulated GH levels and 24-hour GH secretion are also low. They are considered not to have GH deficiency, but to have a down-regulated GH secretion, which is reversible after weight loss [53]. Therefore the question was raised whether the low GH secretion in PWS was the result of true GH deficiency, or secondarily to obesity. In healthy obese children and adolescents, IGF-I levels are high or normal [54,55]. In contrast, in PWS children, IGF-I levels are low, which is similar to patients with GH deficiency [56]. Other arguments for GH deficiency in PWS are short stature, small hands, feet and abnormal body composition, low insulin levels and the dramatic response to GH treatment, resembling the GH deficient state [49,57,58]. Most authors concluded that GH deficiency in PWS is most likely the result of a hypothalamic dysfunction [49,52,57].

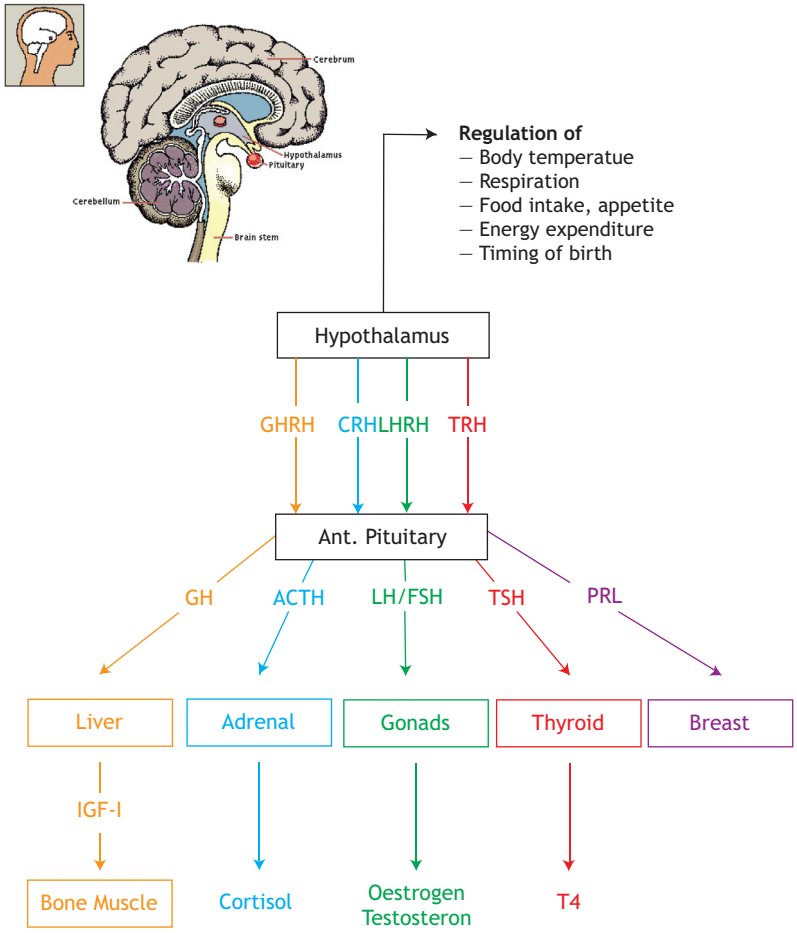


Figure 5. The hypothalamus regulates the release of hormones from the anterior pituitary. GHRH: Growth hormone releasing hormone; CRH: Corticotropin releasing hormone; LHRH: Luteinizing hormone releasing hormone; TRH: Thyrotropin releasing hormone; GH: Growth hormone; ACTH: Corticotropin; LH: Luteinizing hormone; FSH: Follicle stimulating hormone; TSH: Thyrotropin; PRL: Prolactin

1.1.7.1.2 Gonadal dysfunction and cryptorchidism

Puberty is often delayed or incomplete. Cryptorchidism is common in boys with PWS, and they may have hypoplastic external genitalia. Girls often have hypoplastic labia minora, which may be more subtle and easily missed. These abnormalities are compatible with decreased function of the hypothalamus-pituitary-gonadal axis and were generally considered of hypothalamic origin. Alternatively it was suggested that a

lack of abdominal pressure due to extreme hypotonia might play a role in cryptorchidism as well [59]. Several reports demonstrated hypogonadotropic hypogonadism in a subgroup of children, with low response of LH and FSH to GHRH administration [45,60]. However, others reported signs of hypergonadotrophic hypogonadism [61]. Recently, a combination of low LH levels, indicating hypothalamic hypogonadism and high FSH and low Inhibin B, indicating a primary defect in Sertoli and/or germ cell maturation or an early germ cell loss, was reported in PWS boys [62]. Therefore some, but not all, of the PWS patients may have hypogonadotrophic hypogonadism, whereas others, in particular males, may have primary gonadal failure. It is not known whether the latter is the result of uncorrected or late correction of cryptorchidism, which often occurs in PWS boys.

1.1.7.1.3 Thyroid hormone axis

Studies concerning thyroid hormone levels in PWS children are very limited. Congenital hypothyroidism has been reported in one PWS girl [63]. In some studies, however, baseline TSH levels have been reported to be normal, and TSH response to TRH stimuli has been reported to be high-normal [45,60], indicating a mild hypothalamic hypothyroidism in some PWS patients. However, these studies reported thyroid function in mostly obese adult PWS patients.

It has been suggested that GH therapy has an inhibitory effect on the hypothalamo-pituitary-thyroid axis [64,65] and might increase peripheral conversion of T4 to T3 [66-68]. Some studies report low fT4 levels requiring replacement therapy in individual PWS subjects [18,69,70].

1.1.7.1.4 Adrenal axis

Premature adrenarche has been reported in PWS patients [71] and is one of the supportive findings of clinical diagnosis [11]. As a group, PWS children have increased adrenal androgen levels [72]. It has been suggested that this is the result of the PWS associated obesity, which may lead to hyperinsulinism, and increased serum IGF-I levels, which may in turn increase adrenal androgen levels resulting in premature adrenarche. High leptin levels might stimulate CRH. Recently, adrenal androgen levels in PWS were indeed found to be correlated with insulin, leptin and IGF-I [72]. However, in contrast to non-syndromal obesity, PWS children have low insulin and IGF-I levels, probably due to insufficient GH secretion.

1.1.7.2 Carbohydrate metabolism, lipid metabolism and cardiovascular risk factors

As a group, PWS individuals have lower fasting insulin levels, and higher insulin sensitvity than a weight comparable group [73-77]. Suggested mechanisms for the higher insulin sensitivity are GH deficiency [58] and an abnormal body fat distribution compared to simple obesity, as has been described in PWS female adults [78]. In PWS women, insulin sensitivity was higher and hepatic insulin extraction was increased compared to women with simple obesity. Nevertheless, a subgroup of PWS individuals do develop insulin resistance and the absolute prevalence of diabetes mellitus type 2 (DM2) in adult PWS subjects is 25% [79]. The mean age at onset is 20 years.

Adiponectin is an anti-inflammatory adipocytokine that has been inversely related to adiposity and insulin resistance [80-83], and is thought to be protective with regard to DM2 and cardiovascular disease [84]. Adiponectin levels increase during weight loss [85]. Adiponectin levels in obese adults with PWS are high, compared to simple obesity [86-89] and low [86,89] or similar [88] compared to lean adult controls. So far, no published data concerning adiponectin levels in pre-pubertal, not severely overweight PWS children compared to normal-weight healthy controls are available.

1.1.8 Cognition, behaviour and psychotic illness

1.1.8.1 Cognition

IQ tests indicate that IQ in PWS is usually in the ranges of 50-85, with 25% having an IQ over 70 [90,91]. In a UK population study, it was shown that the global ability was near-normally distributed, but shifted downwards by about 40 IQ points for patients with both deletion and UPD. The most likely cause of the intellectual impairment is a genetic defect within the PWS region. It is now generally accepted that, as a group, PWS people have poor short-term memories, deficits in sequential processing, and perform better on visuo-spatial tasks and have exceptional skills with jigshaw puzzles [92,93]. The latter is accepted as one of the supportive findings for the clinical diagnosis of PWS [11]. However, more recently, it was shown that scores on 2 WISC subtests (Object assembly and Block design) were lower in subjects with both deletion and disomy, than in a reference group with mental disability for other reasons. Furthermore, IQ was not related to weight and did not decline with age [93]. Systematic differences between patients with paternal deletions and UPD have been found. Patients with disomy have higher verbal abilities than those with deletions [94], and they have particularly difficulties with the Coding subtest. These differences might result from different expression of genes on chromosome 15, in patients with UPD compared to deletions [94].

1.1.8.2 Behaviour

Many behavioural abnormalities have been reported in PWS, such as temper tantrums, mood instability, self-injury and skin picking, excessive daytime sleepiness, impulsiveness, repetitive speech, obsessional symptoms and unusual routines [95-98]. Skin-picking, in combination with decreased sensitivity to pain, is more frequently observed with increasing age. Some children with PWS have clear signs of autism, some have signs of borderline autism. The obsessive behaviour of children with PWS becomes evident when trivial alterations in routines occur, such events may provoke a tantrum. These symptoms are very specific for PWS and could not be explained by their mental disability and obesity [30-33].

1.1.8.3 Psychosis

Until 1997, several case reports were published of PWS patients who suffered from psychosis. In order to establish whether this was a part of the PWS behavioural phenotype, an international collaboration was proposed and several cases of psychotic PWS patients were collected [99], which indicated a non-chance relationship between PWS and psychoses. The latter has now been confirmed in a population study. This study showed that the prevalence of psychosis in PWS subjects with deletions, only just exceeded the prevalence reported for affective disorders in adults with learning disabilities, but of the adults with UPD, 62% had psychosis. The authors postulated that an abnormal pattern of expression of a maternally or paternally imprinted gene on chromosome 15 might lead to development of psychotic symptoms in PWS [40].

1.1.9 Sleep and breathing abnormalities

Sleep disorders, such as excessive daytime sleepiness and rapid eye movement (REM)-sleep abnormalities have been reported [100]. Respiratory abnormalities, such as hypoventilation [101-103], decreased pulmonary function [104], obstructive and central sleep apnea [105-108], and abnormal ventilatory and arousal response during hypercapnia both during wakefulness and during sleep [100,105,108,109] have been described in PWS. Studies evaluating sleep-related breathing in PWS by overnight complete polysomnography are limited. These studies show a wide range of incidence and nature of sleep-related breathing abnormalities. Most likely, the discrepancies between these studies are caused by the selection of patients: most studies included both children and adults, with or without obesity. Many patients were evaluated after referral to the sleep clinic for symptoms suggesting sleep-related breathing disorders.

A few months after start of the Dutch GH trial, several reports were published on sudden death in PWS children during GH treatment, most of which were related to breathing abnormalities (obstructive apnea, complications of upper respiratory tract infections). A causal relationship with GH treatment was suggested [110-112]. Unexpected death, however, has also been described in non-GH-treated children with PWS (113). In fact, Whittington et al. reported an overall death rate of 3% per year in a PWS population in one of the Health Regions in UK [4].

1.1.10 Associations between sleep-related breathing disorders, cognition, behaviour and psychomotor development

In healthy children, increasing attention has been drawn to the association between sleep-disordered breathing and neurobehavioural abnormalities, in particular symptoms of Attention-Deficit/Hyperactivity Disorder (ADHD). Associations between sleep-disordered breathing and cognition, school performance and psychiatric and behavioural co-morbidities are consistently reported, and seem to be at least partial reversible [114-116]. In PWS patients, no reports on the associations between sleep-related breathing disorders and cognition or behaviour were available at the time we started the study.

1.2 Growth hormone treatment

Twenty years ago, GH treatment has first been suggested to improve growth velocity in children with PWS [117]. Until now, several studies have shown that GH treatment improves height velocity in PWS children [49,51,118-120], resulting in a normal height-for-age, if started before puberty. A few GH-treated patients had reached final height, which was within the ± 2 SDS of their target height [121]. In addition, GH treatment has been shown to improve (but not normalise) body composition, with an increase in LBM and a decrease in body fat percentage, as had been measured with doubly labelled water [118] or DXA [49,70,122]. In 2000, GH was registered for PWS children.

It might well be that the anabolic and lipolytic effects of GH treatment are far more important for the children than the increase in height velocity. In previous studies, GH treatment improved muscle strength and physical performance [52,69,122], and parents reported that their child was more active in daily life [50, 52,69,122]. The latter remain to be objectively confirmed. In addition, preliminary reports indicated that GH treatment prevented deterioration of behaviour [123]. In several studies parents

reported that their child was more alert, had a more stable temperament and was easier to handle [69,119,123]. In contrast, it was reported that after discontinuation of GH treatment, children were more unhappy, more tired and less active. After stop of GH they had more temper tantrums and were more difficult to handle [69,123].

Recently, the question was raised whether start of GH treatment in infancy, might stimulate psychomotor development. Due to the increased awareness of paediatricians and neonatologists of the clinical presentation of PWS in infancy combined with the availability of the methylation test for rapid and reliable diagnosis, PWS is now usually diagnosed in infancy. Data on the effects of GH treatment on psychomotor development in PWS infants and toddlers are, however, limited. Eiholzer at al demonstrated a normalisation of LBM after 30 months of GH treatment in 18 PWS infants (<2 years) [124]. These children started walking freely at an earlier age than PWS children without GH, reported in literature [125]. Unfortunately, this study had no randomised controlled design. So far, there is only one controlled study on motor development in PWS infants during GH treatment, demonstrating an improvement in body composition, and suggesting an improvement in motor development if GH is started at an early age [126]. Long-term reports (> 1 year) of controlled GH studies in PWS, are however not available.

Figure 6. (**a**) An adolescent with PWS before the era of early diagnosis and GH treatment. (**b**) A different generation. Adapted from U. Eiholzer, Prader-Willi Syndrome: Coping with the disease – living with those involved, Karger, Edt, Dr. E. Zangger *(with permission)*

1.3 Growth hormone treatment and safety issues

1.3.1 Sleep-related breathing disorders

After recent reports of unexpected death in children and adults with PWS [110,111,113, 127] renewed attention had been drawn to the described respiratory abnormalities. Some of the unexpected deaths were related to sleep apnea, obstruction or upper respiratory tract infections. Some of the deceased children, were treated with GH. By some clinicians a causal relationship between GH treatment and unexpected death was suggested [110-112]. Pfizer, the pharmaceutical company that has a license for GH treatment of PWS children sent out a safety warning, stating that PWS children with respiratory abnormalities and obesity should be excluded from GH treatment. At the start of this study, only 3 reports were available concerning the effects of GH treatment on respiratory parameters in PWS children. Lindgren et al found improved CO_2-responsiveness in 9 PWS children after 6-9 months of GH compared with baseline [128]. Myers et al demonstrated that inspiratory and expiratory muscle strength improved in 20 PWS children, aged 4 to 16 years after 12 months of GH compared with 10 controls [70]. Haqq et al reported in a double blind placebo-controlled cross-over GH study a decrease in apnea hypopnea index after 6 months of GH in 12 PWS children, aged 4.5 to 14.5 years, although this did not reach statistical significance [129]. More data are however required on the effects of GH on breathing.

1.3.2 Scoliosis

Scoliosis in PWS is common, starts in childhood and becomes worse during adolescence. Many adolescent patients have orthopaedic problems such as lumbago, walking difficulties, contractures etc. Muscle hypotonia might contribute to the severity of scoliosis. Increased height velocity, as is evidently present during GH therapy, is generally considered as one of the exacerbating factors of scoliosis [130]. On the other hand, increased muscle mass and muscle strength during GH treatment might compensate for the effects of height velocity. Preliminary retrospective studies showed that GH therapy does not induce scoliosis, and that early start of GH therapy may not be an exacerbating factor of scoliosis [131]. However, prospective studies on the effects of GH on progression of scoliosis are required.

1.3.3 Thyroid hormone levels

Growth hormone might interact with the thyroid hormone axis, either via a direct inhibition of TSH secretion or via an increased peripheral conversion of T4 to T3.

Prospective studies on the effects of GH treatment on thyroid hormone function in children with PWS are, however, not available, but some studies report low fT4 levels requiring replacement therapy in individual PWS subjects during GH [18,69,70].

1.3.4 Risk factors for DM2

GH treatment has been associated with decreased insulin sensitivity [132]. Recently, a higher incidence of insulin resistance and DM2 has been reported in GH-treated children, compared to the normal references [133]. These authors warned that children with a higher risk for developing DM2, such as PWS children, are more prone to develop DM2 during GH treatment. Monitoring of glucose homeostasis during GH treatment in these children is therefore warranted.

1.4 Aims of the study and outline of the thesis

This thesis presents a detailed description of several studies on growth, metabolism, psychomotor development, cognition, behaviour and breathing in PWS children. These children were enrolled in the Dutch GH study in PWS children. For a detailed description of the study, the reader is referred to Appendix A

Growth and body composition *(Chapter 2)*

We aimed to investigate whether GH improves growth velocity and contributes to the maintenance of normal weight-for-height in PWS children compared with randomised controls. In addition, we evaluated the effects of GH on body composition and body proportions.

Psychomotor development in infants with Prader-Willi Syndrome *(Chapter 3)*

We aimed to evaluate if GH treatment improves mental and/or motor development, when started at a very early age. We hypothesized that initiation of GH treatment in infancy stimulates acquisition of gross motor skills, such as sitting and standing independently, walking, and climbing stairs. In addition, we postulated that an improvement of motor development stimulates the infant to explore his/her environment, and will subsequently improve mental development. We performed Bayleys Scales of Infant Development (BSID II) to evaluate mental and motor development.

Breathing disorders *(Chapter 4)*

The aim of this part of the study was to determine whether sleep-related breathing disorders do occur in young, pre-pubertal PWS children, and if so, how frequently. In addition, we evaluated whether GH treatment worsens sleep-related breathing disorders. We performed complete nocturnal polysomnography both before and during GH treatment. In addition, we reviewed literature on respiratory abnormalities in PWS, and the effects of GH on breathing disorders, and sleep-related breathing during upper respiratory tract infections.

Associations between sleep-related breathing, cognition, psychomotor development and neurobehavioural abnormalities *(Chapter 5 and 6)*

In healthy children and adults consistent relations have been found between sleep-disordered breathing and neurobehavioural abnormalities. Since sleep-disordered breathing, neurobehavioral abnormalities, and cognitive deficits are common in PWS, we evaluated the associations in PWS.

In addition, we evaluated in infants whether psychomotor development is affected by sleep-disordered breathing, particularly obstructive sleep apnea syndrome (OSAS), as was recently shown for healthy infants.

Thyroid hormone function *(Chapter 7)*

The aim of this part of the study was to evaluate the thyroid hormone function in a large group of pre-pubertal PWS children, and whether GH treatment affects thyroid hormone function.

Adiponectin levels and insulin sensitivity *(Chapter 8)*

As GH treatment induces insulin resistance, we aimed to evaluate serum adiponectin levels both before and during GH treatment. Adiponectin was chosen because it is strongly related to insulin sensitivity and is considered cardioprotective.

In **chapter 9**, the general discussion, our data are discussed in relation to the present literature. **Chapter 10** summarizes our findings in English, and **chapter 11** presents a Dutch summary.

Appendix A

In- and exclusion criteria and design of the study, entitled *"Multicenter, randomised controlled growth hormone study in children with Prader-Willi Syndrome: effects on growth, body composition, activity level and psychosocial development"*

A.1 Patients

Inclusion criteria:

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

Exclusion criteria:

- Severe degree of scoliosis >15-20°
- Non cooperative behaviour
- Extremely low dietary intake of less than minimal required intake according to WHO
- Medication to reduce weight (fat)
- If female >13 years of age: a positive pregnancy test
- In children over 3 years of age: height > 0 SDS unless weight-for-height is > 2 SDS.
- Previously treated with GH

A.2 Study Design

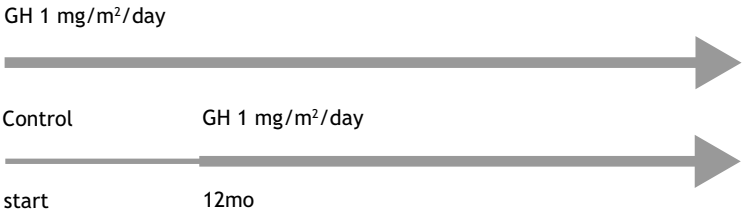
Group 1

Infants

Infants, with age between 6 months and 3 years at start of study, after randomisation for age, were divided into 2 groups

1.a. GH treatment group (50% of infants)
Infants were treated with GH 1 mg/m^2/day s.c. from start of the study.

1.b. Control group (50% of infants)
Infants were followed as control patients during the first year of the study. After one year of follow-up, GH treatment 1 mg/m^2/day s.c. was started.



Group 2

Pre-pubertal children

Pre-pubertal children with PWS, aged 3-12 years in girls, 3-14 years in boys, after randomisation for BMI, were divided into 2 groups

2.a. GH treatment group (50% of children)
Children were treated with GH 1 mg/m²/day s.c. + dietary advice + training. Three months prior to the study dietary advice and training were started.

2.b. Control group (50% of children)
No GH treatment, but children received dietary advice + training i.e. control group for 24 months. After 3+24 months children in group 2.b started GH treatment in a dose of 1 mg/m²/day s.c.

Group 3

Pubertal children

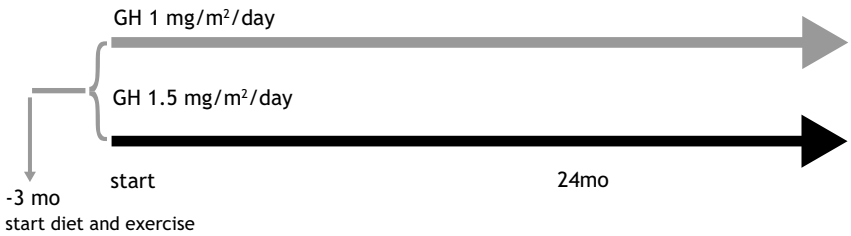
If puberty did not start spontaneously in children with PWS with chronological age at or above 12 years in girls and at or above 14 years in boys **puberty was induced.** All pubertal children were treated with GH.

3.a. GH treatment group 1 mg/m²/day (50% of adolescents)
Pubertal children were treated with GH 1 mg/m²/day s.c. + dietary advice + training.
Three months prior to the study dietary advice and training were started.

3.b. GH treatment group 1.5 mg/m^2/day (50% of adolescents)

Pubertal children were treated with GH 1.5 mg/m^2/day s.c. + dietary advice + training.

Three months prior to the study dietary advice and training were started.



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Chapter 2

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Submitted

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

2.1 Abstract

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

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

Design/Patients: We performed a randomised controlled GH trial in 91 pre-pubertal PWS children (42 infants, 49 children, aged 3-14 years). Infants were randomly assigned to GH treatment (GH group; 1 mg/m^2/day; n=20), or no treatment (control group; n=22) for 1 year. Children >3 years were randomly assigned to GH treatment (GH group; 1 mg/m^2/day; n=27) or no treatment (control group; n=22) for 2 years.

Methods: Anthropometric parameters were assessed 3-monthly. Body composition was measured by Dual Energy X-ray Absorptiometry.

Results: Height, body mass index (BMI), and head circumference completely normalised during 1 and 2 years of GH in infants and children, respectively. Body fat percentage and body proportions improved in GH-treated children, but did not completely normalise. Lean body mass, for height and sex, did improve compared with the control group. Insulin like growth factor I (IGF-I) levels increased to levels above the normal range in most children.

Conclusions: Thus, 2 years of GH in PWS children normalises height, BMI and head circumference, and improves body composition, and body proportions. PWS children are highly sensitive to GH, suggesting that monitoring of serum IGF-I is indicated.

2.2 Introduction

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

Several endocrine abnormalities have been reported, including growth hormone (GH) deficiency and hypogonadotropic hypogonadism [4]. Twenty years ago, GH treatment was suggested to improve growth velocity in children with PWS [5]. Nowadays, studies are available, showing that GH treatment improves growth velocity notably in GH-deficient PWS children [6-8], or in PWS children treated irrespective of their GH-status [9]. In addition, body composition improved, i.e. body fat percentage decreased and lean body mass (LBM) (expressed as kg) increased during 1 year of GH treatment [7,10]. From the methodological point of view, it is striking that those studies did not adjust LBM for sex- and age dependency or, more importantly, for height at baseline and during GH treatment. Consequently, these studies might have reported an underestimation of LBM for height and an overestimation of the effect of GH treatment on LBM. There is only one study describing LBM adjusted for height in PWS children, but that was a non-controlled trial [11]. In previous studies, the incidence of overweight is high. Today, due to early diagnosis and intervention, severe obesity can often be prevented in PWS children, resulting in a different clinical presentation.

We therefore evaluated a large group of 91 PWS children, according to a one- and two-year controlled design in infants and children, respectively. We investigated effects of GH on growth, LBM adjusted for height, and body fat percentage, head circumference, and body proportions.

2.3 Patients and Methods

Patients

Ninety-one patients were included in our randomised controlled GH trial. Forty-two children below 3.5 years at start of study *("PWS infants")* were treated in a one-year controlled study. Forty-nine pre-pubertal children over 3.5 years at start of study *("Pre-pubertal PWS children")* were treated in a two-year controlled study. All participants fulfilled the following inclusion criteria: (1) genetically confirmed diagnosis of PWS; (2) age between 6 months and 14 years at start of study; (3) bone age less than 14 years (girls) or 16 years (boys); (4) pre-pubertal at start of study, defined as Tanner breast stage < 2 for girls and testicular volume less than 4 ml for boys [12], or with age below 12 or 14 years in girls or boys, respectively. Patients with non-cooperative behaviour or patients receiving medication to reduce fat were excluded. Children were regularly seen by a dietician and a physiotherapist. The caloric intake and activity level of all children were standardised at 3 months prior to start of study, and recommendations were given. Compliance to diet and exercise was evaluated by the research nurse (MvE), in close collaboration with the dietician and, if indicated, the physiotherapist. All children were naïve to GH treatment at the start of study. They were included irrespective of their GH secretory status. After stratification for age and BMI, all participants were randomly assigned to GH treatment (GH group) or no GH treatment (control group). The GH group received Genotropin® (somatropin) GH 1 mg/m^2/day s.c. (Pfizer).

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

Methods

Anthropometry

At baseline, and at 3-monthly intervals, anthropometric measurements were performed. Standing height (or supine length, when appropriate) and sitting height (only in children >3.5 years) were obtained using a Harpenden stadiometer and a sitting height table. Weight was measured on an accurate scale. Left foot length, left tibia length and span width were measured according to Cameron [13], using a Harpenden anthropometer. The mean of 3 measurements was used for analysis. All

measurements were obtained by two observers (DF, MvE). Height, body mass index (BMI), and head circumference were expressed as standard deviation (SD) scores, adjusting for age and sex [14,15]. BMI and SD scores of BMI, height, sitting height, head circumference, foot length, span width, and tibia length were calculated with Growthanalyser, Version 3.0. (www.growthanalyser.org)

Body composition

Dual Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare) was performed in all children and total fat mass, fat percentage, and LBM were measured. Fat mass, fat percentage and LBM were transformed into SD scores adjusting for sex and age (LBM_{age}) [16]. Since body composition, particularly LBM, is strongly related to height, LBM expressed as SD-score for age and sex might result in an underestimation in short stature. Besides, we were interested to know whether GH had an effect on LBM beyond the catch-up in height. For these reasons, LBM was also expressed as SDS for height and sex (LBM_{height}). The LBM_{height} SD scores were computed by comparing LBM of PWS children with LBM of healthy children with the same height and sex. Reference values were obtained using the same instrumentation and software [16]. Because reference data for DXA (Lunar Prodigy) were not available for children aged 0-4 years, only children >4 yrs were included in this analysis.

IGF-I and IGFBP-3

Blood samples were collected in the morning after a 12h overnight fast, immediately centrifuged and stored at -20° until assayed. Samples were pooled, and assayed in one session, to minimize variation.

Serum insulin like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) were measured using a specific RIA in one laboratory [17]. The intra-assay coefficient of variation (CV) was 4% and the inter-assay CV was 6%. For IGF-I and IGFBP-3, sex- and age-matched Dutch references were available. Because of the age- and sex dependency IGF-I and IGFBP-3 levels were transformed to SD scores.

Statistical analysis

Statistical analysis was performed by the Statistical Package for Social Sciences (Version 11.0, Chicago, IL). Most of our data were not Gaussian distributed. We therefore expressed data as median (interquartile range, iqr), and we used non-parametric tests. Differences compared with baseline within groups were calculated using Wilcoxon signed rank test. Differences in change compared with baseline between

groups were calculated using Mann Whitney U tests. We used Bonferroni's correction for multiple testing for both inter- and intra-individual testing. We used the binomial test to compare results of SD scores in PWS children with healthy reference data (0 SDS). Values between -2 SDS and +2 SDS are considered within the normal range. Correlations were calculated using Spearman's rank correlation coefficient (rho).

2.4 Results

Infants

Clinical characteristics

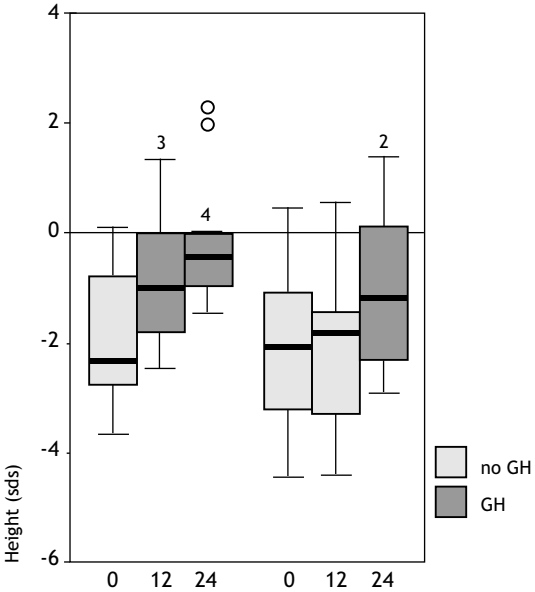
Table 1 lists the clinical data of 42 PWS infants and toddlers. After 1 year, 31 of them were re-evaluated (11 did not yet complete 1 year of study). After 1 year the control group also started with GH. After 2 years, 24 of them were re-evaluated (7 did not yet complete the second year of study). Infants with repeated measurements had similar SD scores of BMI and height compared with infants who did not finish 2 years of study yet. Infants with repeated measures were, however, older (p=0.025), which might reflect early diagnosis of PWS during recent years. One infant of the GH group was treated with thyroxin replacement therapy to correct low fT4 levels prior to start of GH. Fifteen infants had a paternal deletion, 13 a uniparental maternal disomy, and in 14 PWS was confirmed by a positive methylation test, albeit the underlying genetic defect was yet unknown.

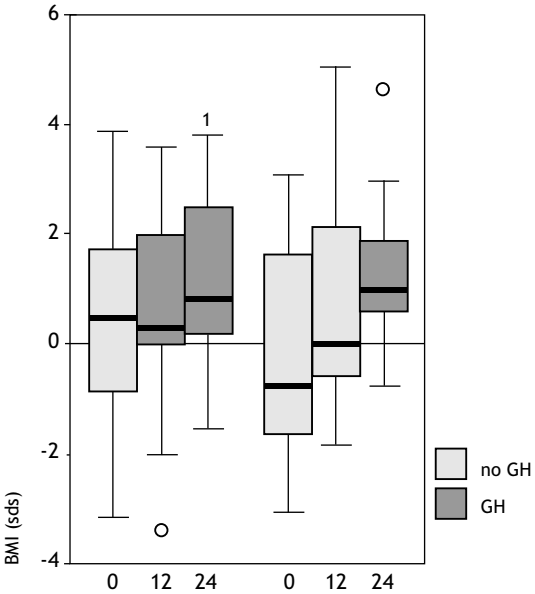
Height, BMI and head circumference

At start, BMI was not significantly different from 0 SDS, but all other median SD scores were significantly below 0 (Table 1). Anthropometric parameters were similar in the 2 groups (Figure 1 a-c). Median head circumference SDS was relatively high compared to height SDS; -0.8 (-1.6 to -0.3) versus -2.3 (-2.8 to -0.7) in the GH group, and -1.1 (-1.8 to -0.5) versus -2.1 (-3.2 to -1.0) in the control group.

In the GH group, height SDS increased from -2.3 (-2.8 to -0.7) to -1.0 (-1.9 to 0.10) ($p < 0.005$) after 1 year, and to -0.4 (-1.1 to 0.0) ($p < 0.001$) after 2 years. After 2 years of GH, all infants had a height SDS above -2 SDS. In the control group, height SDS remained low in the first year, at start -2.1 (-3.2 to -1.0), and after 1 year -1.8 (-3.5 to -1.4) (not significant), but height increased to -1.2 (-2.3 to 0.1) ($p < 0.01$), when GH was started in the second year (Figure 1a). Head circumference SDS showed a similar trend from -0.8 (-1.6 to -0.3) to 0.0 (-0.9 to 0.7) ($p<0.005$) to 0.4 (-0.5 to 1.1) ($p<0.005$)

in the GH group, and from -1.1 (-1.8 to -0.5) to -0.8 (-1.6 to -0.3) ($p<0.05$) to 0.0 (-0.6 to 0.6) ($p<0.01$) in the control group (Figure 1c). BMI SDS increased progressively in both groups, but remained within the normal range in most patients (Figure 1b).





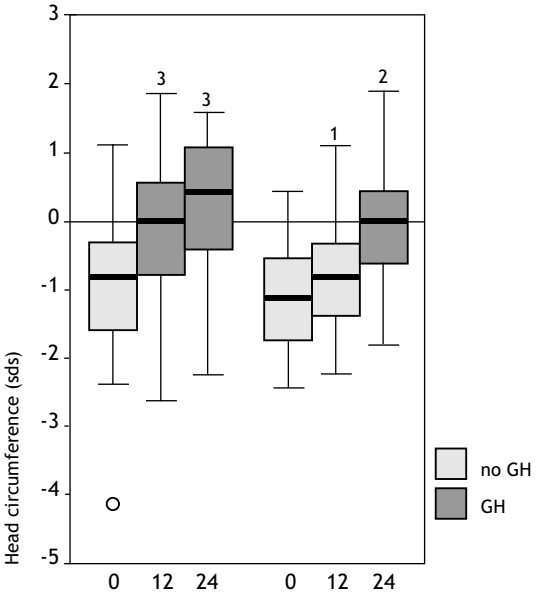


Figure 1 a-c: Anthropometric data in PWS infants, during 2 years follow-up. In the first year only the GH group received GH, during the second year both groups were treated. Data are expressed in SDS for age and sex and represented by boxplots. The lower boundary is the 25th percentile and the upper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

Table 1. Clinical characteristics of PWS infants (6 months to 3 years)

	GH Group			Control Group		
	0	*1* **[GH]**	*2* **[GH]**	*0*	*1* **[-]**	*2* **[GH]**
N	20	16	12	22	15	12
Sex (m/f)	12/8			16/6		
Age (yrs)	2.0 (1.6 to 3.1)	3.1 (2.6 to 4.1)	4.5 (3.6 to 5.1)	1.3 (1.0 to 2.8)	2.6 (2.3 to 4.2)	4.2 (3.3 to 5.2)
Height (SDS)	-2.3 (-2.8 to -0.7)	-1.0 (-1.9 to 0.1)[4d]	-0.4 (-1.1 to 0.0)[3a]	-2.1 (-3.2 to -1.0)	-1.8 (-3.5 to -1.4)[d]	-1.2 (-2.3 to 0.1)[2a]
BMI (kg/m^2)	16.4 (15.1 to 18.6)	16.3 (15.7 to 18.2)	16.7 (15.7 to 21.2)	16.1 (14.7 to 18.2)	16.4 (15.4 to 19.8)	17.1 (16.4 to 18.7)
BMI (SDS)	0.5 (-0.9 to 1.9)	0.3 (-0.1 to 1.6)[1]	0.8 (0.1 to 2.8)[3]	-0.8 (-1.7 t0 1.6)	0.1 (-0.7 to 2.6)[2]	0.9 (0.5 to 1.9)[3]
Head circ (SDS)	-0.8 (-1.6 to -0.3)	0.0 (-0.9 to 0.7)[3d]	0.4 (-0.5 to 1.1)[3]	-1.1 (-1.8 to -0.5)	-0.8 (-1.6 to -0.3)[1d]	0.0 (-0.6 to 0.6)[2]
IGF-I (ng/ml)	27.0 (22.0 to 35.0)	179.0 (119.5 to 241.0)	287.0 (227.0 to 419.0)	47.0 (17.0 to 52.0)	33.0 (22.5 to 47.8)	259.0 (164.0 to 292.0)
IGF-I (SDS)	-1.9 (-2.8 to -1.3)	2.5 (1.6 to 3.0)[3]	3.2 (1.9 to 4.3)	-1.6 (-2.6 to -0.4)	-2.1 (-3.1 to -0.5)	2.4 (2.2 to 3.0)
IGFBP-3 (ng/ml)	0.8 (0.7 to 1.1)	2.2 (1.6 to 2.4)	2.7 (2.2 to 3.6)	1.1 (0.8 to 1.3)	0.9 (0.7 to 1.3)	2.5 (1.9 to 2.6)
IGFBP-3 (SDS)	-2.6 (-3.3 to -2.0)	0.5 (0.0 to 1.2)[2]	1.5 (0.4 to 2.3)[1]	-1.5 (-2.6 to -0.7)	-2.4 (-3.5 to -1.2)	0.7 (0.5 to 1.1)
IGF-I/BP3 (SDS)	-0.9 (-2.0 to -0.4)	2.3 (1.7 to 3.4)[2d]	2.4 (1.7 to 3.3)	-0.3 (-1.7 to 0.6)	-1.1 (-2.1 to 0.0)[d]	2.5 (2.0 to 3.5)

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

IGF-I and IGFBP-3 levels

IGF-I levels rapidly increased during GH treatment to levels with a median above +2 SDS (Table 1). After 1 year of GH treatment, 8/13 infants (62%) had an IGF-I level > +2 SDS, and after 2 years, 5/7 (71%). In the control group, IGF-I levels only increased during GH in the second year, with 6/7 (86%) infants having an IGF-I level > +2 SDS. IGFBP-3 levels increased during GH treatment and remained low during the first year in the control group. However, as IGFBP-3 levels were within the normal range during GH, the IGF-I/IGFBP-3 ratio increased from -0.9 (-2.0 to -0.4) to 2.3 (1.7 to 3.4) ($p<0.01$) to 2.4 (1.7 to 3.3) ($p=0.056$) in the GH group and from -0.3 (-1.7 to 0.6) to -1.1 (-2.1 to 0.0) to 2.5 (2.0 to 3.5) ($p= 0.056$) in the control group.

Pre-pubertal children (3-14 years)

Clinical characteristics

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

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

Height, BMI and body proportions

At start of study, BMI was significantly higher than 0 SDS (Table 2). Ten out of 47 children (21%) were considered overweight. All other median SD scores were significantly below zero. Head circumference SDS, however, appeared to be relatively high compared to other anthropometric parameters. At start, height SDS and BMI SDS were highly correlated ($r = 0.44$, $p<0.005$), but neither height SDS nor BMI SDS were related to IGF-I SDS. Baseline anthropometric parameters were not significantly different between the GH- and control group.

Table 2. Clinical characteristics of pre-pubertal PWS children (3-14 years)

	GH Group			Control Group		
	0	*1* **[GH]**	*2* **[GH]**	*0*	*1* **[-]**	*2* **[-]**
N	25	21	20	22	21	20
Sex (m/f)	13/12			8/14		
Age (yrs)	6.8 (5.4 to 8.8)	7.8 (6.4 to 9.7)	8.7 (7.3 to 11.2)	5.9 (4.7 to 7.4)	7.0 (5.6 to 8.5)	8.0 (6.6 to 9.5)
Height (SDS)	-2.0 (-3.1 to -1.7)	-1.2 (-1.5 to -0.6)[4d]	-0.6 (-1.1 to -0.1)[4d]	-2.5 (-3.3 to -1.9)	-2.6 (-3.4 to -2.3)[d]	-2.6 (-3.4 to -2.3)[d]
BMI (kg/m^2)	17.7 (16.0 to 22.3)	17.5 (15.3 to 19.8)	17.5 (16.1 to 21.1)	18.1 (17.2 to 19.9)	18.6 (17.6 to 19.7)	19.1 (17.8 to 20.8)
BMI (SDS)	1.2 (0.1 to 2.2)	0.8 (-0.3 to 1.4)[4d]	0.6 (-0.4 to 1.6)[3c]	1.3 (1.1 to 1.6)	1.4 (1.0 to 1.6)[d]	1.3 (1.1 to 1.6)[c]
Head circ (SDS)	-0.8 (-1.5 to -0.2)	-0.2 (-1.2 to 0.2)[4]	-0.1 (-1.1 to 0.5)[3]	-0.6 (-1.2 to -0.1)	-0.6 (-0.9 to 0.3)[1]	-0.6 (-1.1 to 0.3)[a]
IGF-I (ng/ml)	60.0 (46.5 to 96.5)	337.0 (274.3 to 474.3)	424.0 (313.0 to 570.0)	56.0 (42.0 to 88.0)	55.0 (42.5 to 94.8)	92.0 (61.8 to 130.0)
IGF-I (SDS)	-1.7 (-2.3 to -1.2)	2.3 (1.5 to 2.7)[4d]	2.4 (2.1 to 2.8)[4d]	-1.9 (-2.6 to -1.2)	-2.5 (-2.8 to -1.4)[d]	-1.8 (-2.6 to -1.0)[d]
IGFBP-3 (ng/ml)	1.3 (0.9 to 1.5)	2.5 (2.2 to 2.9)	2.8 (2.6 to 3.2)	1.2 (0.9 to 1.5)	1.3 (0.8 to 1.5)	1.5 (1.2 to 1.8)
IGFBP-3 (SDS)	-1.9 (-2.8 to -1.2)	0.4 (-0.1 to 0.8)[4d]	0.6 (0.3 to 1.1)[4d]	-2.2 (-3.1 to -1.4)	-2.4 (-3.5 to -1.8)[d]	-1.7 (-2.3 to -1.2)[d]
IGF-I/BP3 (SDS)	-0.5 (-1.0 to 0.5)	2.5 (2.0 to 3.0)[4d]	2.5 (1.8 to 2.9)[4d]	-0.6 (-1.6 to 0.3)	-0.8 (-1.4 to -0.2)[d]	-0.6 (-1.2 to -0.1)[d]

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

[1]p<0.05 [3]p<0.005 [4]p<0.001 compared with baseline levels, [a]p<0.05 [c]p<0.005 [d]p<0.001 change GH group vs. control group

During GH treatment, median height SDS increased compared with baseline from -2.0 (-3.1 to -1.7) to -1.2 (-1.5 to -0.6) ($p<0.001$) after 1 year, and to -0.6 (-1.1 to -0.1) ($p<0.001$) after the second year. In the control group, who did not receive GH for 2 years, height SDS remained low (GH group vs. control group $p<0.001$ and $p<0.001$, after 1 and 2 years, respectively) (Figure 2a). BMI SDS decreased significantly ($p<0.001$) during the first year of GH treatment and then stabilized, at a level that was not significantly higher than 0 SDS ($p=0.08$ and $p=0.12$ after 1 and 2 years respectively), whereas in the control group BMI remained significantly higher than 0 SDS (Figure 2b). Head circumference increased significantly to completely normal values during GH treatment ($p<0.001$ and $p<0.005$ compared with baseline, after 1 and 2 years respectively) (Figure 2c), whereas tibia length, foot length, arm span, and sitting height significantly improved, but remained significantly lower than 0 SDS (Table 3).

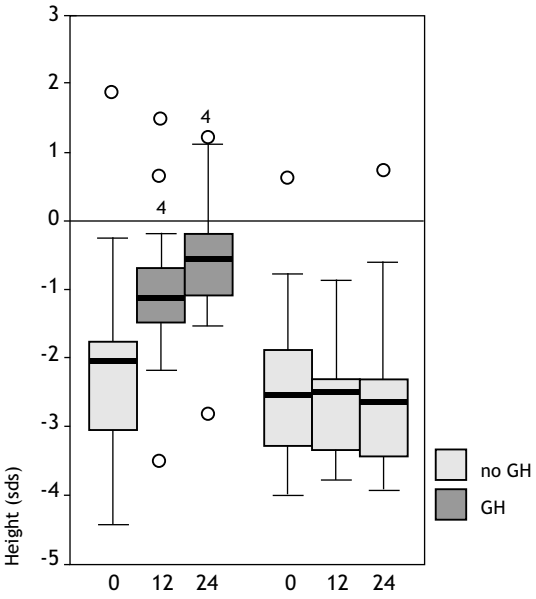
Body composition

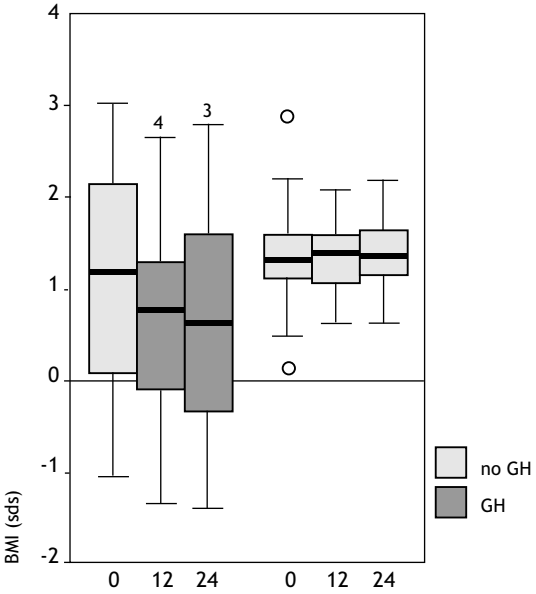
At start of study, lean body mass corrected for age and sex (LBM_{age}), and for height and sex (LBM_{height}) were significantly below 0 SDS and body fat percentage was significantly higher than 0 SDS in the total group. During GH treatment, LBM_{age} SDS increased from -1.7 (-3.0 to -1.0) to -0.5 (-1.3 to 0.7) ($p<0.005$) after 1 year, and to -0.1 (-1.3 to 0.6) ($p = 0.10$ compared with baseline) after 2 years, resulting in a LBM_{age} not significantly below 0 SDS after 1 and 2 years of GH treatment (Table 3, Figure 3a). In the control group, LBM_{age} SDS declined over time from -1.9 (-3.4 to -1.2) to -2.1 (-4.1 to -1.3) ($p<0.05$) to -2.5 (-3.8 to -1.4) ($p<0.005$) after 2 years and body fat percentage remained high. However, LBM_{height} SDS did not significantly increase in the GH group from -1.7 (-3.8 to -0.6) to -1.5 (-2.3 to -0.7) (NS) to -1.9 (-2.4 to -1.4) (NS). Notably, in the control group there was a progressive and significant decrease in LBM_{height} SDS from -1.4 (-2.9 to 0.9) to -1.9 (-2.9 to 0.0) ($p<0.05$) to -2.3 (-2.7 to -1.3) ($p<0.005$), resulting in a significantly different change in LBM_{height} between the GH group and the control group after 1 ($p < 0.05$) and 2 ($p < 0.05$) years. Body fat percentage SDS decreased significantly from 2.1 (1.7 to 2.7) to 1.5 (0.7 to 2.1) ($p<0.005$) to 1.9 (0.7 to 2.3) ($p < 0.005$), but body fat percentage was still significantly higher than 0 SDS, after 1 and 2 years of GH. Trunk fat (%) significantly decreased in the first year of GH, and increased in the second year to a level still significantly below baseline. In contrast, in the control group, trunk fat increased gradually, resulting in significantly higher levels after 2 years.

Table 3. Body proportions and body composition of pre-pubertal PWS children (3-14 years)

	GH Group			Control Group		
	0	1 [GH]	2 [GH]	0	1 [-]	2 [-]
Tibia length (SDS)	-1.2 (-1.9 to -0.5)	-0.9 (-1.4 to -0.2)[1a]	-0.5 (-0.9 to -0.3)[1d]	-1.8 (-2.6 to -1.4)	-2.0 (-2.7 to -1.2)[a]	-2.4 (-3.0 to -1.7)[2d]
Foot length (SDS)	-1.8 (-3.3 to -1.6)	-1.3 (-2.4 to -0.9)[4d]	-1.4 (-2.1 to -0.5)[3d]	-2.2 (-2.7 to -1.6)	-2.5 (-3.4 to -1.7)[3d]	-3.2 (-3.7 to -2.2)[3d]
Sitt height (SDS)	-1.3 (-1.7 to -0.8)	-0.4 (-1.0 to 0.2)[4d]	-0.4 (-1.2 to 0.0)[4d]	-1.6 (-2.0 to -0.8)	-1.5 (-2.0 to -0.7)[d]	-2.1 (-2.5 to -0.8)[1d]
Arm span (SDS)	-1.6 (-2.3 to -0.8)	-1.0 (-1.4 to -0.5)[2d]	-0.9 (-1.6 to -0.5)[1d]	-1.8 (-2.8 to -1.2)	-2.0 (-3.3 to -1.6)[1d]	-2.4 (-3.6 to -1.7)[1d]
Fat % (SDS)	2.1 (1.7 to 2.7)	1.5 (0.7 to 2.1)[3d]	1.9 (0.7 to 2.3)[3d]	2.3 (1.9 to 2.6)	2.3 (2.0 to 2.6)[d]	2.4 (2.1 to 2.7)[d]
Fat (SDS)	1.2 (0.8 to 2.0)	0.9 (0.2 to 1.4)[3d]	1.1 (0.6 to 2.0)[1b]	1.2 (0.7-1.6)	1.3 (0.7 to 1.9)[d]	4.5 (0.9 to 2.0)[b]
LBMage (SDS)	-1.7 (-3.0 to -1.0)	-0.5 (-1.3 to 0.7)[3d]	-0.1 (-1.3 to 0.6)[d]	-1.9 (-3.4 to -1.2)	-2.1 (-4.1 to -1.3)[1d]	-2.5 (-3.8 to -1.4)[3d]
LBMheight (SDS)	-1.7 (-3.8 to -0.6)	-1.5 (-2.3 to -0.7)[a]	-1.9 (-2.4 to -1.4)[a]	-1.4 (-2.9 to 0.9)	-1.9 (-2.9 to 0.0)[2a]	-2.3 (-2.7 to -1.3)[1a]
Trunk fat (%)	36.0 (24.8 to 46.2)	28.0 (16.9 to 36.7)[4d]	33.3 (17.3 to 40.9)[3d]	36.0 (29.2 to 41.2)	37.2 (32.0 to 42.5)[d]	37.9 (35.0 to 45.7)[1d]

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


Height (sds)
3
2
1
0
-1
-2
-3
-4
-5
4
4
0
12
24
0
12
24
no GH
GH


BMI (sds)
4
3
2
1
0
-1
-2
4
3
0
12
24
0
12
24
no GH
GH

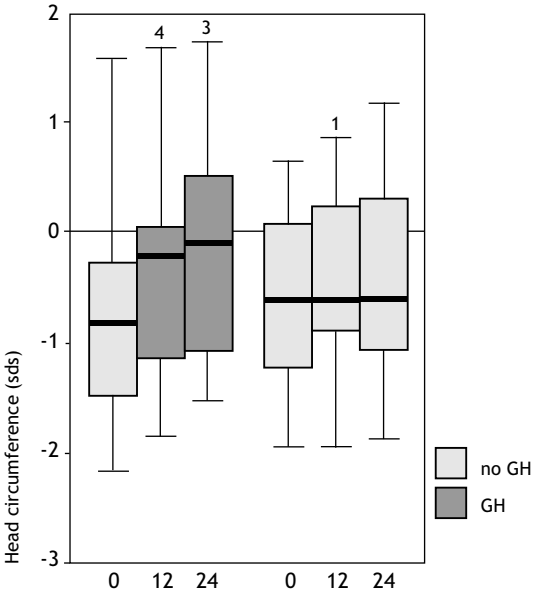


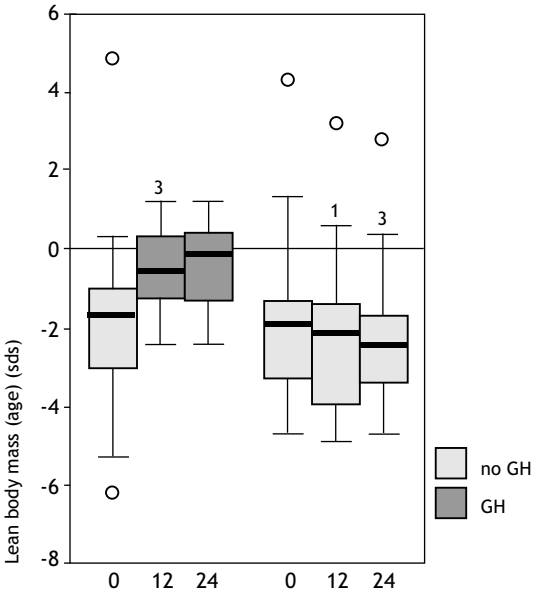
Figure 2 a-c: Anthropometric data in pre-pubertal PWS children, during the 2-year controlled study. Data are expressed in SDS for age and sex and represented by boxplots. Significantly different compared with baseline [1]p<0.05, [2]p<0.01, [3]p<0.005, [4]p<0.001. Significantly different change in GH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

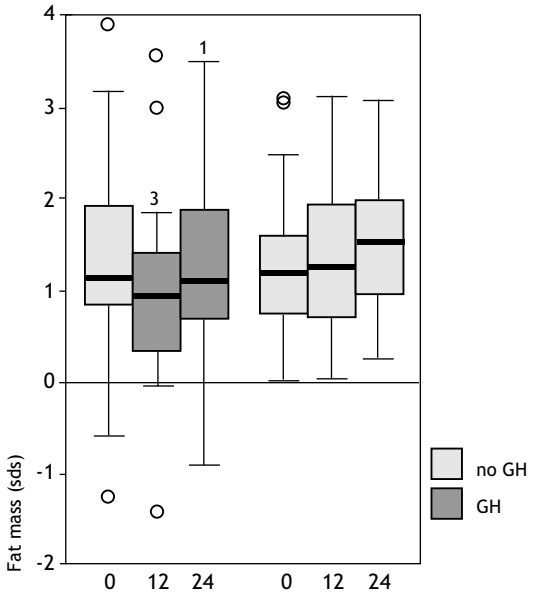
IGF-I and IGFBP-3

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

Safety aspects

GH was well tolerated. Compared with randomised controls, GH treatment did not induce disadvantageous effects on carbohydrate metabolism, sleep-related breathing disorders and thyroid hormone levels. The results of these safety aspects have been published separately [18-20].


Lean body mass (age) (sds)
6
4
2
0
-2
-4
-6
-8
3
1
3
0
12
24
0
12
24
no GH
GH


Fat mass (sds)
4
3
2
1
0
-1
-2
1
3
0
12
24
0
12
24
no GH
GH

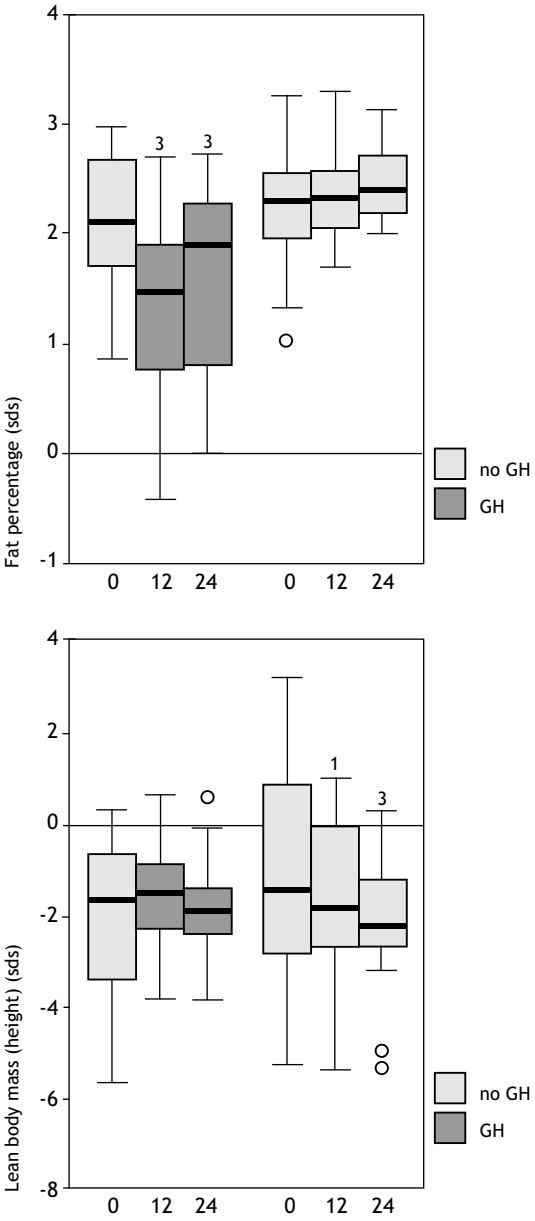


Figure 3 a-d: Body composition in pre-pubertal PWS children, during the 2 years controlled study. Data are expressed in SDS for age and sex or SDS for sex and height and represented by boxplots. Significantly different compared with baseline [1]p<0.05, [2]p<0.01, [3]p<0.005, [4]p<0.001. Significantly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

2.5 Discussion

Our study demonstrates that height, BMI, and head circumference significantly increased in GH-treated infants and children, resulting in a complete normalisation after 2 years of GH in pre-pubertal PWS children. Also length of foot and tibia, arm span, sitting height and body fat percentage improved in GH-treated children. LBM, corrected for sex and age, significantly improved. LBM corrected for height and sex (LBM_{height}) did not increase during GH treatment, but significantly declined in non-GH-treated controls, resulting in a significantly positive effect on body composition in the GH group compared with the control group. During GH treatment, IGF-I levels increased to levels above normal ranges in many patients. Also, IGFBP-3 increased, but to a lesser extent, resulting in an increased IGF-I/IGFBP3 ratio.

At baseline, we found a strong positive correlation between height SDS and BMI SDS. This is in favour of the hypothesis that growth in PWS represents a combination of 2 opposite mechanisms: an acceleration of growth by obesity and a growth retardation due to insufficient GH secretion [21].

Median height SDS and IGF-I SDS, prior to start of GH treatment, were relatively low compared with previously reported levels [7,10,22]. This may be explained by the fact that our PWS children were not severely overweight in contrast to previously reported studies. In non-syndromal obese children, stimulated GH values are low as well as the 24-hour GH secretion, but IGF-I levels are high or normal [23,24]. It might be that in more severely obese PWS children, the low IGF-I levels are counteracted by the obesity-related IGF-I increase.

Head circumference SDS was less depressed than median SD scores of height, foot length, arm span and sitting height. This was in contrast to our expectations, because usually, in syndromal short stature, head circumference and other anthropometric parameters are equally impaired. Our results are in line with previous studies in young PWS children [25,26].

Two years of GH treatment resulted in a normal median height SDS, whereas height SDS in non-GH-treated children remained low. This is in line with previous studies [10, 21,27], although so far, no 2-year controlled studies were available.

The most important effect of GH, in our opinion, is the improvement of LBM. The low LBM in PWS most likely reflects a reduced muscle mass, and may therefore contribute to clinical hypotonia, poor physical performance, and as a result, reduced energy expenditure [4]. LBM corrected for age and sex (LBM_{age}) increased during GH treatment from levels below normal to levels not significantly different from 0 SDS.

Although LBM corrected for height and sex (LBM_{height}), did not increase during GH treatment, LBM_{height} did significantly decrease in the control group, which means that GH is beneficial for improving LBM. The decrease in LBM in non-GH-treated PWS children is in line with previous studies [28]. In previous GH studies, LBM was expressed in kg, not taking into account height-dependency of LBM. Only one study expressed LBM in SDS (for both height and age) in GH-treated PWS subjects compared with baseline [11]. The authors suggested that the increase in LBM in 12 overweight PWS subjects was growth-related and that the initial deficit of LBM cannot be compensated for by GH. This study was, however, a non-controlled study. Our study is the first one showing that, although LBM_{height} did not increase in the GH group, the LBM_{height} did improve compared with the control group, indicating that GH is useful for improving LBM, taking into account the catch-up in height, in PWS children.

Trunk fat percentage decreased during GH treatment and increased in the control group, indicating that GH decreases trunk fat percentage. However, trunkal fat percentage includes both subcutaneous trunk fat and visceral fat. It remains to be established whether GH treatment is beneficial for decreasing visceral fat in PWS children.

It has previously been suggested that a normalisation of small hands and feet during GH treatment, would suggest that those features are the result of GH deficiency rather than directly caused by the genetic defect [21]. Our results, however, show that the size of the feet, arm span and sitting height do not normalise during GH treatment, indicating that GH deficiency cannot fully explain these features. We did not measure hand length, because in our experience, a reliable assessment of hand length is difficult in PWS subjects. Although we cannot exclude that further catch-up takes place after the first 2 years of GH, it seems that increased growth stabilizes after the first year.

During GH, median IGF-I levels rapidly increased to levels above the upper limit of normal, whereas IGFBP-3 increased more gradually to levels completely within the normal range. IGFBP-3 is the major carrier protein of IGF-I and binds 70-95% of IGF-I as a binary complex or as a ternary complex together with the acid labile subunit (ALS) [29]. Only a minor fraction of IGF-I circulates in its free form, which is considered the biologically active form [30]. An increase of the IGF-I/IGFBP-3 ratio therefore suggests a large increase in free IGF-I. IGF-I levels in the supraphysiological range are in line with those reported by others in pre-pubertal PWS children and infants/toddlers [7, 22,31]. In one of our patients, the GH dose had to be decreased in order to keep IGF-I levels within reasonable limits. It is not known why PWS patients seem more

responsive in terms of IGF-I than GH deficient children [32]. In our study, samples were collected and stored, as all assessments were performed afterwards. We therefore did not adjust GH dose in most children. Nevertheless, we do now recommend to monitor IGF-I levels regularly, to prevent extremely high levels of IGF-I. Based on our data, one might consider treating PWS subjects with a lower GH dose. However, it has been shown previously that in order to optimally benefit the metabolic effects of GH, PWS children should be treated with at least 1 mg/m^2/day [33]. Future studies should evaluate the optimal GH dose in PWS children.

In conclusion, we found a normalisation of median SD scores of height, BMI, and head circumference, in pre-pubertal PWS children. Foot length, arm span, tibia length, sitting height and body fat percentage improved significantly, but did not completely normalise. LBM_{age} SDS improved to levels within the normal range, but LBM_{height} SDS did not change in the GH group. However, we found that LBM_{height} SDS decreased in controls, and therefore GH may still be considered beneficial to optimize LBM in PWS. IGF-I levels increased rapidly to supraphysiological levels in most PWS children, indicating that PWS children are highly sensitive to GH and that monitoring of IGF-I is warranted to keep IGF-I levels within reasonable limits, arbitrarily below +3 SDS.

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Nagler, Medisch Centrum Leeuwarden, Leeuwarden; D. Haring, Diaconessen Hospital, Leiden; F. Versteegh, Groene Hart Hospital, Gouda; J.W. Pilon, Ijsselmeerziekenhuizen, Lelystad.

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Chapter 3

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Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome

3.1 Abstract

Background: Prader-Willi Syndrome (PWS) is a neurogenetic disorder characterised by muscular hypotonia, psychomotor delay, feeding difficulties and failure to thrive in infancy. Growth hormone (GH) treatment will improve growth velocity and body composition. Research on the effects of GH on psychomotor development in infants with PWS is limited.

Objective: To evaluate psychomotor development in PWS infants and toddlers during GH compared with randomised controls.

Design/Patients: 43 PWS infants were evaluated at baseline. Twenty-nine of them were randomised into a GH group (n=15) receiving 1 mg/m^2/day GH treatment or a non-GH-treated control group (n=14). At baseline and after 12 months of follow-up, Bayley Scales of Infant Development (BSID II) were performed. Data were converted to percentage of expected development for age (%ed), and changes during follow-up were calculated.

Results: Infants in the GH group had a median age of 2.3 years (interquartile range (iqr) 1.7 to 3.0) and in the control group of 1.5 years (iqr 1.2 to 2.7) (p=0.17). Both mental and motor development significantly improved during the first year of study in the GH group vs. the control group. Median (iqr) change in mental development was +9.3% (-5.3 to 13.3) vs. -2.9% (-8.1 to 4.9), $p<0.05$, and in motor development was +11.2% (-4.9 to 22.5) vs. -18.5% (-27.9 to 1.8), $p<0.05$, respectively.

Conclusion: One year of GH treatment significantly improved mental and motor development in PWS infants compared with randomised controls.

3.2 Introduction

PWS is characterised by muscular hypotonia and failure to thrive in infancy, hypogonadism, psychomotor delay, cognitive deficits, short stature, and obesity. Children with PWS have an abnormal body composition with a relatively high body fat percentage and a low lean body mass (LBM). Even in infants, who are still underweight, body fat percentage is high [1,2]. Accumulating evidence shows that hypothalamic dysfunction is responsible for many of the features in PWS, including hypogonadism, excessive appetite with decreased satiety, respiratory dysfunction, dysfunctional thermoregulation and growth hormone (GH) deficiency [3,4].

The cause of PWS is a paternal deletion or a uniparental maternal disomy of chromosome 15q11-13, and in 1-5%, an imprinting center mutation, which causes genes in the paternally inherited chromosome 15q11-13 to be silenced [5,6].

Reports have demonstrated that GH treatment in older PWS children not only resulted in a remarkable growth response, but also in a dramatic improvement of body composition, with a decline in fat percentage and an increment in LBM, which resulted in an increased muscle strength and agility [7-10]. Preliminary studies suggested that GH might improve psychosocial development in PWS [11,12]. Due to the increased awareness of paediatricians and neonatologists of the clinical presentation of PWS in infancy combined with the availability of the methylation test for rapid and reliable diagnosis of PWS [13,14], diagnosis is now usually confirmed in infancy. So far, there is only one controlled study on psychomotor development in PWS infants during GH treatment, demonstrating an improvement in body composition and motor development (mobility) [15] and an improvement of cognitive development [16].

The aim of our randomised, controlled GH-study was to evaluate in a group of PWS infants and toddlers whether start with GH treatment at a very early age could contribute to improvement of mental and motor development.

3.3 Patients and Methods

Patients

The study group consisted of 32 Dutch and 11 Swedish PWS infants and toddlers. They fulfilled the following inclusion criteria: (1) genetically confirmed diagnosis of PWS; (2) age between 6 months and 3 years at start of the protocol. Patients with severe degree of scoliosis (> 20°) or extremely low dietary intake were excluded from the study. The caloric intake of all infants and toddlers was evaluated at start of study and recommendations were given based on their height and weight. Compliance to the diet was evaluated 3-monthly by the research nurse.

Baseline group

We included 43 infants and toddlers aged up to 3 years at start of study (24 boys). Twenty-one patients had a paternal deletion, 13 had a uniparental maternal disomy, one had an unbalanced translocation x 15, and in 8 patients, the diagnosis of PWS was confirmed by a positive methylation test, but the underlying genetic defect was not yet specified. After stratification for age, children were randomised into a GH group receiving Genotropin® (somatropin) 1 mg/m^2/day (Pfizer), or into a non-GH-treated control group.

After 12 months of follow-up

For repeated measurement analysis only children were included with two Bayley Scales of Infant Development – II (BSID II) scores available. BSID II can only be used if "developmental age" is maximally 3.5 years. Overall, 29 infants and toddlers were eligible for repeated BSID II analysis. Of these, 15 were randomised into the GH group (7 boys) and 14 in the control group (8 boys). Fifteen patients had a paternal deletion, 9 had a uniparental maternal disomy, 1 had an unbalanced translocation of chromosome 15, and in 4 the diagnosis was confirmed, but the underlying defect not yet specified. Results of 14 patients were not analysed due to the following reasons: Five children did not reach one year of study yet, 1 was excluded from the study because he had thyroid hormone deficiency, and 8 had already passed the upper limit of BSID II after 1 year of follow-up (equally divided over the GH group and the control group).

The study protocol was approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam, The Netherlands and the Medical Ethics Committee of Karolinska Institute, Stockholm, Sweden. Written informed consent was obtained from the parents.

Methods

Anthropometric parameters

Height (supine height below the age of 2.5, thereafter standing height, measured with a Harpenden stadiometer), weight and head circumference were measured before start of study, and after 12 months. Median height, body mass index (BMI) and head circumference were expressed as standard deviation scores (SDS) for age and sex, according to Dutch references [17]. Growth Analyser Version 3.0 software was used to calculate BMI, and SD scores of height, BMI and head circumference (www.growthanalyser.org).

Psychomotor Development

Psychomotor development was assessed at baseline and after 12 months with BSID-II. BSID II results were compared to reference data derived from healthy infants and toddlers of comparable age [18,19]. BSID II yields 2 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 gross and fine motor skills. The mental scale was performed in all children, and the motor scale was only performed in the Dutch children (of 32 Dutch infants, 30 underwent motor evaluation). All psychomotor tests were conducted by a qualified psychologist (M.W., B.B.).

Body composition

Body composition was assessed at baseline and after 12 months in the Dutch participants, using dual energy X-ray absorptiometry (DXA, type Lunar Prodigy, GE Healthcare). Total fat mass (in gram), fat (%), and lean body mass (LBM in gram) were measured at start of the study and after 12 months. Since reference values for DXA in this age-group are not yet available, we expressed LBM and body fat as percentage of total body mass.

Insulin-like growth factor (IGF) I and IGF binding protein 3 (IGFBP-3)

In all children, IGF-I was measured. In Dutch children an immunometric technique on an Advantage Chemiluminescense System (Nicholls Institute Diagnostics, San Juan Capistrano, USA) was used. In Swedish infants a semi-illuminiscent technique on Immulite 1000 analyzer (Los Angeles, USA) was used. Intra- and inter-assay coefficient of variation (CV) for IGF-I are 4% and 6% respectively for the Advantage

chemiluminescence System, and 2.3-3.9% and 3.7-8.1% respectively for the Immulite 1000 analyser. In Dutch infants, serum IGFBP-3 levels were measured using a specific RIA [20] in one laboratory. The intra-assay CV was 4% and the inter-assay CV was 6%. Because of age- and sex-dependency, IGF-I and IGFBP-3 levels were transformed into SDS [20, 21].

Data analysis

Statistical Package for Social Sciences (SPSS, Version 11) was used to perform statistical analysis. Data were expressed as median (interquartile range, iqr). Test results of psychomotor development were expressed as developmental age divided by chronological age, multiplied by 100 reflecting percentage of expected development (%ed) for that age. Most of our data were not Gaussian distributed. We therefore used non-parametric statistics. To compare psychomotor development with normal (100 %ed), binomial test was used. Differences between groups at baseline (2-tailed) were calculated with Mann Whitney U tests. Changes in motor and mental development are expressed as percentage of change over 12 months (development at 12 months minus development at start, divided by development at start, multiplied by 100). The use of change scores, rather than absolute measures enabled to adjust for age and development at start of study. For analysis of the data, we used analysis of covariates (ANCOVA) (1-tailed) with age and mental/motor development at start of study as covariates to test the change in mental and motor development between the GH group and the control group. Spearman non-parametric correlations were used to evaluate associations between mental and motor development within patients, and associations between psychomotor development and anthropometric parameters. We investigated if infants with less motor or mental abilities at start, would benefit more from GH treatment. We divided all infants in 4 groups, based on dichotomised variables: (1). Initial mental and motor development, and (2). GH group vs. control group, in order to achieve an equal number of patients in each of the four groups.

3.4 Results

At baseline

At baseline, 43 infants (24 boys) were evaluated. Median (iqr) age was 2.0 years (1.3 to 3.1): 2.3 years (1.6 to 3.0) and 1.6 years (1.2 to 2.9) (p=0.17) in the GH group and in the control group, respectively. Clinical characteristics are listed in Table I. Height,

BMI, head circumference, IGF-I and IGFBP-3, all expressed as SDS, were significantly below 0 SDS. Results of mental and motor development are presented in Table 2 and Figure 1. At baseline, median mental developmental age was 1.5 years (1.2 to 2.1) and 1.2 years (1.0 to 2.0) ($p=0.08$) and median motor developmental age was 1.2 years (0.8 to 1.8) and 0.9 years (0.8 to 1.8) ($p=0.22$) for the GH group and the control group, respectively. Mental development in the total group was 71.6% (66.3 to 80.0) of the expected mental development and motor development was 56.8% (51.6 – 64.7) of the expected motor development. Mental development was significantly higher than motor development ($p<0.001$) and they were both significantly lower in PWS than in healthy children (100%) ($p<0.001$). Mental and motor development were significantly correlated ($r=0.45$, $p<0.05$). We found no significant correlation between psychomotor development and the anthropometric parameters and no significant association between age and development at start of study (mental development: $r=-0.02$, $p=0.91$, motor development: $r=0.16$, $p=0.41$).

After 12 months of follow-up

Anthropometric parameters (Table 3)

At start of study, the GH group and the control group were not significantly different with regard to height SDS ($p=0.75$), BMI SDS ($p=0.51$), and head circumference SDS ($p=0.29$). After 12 months of follow-up, height SDS had significantly increased from -2.6 SDS (-3.3 to -1.8) to -1.6 (-2.1 to -0.8) ($p<0.005$) in the GH group and remained low in the control group. Notably, head circumference SDS significantly increased from -1.0 (-1.7 to -0.3) to -0.2 (-1.2 to 0.6) ($p<0.005$) in the GH group, and remained low in the control group.

Table 1. Clinical characteristics at baseline (total group)

		Median	iqr
n		43	
Sex	(m/f)	24/19	
Age	(yr)	2.0	(1.3 to 3.0)
Height	(SDS)	-2.0[1]	(-2.9 to -1.0)
BMI	(kg/m^2)	16.3	(15.1 to 18.4)
BMI	(SDS)	-0.1	(-1.2 to 1.6)
Head circ.	(SDS)	-1.0[1]	(-1.7 to -0.3)
Body fat*	(%)	27.8	(23.7 to 33.5)
LBM*	(%)	70.9	(65.2 to 74.7)
IGF-I	(µg/L)	28.5	(21.0 to 47.3)
IGF-I	(SDS)	-1.9[1]	(-2.6 to -1.3)
IGFBP-3	(µg/L)	1.0	(0.7 to 1.2)
IGFBP-3	(SDS)	-2.1[1]	(-3.1 to -1.4)

IGF-I was available in 34, and IGFBP-3 in 23 patients.
[1] $p<0.001$, compared to 0 SDS.
*Only available in Dutch patient group (n=32).

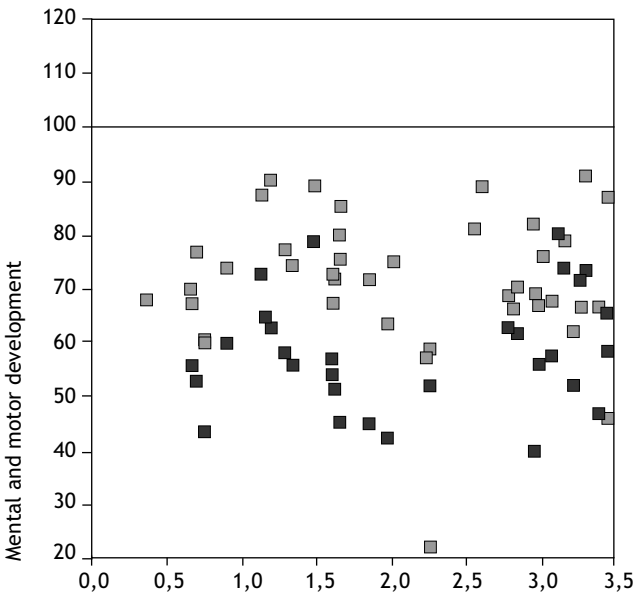

Figure 1. Mental and motor development at baseline (total group). Open squares represent mental development and solid squares represent motor development. Reference line represents normal motor and mental development (100%).

Table 2. Psychomotor development at baseline (total group)

	Median	iqr
n	43	
Chronological age (yrs)	2.0	(1.3 to 3.0)
Mental developmental age (yrs)	1.3	(1.1 to 2.1)
Mental development (%ed)	71.6	(66.3 to 80.0)
Motor developmental age* (yrs)	1.0	(0.8 to 1.8)
Motor development* (%ed)	56.8	(51.6 to 64.7)

*Only available in Dutch patient group (n=30).
Motor development (%ed) is significantly lower than mental development (%ed) ($p<0.001$). Motor and mental development are both significantly lower than in healthy children (100%ed) ($p<0.001$) and both significantly correlated ($r=0.45$, $p<0.05$).

Table 3. Baseline and 1 year data of infants participating in controlled GH study

	GH Group				Control Group			
	Baseline		12 months		Baseline		12 months	
n	15		15		14		14	
Sex (m/f)	7/8		7/8		8/6		8/6	
Age (yrs)	2.3	(1.7 to 3.0)	3.3	(2.7 to 4.0)	1.5	(1.2 to 2.7)	2.6	(2.3 to 3.8)
Height (SDS)	-2.6	(-3.3 to -1.8)	-1.6[2]	(-2.1 to -0.8)	-2.3	(-3.3 to -1.1)	-2.3	(-3.9 to -1.5)
BMI (kg/m^2)	16.3	(14.5 to 17.8)	16.4	(15.2 to 18.5)	15.9	(14.7 to 16.8)	15.5	(14.9 to 17.6)
BMI (SDS)	-0.3	(-1.1 to 1.3)	0.3	(-0.9 to 1.8)	-0.9	(-1.8 to -0.8)	-0.4[1]	(-0.8 to 1.3)
Headc (SDS)	-1.0	(-1.7 to -0.3)	-0.2[23]	(-1.2 to 0.6)	-1.1	(-1.8 to -0.9)	-1.1[3]	(-1.6 to -0.6)
Body fat (%)	26.2	(22.2 to 28.9)	22.5	(11.3 to 33.2)	25.8	(23.1 to 27.7)	22.8	(19.5 to 32.9)
LBM (%)	72.1	(69.8 to 75.7)	74.8	(63.7 to 82.3)	73.3	(70.9 to 75.2)	73.6	(61.6 to 75.9)
IGF-I (SDS)	-2.1	(-2.7 to -1.7)	1.7[24]	(0.1 to 2.5)	-2.0	(-2.6 to -0.3)	-2.6[4]	(-4.1 to -0.4)
IGFBP-3 (SDS)	-2.8	(-3.5 to -2.4)	0.4[13]	(-0.3 to 1.1)	-1.8	(-3.4 to -0.9)	-3.1[3]	(-4.0 to -2.2)

Data are expressed as median (interquartile range)
[1]p<0.05, [2]p<0.005: 12 vs. 0 months
[3]p<0.05, [4]p<0.001: GH group vs. Control group

Psychomotor development

We found a significant improvement during 1 year of follow-up in mental development for the GH group +9.3% (-5.3 to 13.3) compared with the control group, -2.9% (-8.1 to 4.9) (p =0.04). Also motor development improved significantly in the GH group, +11.2% (-4.9 to 22.5), compared with the control group, -18.5% (-27.9 to 1.8) (p=0.04). For both motor and mental development in the GH group and control group, there was a large individual variation in change during follow-up (Figure 2). When infants were subdivided depending on their initial developmental age, the improvement in motor development was only significant in infants with lower initial motor development (i.e. motor developmental age below 0.85 years) (Figure 3). We did not find such differences for mental development. There was a weak correlation between the change in mental development and change in head circumference, but this did not reach significance (r=0.31, p=0.11). The change in motor development did not significantly correlate with the change in LBM % (r=0.27, p=0.31).

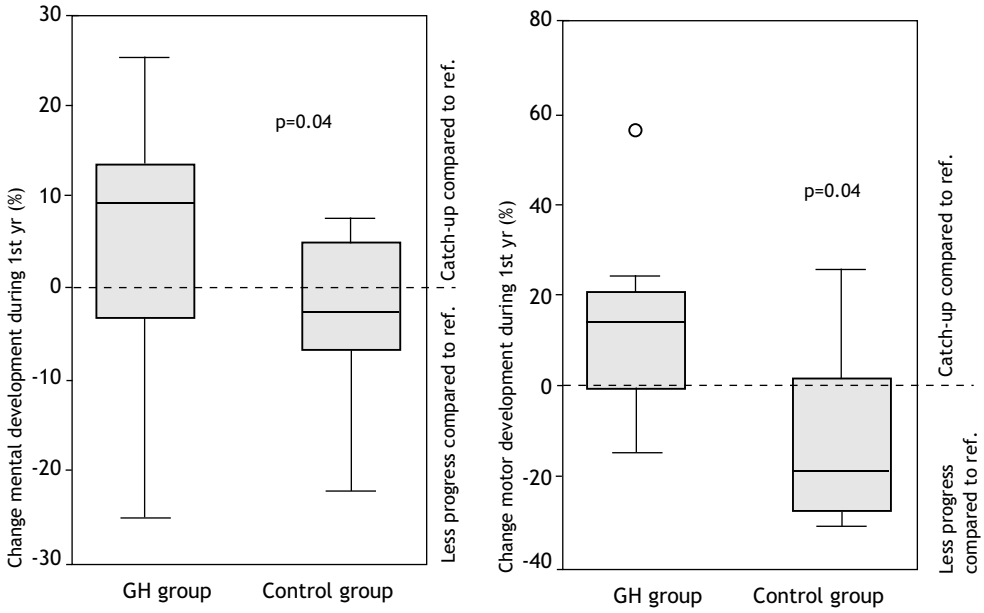

Figure 2. Changes in mental and motor development during 12 months of study. The lower boundary of the boxplot is the 25th percentile, and the higher boundary is the 75th percentile. The line in the box represents the median. Lines are drawn from the smallest to the largest observed value that is not an outlier. Horizontal line represents no change compared to normal children (change of 0%). A positive value represents a catch-up, compared to normal children, whereas a negative value represents less progress compared to normal children.

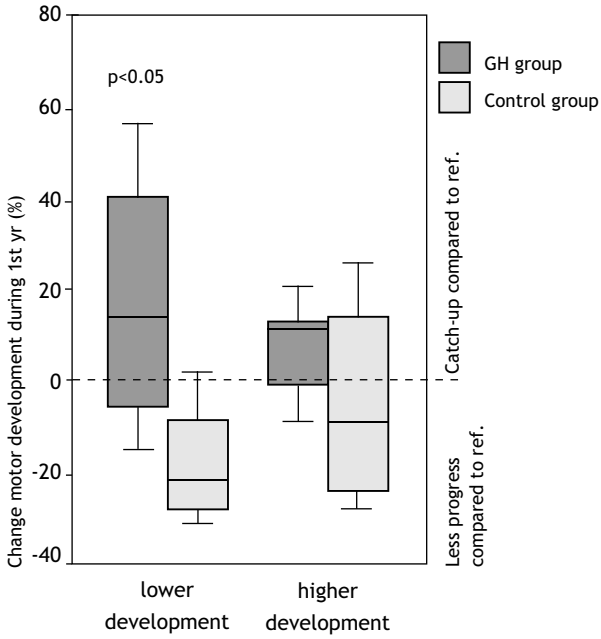

Figure 3. Motor development for children with lower and higher developmental age at start of study. Horizontal line represents no change compared to normal children (change of 0%). A positive value represents a catch-up, compared to normal children, whereas a negative value represents less progress compared to normal children.

Body composition

LBM and body fat (both expressed as percentage of total body mass) are listed in Table 3. Body composition was similar in both groups at start of study, and body composition did not significantly change during follow-up in both groups.

IGF-I and IGFBP-3 (Table 3)

The median (iqr) IGF-I SDS at start of study was -2.1 (-2.7 to -1.7) for the GH group and -2.0 (-2.6 to -0.3) for the control group. Median IGFBP-3 SDS was -2.8 (-3.5 to -2.4) and -1.8 (-3.4 to -0.9) respectively, which was not significantly different. After 12 months compared with baseline, median IGF-I in the GH group had significantly increased to 1.7 SDS (0.1 to 2.5) ($p<0.005$) with IGFBP-3 levels of 0.4 (-0.3 to 1.1) ($p<0.05$), whereas in the control group, IGF-I and IGFBP-3 levels remained low. This resulted in a significantly higher IGF-I, and IGFBP-3 levels in the GH group, compared with the randomised controls after 1 year of study ($p<0.001$ and $p<0.05$, respectively).

Safety aspects

GH was well tolerated. Compared with randomised controls, GH did not induce disadvantageous effects on carbohydrate metabolism, sleep-related breathing disorders, and thyroid hormone levels. The results of these safety aspects have been published separately [22-24].

3.5 Discussion

We assessed psychomotor development in relation to anthropometry in 43 infants with genetically confirmed diagnosis of Prader-Willi Syndrome. Both mental and motor development were impaired in the PWS infants, compared to the normal population. We did not find associations between psychomotor development, and anthropometric parameters. In the randomised GH study, we found a significant improvement of both mental and motor development in the GH group compared with the control group. Infants with lower developmental age had the greatest improvement in motor development, suggesting that GH treatment might be considered at an early developmental age to optimize the GH effect on motor development.

Mental development improved after 1 year in the GH group compared with the control group. This is in line with previous data, in 7 GH-treated PWS infants compared with 5 randomised controls, showing an improvement of cognitive development and language development, combined with an improvement of head circumference [16]. We could not find a significant association between mental development and head circumference, neither at baseline nor after 1 year of follow-up (data not shown), but there was a weak correlation between the change in mental development and head circumference after 1 year of follow-up ($r=0.31$, $p=0.11$), which did not reach statistical significance. Our sample size might have been too small to identify such an association. The increased head circumference might reflect involvement of the central nervous system in the cognitive effects of GH treatment.

Head circumference SDS was in our study in the low-normal range, which was relatively high compared to height SDS at start of the study. In syndromal short stature, height and head circumference are usually equally affected. Our data are however consistent with previous studies in which also relatively larger head circumferences were reported for PWS infants [16,25]. After one year of follow-up, we found a normalisation of head circumference, and a significant increase of height SDS in the GH group, but not in the control group. The increase in height was similar

to the response reported by others in infants and toddlers with PWS and idiopathic GH deficiency [26,27].

In the general population, there is evidence that intelligence tends to be higher in subjects whose head circumference is higher, and brain growth during infancy and early childhood seems to be more important than during foetal life in determining cognitive function [28]. Long-term data are needed in order to evaluate the effects of improved growth of head circumference in early infancy on cognition in PWS children and adults.

In our study, IGF-I levels rapidly increased during GH treatment from below the normal range to the high-normal range. These levels were similar to those found by others [15]. IGF-I receptors have been localized in several areas in the human brain, indicating that IGF-I may have a neuroregulatory role in the central nervous system [29]. Theoretically, IGF-I may directly influence the central nervous system or GH might induce local IGF-I expression in brain tissue, thereby improving psychomotor development.

Another possible explanation for the improvement of mental development during GH, might be that due to the improved motor development, children are able to sit, stand and walk independently, which enables them to explore and interact with the environment, resulting in a subsequent improvement of mental development.

We found a significant improvement of motor development in the GH group compared with the control group, particularly in children with initially lower developmental age. This might be explained by the effects of GH treatment on LBM. Differently from what observed in literature [15,26], body composition did not significantly change in GH-treated patients. Consequently, it is unlikely that significant improvement of motor development in the GH group might be attributed to the effects of GH treatment on LBM. In addition, we did not find associations between the change in LBM and change in motor development. This might be explained by the lack of sex- and age adjusted SD scores, because reliable reference data for DXA (by Lunar Prodigy) in infants and toddlers do not exist. Only one controlled study is available concerning motor development in PWS infants during GH treatment [15]. This study showed an improvement of mobility (defined as the ability to move the body from one position to another), but not stability in PWS infants.

A limitation of our study is that motor development in these developmentally delayed infants with PWS, was below the reference data for normal children. We, therefore, were not able to express data as SDS for age. We addressed this issue by using the % of expected development, i.e. the ratio between developmental

age and chronological age. In addition, we could not use changes in SD scores to evaluate changes in mental and motor development in the GH group vs. the control group. Although there were at start no statistically significant differences in age and mental or motor development between the GH group and the control group, baseline differences between groups might be of clinical relevance. For that reason we used relative changes and we also adjusted for age and development at start of study by using analysis of covariates.

In conclusion, we found after 1 year of GH treatment a significant improvement in both mental and motor development in PWS infants and toddlers compared with randomised controls. Head circumference increased from low-normal to normal during GH treatment. Children with an initially lower motor developmental age, did improve more than children with a higher motor developmental age at start, which suggests that early start with GH might be beneficial. Long-term studies are warranted to evaluate the effects of very early start with GH on cognition in childhood and adulthood.

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Chapter 4

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Sleep-related breathing disorders in pre-pubertal children with Prader-Willi Syndrome and effects of growth hormone treatment

4.1 Abstract

Background: Recently, several cases of sudden death in growth hormone (GH)-treated and non-GH-treated, mainly young Prader-Willi Syndrome (PWS) patients, were reported. GH treatment in PWS results in a remarkable growth response, and an improvement of body composition and muscle strength. Data concerning effects on respiratory parameters, are however limited.

Objective: To evaluate effects of GH on respiratory parameters in pre-pubertal PWS children.

Design/Patients: Polysomnography (PSG) was performed before GH in 53 children and repeated after 6 months of GH treatment 1 mg/m^2/day in 35 of them.

Patients: 53 pre-pubertal PWS children (30 boys), with median (interquartile range, iqr) age of 5.4 (2.1 to 7.2) years and body mass index (BMI) of +1.0 standard deviation score (-0.1 to 1.7).

Results: Median (interquartile range) Apnea Hypopnea Index (AHI) was 5.1/h (2.8 to 8.7) (normal 0–1/h). Of these, 2.8/h (1.5 to 5.4) were central apneas and the rest mainly hypopneas. Duration of apneas was 15.0 sec (13.0 to 28.0). AHI did not correlate with age and BMI, but central apneas decreased with age (r=-0.34, p=0.01). During 6 months of GH treatment, AHI did not significantly change, from 4.8 (2.6 to 7.9) at baseline to 4.0 (2.7 to 6.2; p=0.36). One patient died unexpectedly during mild upper respiratory tract infection (URTI), although he had a nearly normal PSG.

Conclusions: PWS children have a high AHI, mainly due to central apneas. Six months of GH does not aggravate the sleep-related breathing disorders in young PWS children. Our study also shows that monitoring during URTI in PWS children should be considered.

4.2 Introduction

Prader-Willi Syndrome (PWS) is characterised by muscular hypotonia, hypogonadism, psychomotor delay, obesity that may become extreme after the age of 2-4 years, and short stature. Sleeping disorders and respiratory disorders such as hypoventilation, decreased pulmonary function, obstructive and central sleep apnea and abnormal ventilatory and arousal response during hypercapnia may also occur [1-5]. Hypothalamic dysfunction is thought to be responsible for many features of PWS [6].

The underlying cause of PWS is a paternal deletion or a uniparental maternal disomy of 15q11-13. In 1-5%, PWS is the result of an imprinting-center mutation, which causes genes in the paternally inherited chromosome 15q11-13 to be silenced [7, 8].

Several reports have demonstrated that GH treatment results in a remarkable growth response, but also in an impressive improvement of body composition, with decline in fat-percentage and increment in lean body mass, muscle strength and agility [9-11]. Preliminary studies suggested that GH might improve psychosocial development in PWS [12]. Data on effects of GH on respiratory parameters in young, pre-pubertal PWS children are however very limited. Haqq et al found after 6 months of GH a slight reduction in sleep apnea incidence in 12 PWS children, aged 4.5 to 14.5 years [13]. Lindgren et al found improved CO_2-responsiveness in 9 children with PWS after 6-9 months of GH compared with baseline [14]. Recently, several reports have been published on sudden death in children with PWS during GH treatment [15,16]. Unexpected death, however, has also been described in non-GH-treated children with PWS [17,18]. In fact, Whittington et al reported an overall death rate of 3% per year for PWS patients in one UK Health Region [19].

In our study we evaluated the occurrence of sleep-related breathing disorders (SRBD) in 53 young, pre-pubertal children with PWS and the effects of 6 months of GH treatment in 35 of them.

4.3 Patients and Methods

Patients

In April 2002, a multicenter, randomised, controlled, prospective GH trial in PWS children was started investigating the effects of GH treatment versus no GH on growth, body composition, activity level and psychosocial development. Participants fulfilled the following inclusion criteria: (1) genetically confirmed diagnosis of PWS by positive

methylation test; (2) age between 6 months and 16 years; (3) bone age less than 14 years (girls) or 16 years (boys); (4) in children over 3 years: height standard deviation score (SDS) for age below zero (5) in children over 3 years: if height is > 0 SDS, weight-for-height SDS must be over +2 SDS, according to Dutch standards [20,21]. Patients with non-cooperative behaviour or patients receiving medication to reduce fat were excluded. All patients over 3 years started a diet and exercise program 3 months prior to start of the study. Children were enrolled in the study irrespective of their GH status. Patients received Genotropin® (somatropin) in a dose of 1 mg/m^2/day (Pfizer). The first 4 weeks of treatment, they received only 0.5 mg/m^2/day in order to prevent fluid retention.

In April 2003, we started a polysomnography (PSG) study in addition to the original protocol. For the PSG study we used the following inclusion criteria: (1) pre-pubertal at baseline and at repeated PSG (2) no upper respiratory tract infection (URTI) during PSG (3) no previous GH treatment. On November 11, 2005, 83 patients had been included in the original study. Twenty-five were excluded from the PSG study, because they received GH treatment, before start of the PSG study. For one patient, parents refused PSG, 3 were pubertal at repeated PSG and one was excluded because of treatment with nasal continuous positive airway pressure. As a result, 53 patients were eligible for analysis of baseline PSG. Thirty-nine children had a PSG repeated after 6 months of GH treatment. Fourteen patients were followed in the control group of the original study. Their PSG will be repeated at 6 months after start of GH treatment. As all patients were stratified for age and BMI before randomisation in the original study, these patients were not different from those who had repeated PSG. Of the 39 patients with repeated PSG, 4 had URTI during second PSG and were therefore excluded from group analysis.

The study protocol was approved of the Medical Ethical Committee of Erasmus Medical Center, Rotterdam, Netherlands. Informed consent was obtained from the parents.

Methods

Anthropometry

Supine length was recorded below the age of 2.5 years, and thereafter standing height, measured with a Harpenden stadiometer. Weight was assessed on an accurate scale, and body mass index (BMI) (kg/m^2) was calculated. Height and BMI were converted into SDS according to Dutch references for age [20,21]. Calculations were performed with Growth Analyser Version 3.0 (www.growthanalyser.org).

Polysomnography

PSG was performed before and after 6.6 (6.1-7.3) months of GH treatment. All PSGs were performed in one specialized sleep center (A.W., sleep specialist). Children were admitted to the sleep center at 5.00 p.m., accompanied by one parent. They underwent complete overnight PSG. 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 strain gauges and oxygen saturation (SaO_2) by pulse oximetry. All PSG studies were evaluated independently by two persons, both certified in PSG analysis. In case of major discrepancies between both assessments a third expert opinion was asked. The polygraphic records were scored according to standard criteria of Rechtschaffen and Kales [22]. A period of apnea or hypopnea was defined as more than 90% (apnea) or 50% (hypopnea) reduction of airflow for 3 breaths or longer. For hypopneas, the additional criterion was a reduction of SaO_2 of 4% or more. Periods of apnea and hypopnea were counted over the period of sleep during the night and calculated as mean per hour of sleep (apnea hypopnea index, AHI). An AHI above 1/hour is considered pathological [23]. Apneas were considered obstructive when absence of airflow occurred without a decrease in respiratory effort and central, when thoracic movements were absent. Abnormal SaO_2 was defined as SaO_2 below 92% or more than 4% below baseline values during 3 breaths or longer. Otorhinolaryngologic examination consisted of 3-monthly tonsillar inspection according to Brodsky staging system [24]. Snoring was recorded in a structured interview with parents. When snoring or obstructive sleep apnea syndrome (OSAS) was diagnosed, fiberoptic endoscopy was performed by an ear-nose-throat (ENT) surgeon. If adenoid or tonsillar hypertrophy was found, adenotonsillectomy was performed.

Data analysis

Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS Version 11). Most of the data obtained in our patients were not Gaussian distributed. We therefore expressed our data as median and interquartile range (iqr). Nonparametric tests (Wilcoxon signed ranks test) were used to compare results before and after start of GH. Correlations were calculated using Spearman correlation coefficients. Chi Square test was used to evaluate whether OSAS is more common in children with BMI over +2 SDS compared to normal weight children. Associations between snoring, tonsillar size and AHI were calculated with Kruskal-Wallis tests. P-value <0.05 was considered statistically significant.

4.4 Results

Clinical characteristics at baseline

Fifty-three pre-pubertal PWS children (30 boys) participated in the PSG study. The median (iqr) age was 5.4 years (2.1 to 7.2) and the median (iqr) BMI was 1.0 SDS (-0.1 to 1.7). Sixteen patients had paternal deletion, 21 had maternal disomy, 4 had an imprinting center mutation. In 12 patients diagnosis was confirmed by a positive methylation test for PWS, but was not yet further specified.

Thirty-nine patients (23 boys) started GH at a dose of 1 mg/m^2/day. The first month of GH, they received only 0.5 mg/m^2/day, to avoid fluid retention.

Respiratory parameters at baseline

At baseline, the median (iqr) AHI was 5.1/h (2.8 to 8.7). Of these, 2.8/h (1.5 to 5.4) were identified as central apneas, 0.0/h (0.0 to 0.3) as obstructive apneas and 0.9/h (0.0 to 2.7) as hypopneas. The longest median (iqr) duration was 15.0 sec (13.0 to 28.0). In all children, the AHI exceeded the normal range of 0-1/hr, indicating that SRBD do frequently occur, even in normal-weight pre-pubertal children with PWS. In the total patient group, no correlation was found between BMI SDS and AHI. Forty-five of our 53 patients were not obese. Of them, only 9% had OSAS (4/45), defined as obstructive apnea index over 1/h. In contrast, in our 8 patients who were obese (i.e. BMI over +2 SDS) 50% had OSAS (4/8) (prevalence of OSAS in normal weight versus obese patients, p=0.01). We found a negative correlation between both age and BMI and the number of central apneas (r=-0.34, p=0.01 and r=-0.33, p=0.017 respectively). There was no significant difference in AHI with regard to sex or genetic defect. Tonsil size as assessed by Brodsky staging system, was not associated with the AHI (data not shown).

Respiratory parameters after 6 months of GH

Thirty-five pre-pubertal children had PSG repeated after 6 months of GH treatment. (Table 2) This group of 35 children had a median (iqr) age of 6.0 years (2.4 to 8.6), and median (iqr) BMI of 0.8 SDS (-0.1 to 1.5) before GH. At baseline, median (iqr) AHI in this group was 4.8/h (2.6 to 7.9), of which 2.9/h (1.5 to 5.2) were indicated as central and 0.0/h (0.0 to 0.3) as obstructive. After 6 months of GH (1 mg/m^2/day), a non-significant decline in the AHI was found to 4.0/h (2.7 to 6.2). This decline was mainly due to a reduction in central apneas to 2.2/h (0.8 to 4.1). In 5, adenoidectomy and/or tonsillectomy was performed because adenoidal and/or tonsil hypertrophy developed

during the follow-up period. There was no association between changes in AHI and changes in number of awakenings or REM sleep-percentage (data not shown).

Table 1: Clinical parameters of baseline group

	Median	iqr
N	53	
Sex (m/f)	30 / 23	
Age (yrs)	5.4	2.1 to 7.2
BMI (kg/m^2)	17.7	15.9 to 19.4
BMI (SDS)	1.0	-0.1 to 1.7
Apnea hypopnea index	5.1	2.8 to 8.7
Central apnea index	2.8	1.5 to 5.4
Obstructive apnea index	0.0	0.0 to 0.4
Hypopnea index	0.9	0.0 to 2.7
Duration longest apnea (sec)	15.0	13.0 to 28.0
Genetic defect		
Paternal deletion (n)	16	
Maternal disomy (n)	21	
Imprinting center mutation (n)	4	
Unknown (n)	12	

Breathing disorders during illness

Four patients were excluded from analysis because of URTI. The results of their PSGs during health and illness are listed in table 3. In one of them, PSG was repeated after recovery and adenoidectomy. In this particular patient, the AHI before GH treatment was 7.9/h (100% central), during illness after 6 months of GH treatment, the AHI had impressively increased to 38.6/h (1.2 central apneas/h, 12.4 obstructive apneas/h, and 25.1 hypopneas/h), whereas after recovery and adenoidectomy, AHI was 3.4/h (100% central).

One patient in our study died unexpectedly. This 3-year old boy had GH treatment for 13 months. He responded very well in terms of growth and body composition. In this particular patient, PSG was performed before (AHI 1.7/h, 100% central) and after 6 months of GH (AHI 1.4/h, 67% central, 33% hypopnea). Six weeks before his death, BMI was 1.6 SDS and tonsils were assessed as Brodsky I-II. He had mild URTI and was

clinically evaluated by his paediatrician the day prior to his death. At that time he had URTI, but was in good condition, running around and not generally ill. During the night, he suddenly deteriorated and was found dead in the morning. Autopsy did not reveal the cause of death.

Table 2: Clinical and respiratory parameters of GH-treated group

	Before start of GH		After 6 mo of GH		p-value
	Median	iqr	Median	iqr	
N			35		
Sex (m/f)			20/15		
Age (yrs)	6.0	2.3 to 8.6	6.8	3.1 to 9.9	
BMI (kg/m^2)	17.1	15.9 to 19.2	17.6	15.7 to 18.9	0.38
BMI (SDS)	0.8	-0.1 to 1.5	0.8	-0.1 to 1.2	0.19
Apnea hypopnea index	4.8	2.6 to 7.9	4.0	2.7 to 6.2	0.36
Central apnea index	2.9	1.5 to 5.2	2.2	0.8 to 4.1	0.15
Obstructive apnea index	0.0	0.0 to 0.3	0.0	0.0 to 0.2	0.73
Hypopnea index	0.7	0.0 to 1.9	1.0	0.7 to 2.0	0.26
Longest apnea (sec)	15.0	13.0 to 28.0	17.0	14.0 to 23.3	0.92

Table 3: Respiratory parameters during health and URTI (n=4)

	Health		URTI		p-value
	Median	iqr	Median	iqr	
Apnea hypopnea index	5.7	3.1 to 9.5	36.5	18.1 to 39.5	0.07
Central apnea index	2.7	1.8 to 6.6	6.4	1.9 to 6.6	0.27
Obstructive apnea index	0.0	0.0 to 2.5	8.9	2.4 to 21.0	0.07
Hypopnea index	1.1	0.0 to 3.6	7.2	3.6 to 20.3	0.14
Longest apnea (sec)	24.5	18.5 to 33.5	35.5	27.3 to 49.8	0.27

Respiratory parameters as measured by PSG in health and during an episode of upper respiratory tract infection (URTI).

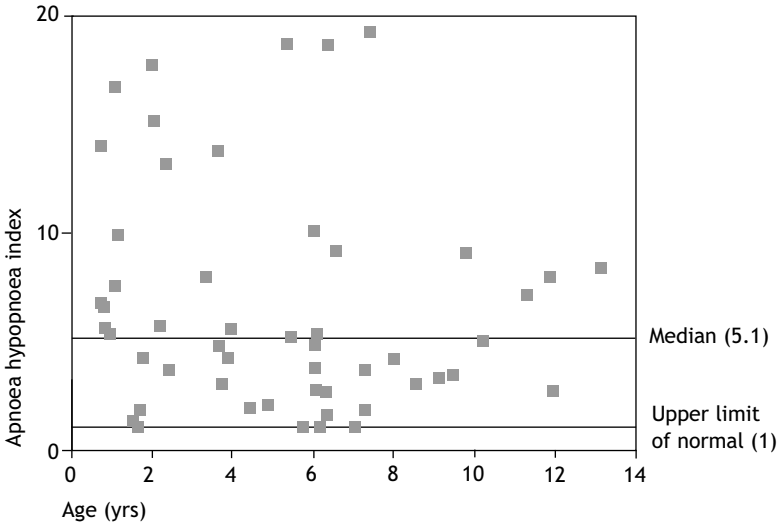

Figure 1: Apnea Hypopnea Index of 53 PWS children before start of GH. Line represents the upper limit of normal i.e. Apnea Hypopnea Index = 1 / h

4.5 Discussion

We found an increased AHI in 53 young, pre-pubertal children with genetically confirmed diagnosis of PWS. The high AHI was mainly due to central apneas and hypopneas. In the total group of mainly non-obese PWS children, obstructive apneas were rare. In contrast, obstructive apneas were found in 4 of the 8 overweight patients. After 6 months of GH treatment a non-significant decrease of AHI was found, mainly due to a decrease in central apneas. No significant change in obstructive apneas was found during GH. Illness or adenoid/tonsil hypertrophy, however did result into a marked increase in sleep-related breathing disorders, and particularly obstructive sleep apnea. Our study also shows that a relatively normal PSG does not exclude the possibility of unexpected death during mild URTI.

The increased number of central apneas, in our young PWS children suggests a central origin of SRBD. A hypothalamic origin of SRBD in PWS was already postulated 20 years ago [25]. A decreased number of oxytocin neurons in the hypothalamic paraventricular nucleus was reported, which might also be involved in reduced neural modulation of breathing [26,27]. Recently, Ren et al [28] proposed that necdin (neurally differentiated embryonal carcinoma-cell derived factor) deficiency may contribute to

the observed respiratory abnormalities in individuals with PWS as the Necdin gene is one of the protein-coding genes that are deficient in PWS [29]. Deficiency of Necdin in mice results in neonatal hypoventilation, which is usually fatal [30].

We found a negative association of both age and BMI, with number of central apneas. Because in PWS children, age and BMI are highly correlated, we cannot distinguish whether this is an effect of age or BMI. From a pathophysiological point of view, we consider it more likely to be an effect of age. In fact, our data are in line with a previous report, indicating that central apneas are more common in younger, healthy children, although within the normal range [31]. The mechanism is unclear, and might be related to a relatively more immature respiratory control in younger children. However, we cannot exclude that underweight in young PWS infants might contribute to as well.

OSAS was uncommon in normal-weight PWS patients. However, 4 of the 8 (50%) overweight (defined as BMI over +2 SDS) patients had signs of OSAS. Increased BMI has been associated with decreased SaO_2 and higher AHI in older PWS children and adults [32]. Harris et al reported an improvement of OSAS and hypoventilation after weight-loss in children and adults with PWS [33]. Tonsillar hypertrophy may also play a role in OSAS. Children with PWS might have a smaller naso- and oropharynx, which could contribute to obstruction [3]. Recently an improvement in AHI and oxygen saturation was reported after adenotonsillectomy in 5 PWS children with OSAS [34].

After 6 months of GH treatment, a non-significant decline in AHI was found compared with baseline, mainly due to a lower number of central apneas. Thus, our study indicates that GH had no adverse effects on the respiration of PWS children. Several publications reported sudden death in infants and children with PWS during GH treatment [15,16,35]. Several ones suggested a causal relationship between GH and sudden death in PWS.

Until now only limited data were available on the effects of GH on PSG. Miller et al recently reported an improvement of AHI after 6 weeks of GH in most of her PWS patients. She performed PSG in children and adults of which 12 were children under the age of 12 years. A subset of patients, however, had an increased AHI after 6 weeks of GH. Most of these patients had URTI during the second evaluation [36]. Haqq et al reported in a cross-over study a decrease in AHI after 6 months of GH in 12 PWS children, aged 4.5 to 14.5 years, although not statistically significant [13]. Myers et al demonstrated that inspiratory and expiratory muscle strength improved in 20 children with PWS, aged 4 to 16 years after 12 months of GH compared with 10 controls [11]. Lindgren et al found improved CO_2-responsiveness in 9 children with PWS after 6-9

months of GH compared to baseline [14]. A number of hormones, including GH and IGF-I, are involved in the physiologic regulation of breathing [37]. IGF-I receptors are located around the central chemoreceptors in the brainstem, and also in the cerebellum where the inputs from chemoreceptors are integrated [38]. GH may therefore theoretically improve breathing via a direct mechanism.

In our study we found only a small number of obstructive apneas both before and during GH treatment. There was no increase in obstructive apneas during GH treatment. Five children had adenotonsillectomy before the second PSG was performed, because of adenoid and/or tonsillar hypertrophy. Unfortunately this might confound our results, but for obvious safety reasons we could not avoid this. The AHI of these patients during both PSGs was not different compared with the rest of the study group. We found no significant association between tonsil size or snoring and the AHI. Sleep apnea, both obstructive and central, occurs more frequently in adults with GH excess (acromegaly) [39] and is associated with thickening of the pharyngeal wall in the acromegalic patients [40]. We cannot rule out that GH might have resulted in some adenoidhypertrophy, as we only performed fiberoptic endoscopy when indicated by snoring or OSAS during PSG. It has been suggested that GH treatment might increase tonsil size, however, to our knowledge, no controlled, prospective study has been performed.

One of our patients died unexpectedly during an episode of URTI. One of the most alarming findings is that this patient had near-normal sleep-related breathing during PSG, both before and during GH treatment. This points out that a near-normal PSG in a healthy PWS child does not guarantee he/she won't die during mild URTI. It might be related to a rise of apneas (both central and obstructive) during illness as shown in 4 of our patients who had a PSG during an episode of mild URTI. Unexpected deaths have been described in PWS children both without and during GH and have been attributed to several possible causes, such as respiratory dysfunction, cardiomyopathy, temperature instability and adrenal insufficiency or combinations of these.

We recommend monitoring of SRBD by PSG and regular ENT-evaluation in all PWS children, both before and during GH treatment. If adenoidhypertrophy or tonsillar hypertrophy occurs, adenotonsillectomy should be considered. It is important to mention that a relatively normal PSG does not exclude the possibility of unexpected death during mild URTI. Based on our results cardiorespiratory monitoring during URTI in children with PWS before and during GH treatment should be considered. Future studies are required for evaluating SRBD in PWS during URTI in order to give recommendations with regard to monitoring during URTI.

In conclusion, our study shows that many pre-pubertal children with PWS have sleep-related breathing disorders, mainly due to central apneas. BMI or age cannot explain the variability in the severity of the SRBD, although OSAS was more prevalent in children with obesity than in normal weight children. After 6 months of GH, a non-significant decrease in AHI was found. Thus our data are reassuring with respect to the effects of GH on SRBD. Our study also shows that a normal PSG does not exclude the possibility of unexpected death during mild upper respiratory tract infections. During URTI, AHI may rise and obstructive apneas may occur.

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Chapter 5

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Psychomotor development in infants with Prader-Willi Syndrome and associations with sleep-related breathing disorders

5.1 Abstract

Background: Prader-Willi Syndrome (PWS) is a neurogenetic disorder with hypotonia, psychomotor delay, obesity, short stature, and sleep-related breathing disorders.

Objective: To evaluate the association between psychomotor development and sleep-related breathing disorders in PWS infants.

Design and Patients: Bayley Scales of Infant Development were performed in 22 PWS infants, with a median (interquartile range, iqr) age of 1.8 (1.1 to 3.4) years, and a body mass index (BMI) standard deviation score (SDS) of -0.5 (-1.3 to 1.6). We evaluated psychomotor development in relation to results of polysomnography.

Results: Median (iqr) mental and motor development was 73.1% (64.3 to 79.6) and 55.2% (46.5 to 63.1) of normal children, respectively. All infants had sleep-related breathing disorders, mostly of central origin. The apnea hypopnea index was not associated with psychomotor development. Only 4 infants had obstructive sleep apnea syndrome (OSAS). They had a significantly delayed mental development of 65.5% (60.0 to 70.3) of normal. They had a median BMI SDS of 1.4 (0.1 to 1.6), which tended to be higher than in those without OSAS.

Conclusions: Our data indicate that psychomotor development in PWS infants is not related to central sleep-related breathing disorders, but infants with OSAS have more severely delayed mental development, suggesting that PWS infants should be screened for OSAS.

5.2 Introduction

Prader-Willi Syndrome (PWS) is characterised by a number of signs and symptoms, including muscular hypotonia, hypogonadism, psychomotor delay, obesity that may become extreme after the age of 2-4 years, and short stature. Children with PWS have an abnormal body composition with a relatively high body fat percentage and a low lean body mass. Even in infants, who are underweight, body fat percentage is high [1,2]. Sleep-related breathing disorders (SRBD) may also occur [3]. The underlying cause of the syndrome is a paternal deletion or a uniparental maternal disomy of chromosome 15q11-13 [4]. In 1-5%, PWS is the result of an imprinting-center mutation [5], which causes genes in the 15q11-13 region of the paternally inherited chromosome to be silenced. Nowadays PWS is mostly diagnosed in infancy [6,7]. This results in optimal management opportunities with regard to diet, exercise and education. As a result, most young PWS patients are currently not severely obese.

In healthy children, associations between sleep-disordered breathing and cognition, school performance and psychiatric and behavioural co-morbidities are well-known [8,9]. In healthy infants, inverse associations between the snoring-related arousal index and standardised mental development assessment scores have been reported [10]. In this study we examined if there was an association between psychomotor development, and sleep-related breathing disorders in infants with PWS.

5.3 Patients and Methods

Patients

All infants, included in the current analysis, participated in a randomised, controlled, growth hormone (GH) trial in children with genetically confirmed diagnosis of PWS. This trial investigated the effects of GH treatment versus no GH on growth, body composition, activity level and psychosocial development. In April 2003, a polysomnography (PSG) study was started in addition to the original protocol, in order to evaluate the effects of GH on sleep-related breathing disorders. The inclusion criteria for infants in the PSG study were: 1) no upper respiratory tract infection during PSG, and 2) no previous GH treatment. We used standardised tests to assess psychomotor development. Data were related with results of PSG. Tests were performed prior to start of GH treatment. Twenty-two infants with PWS were eligible for the present analysis.

The study protocol was approved by the Medical Ethical Committees of Erasmus Medical Center, Rotterdam, The Netherlands, and the other participating centers.

Written informed consent was obtained from the parents.

Methods

Anthropometry

Below the age of 2.5 years, supine length was measured, and thereafter standing height. Height was measured with a Harpenden stadiometer. Weight was assessed on an accurate scale, and body mass index (BMI) (kg/m^2) was calculated. Height and BMI were converted into standard deviation scores (SDS) [11,12] using Growth Analyser Version 3.0 (www.growthanalyser.org).

Psychomotor Development

Psychomotor development was assessed at baseline and after 12 months by the Bayley Scales of Infant Development II, Dutch version (BSID II) [13]. BSID II yields 2 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 psychomotor tests were conducted by a psychologist. Reference data of healthy Dutch children were used to compare the results of our patients [13].

Polysomnography

Patients were admitted to the sleep center at 5.00 p.m., accompanied by one parent. They underwent a complete overnight PSG. 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 strain gauges and oxygen saturation (SaO_2) by pulse oximetry. All PSG studies were evaluated independently by two persons, both certified in PSG analysis. In case of major discrepancies between the two assessments, a third expert opinion was asked. The polygraphic records were scored according to standard criteria of Rechtschaffen and Kales [14]. A period of apnea or hypopnea was defined as more than 90% (apnea) or 50% (hypopnea) reduction of airflow for at least 3 breaths. For hypopneas, the additional criterion was a reduction of oxygen saturation of at least 4%. Periods of apnea and hypopnea were counted over the period of sleep during the night and calculated as mean per hour of sleep (apnea hypopnea index, AHI). An AHI of more than 1 per hour is considered pathological (15). Apneas were considered "obstructive" when absence of airflow occurred without a decrease in respiratory effort and "central", when thoracic movements were absent. Patients with an obstructive sleep apnea index of more than 1/hour were considered to have obstructive sleep apnea syndrome (OSAS). Abnormal SaO_2 was defined as a SaO_2 below 92% or a decrease in SaO_2 of more than 4% compared with baseline values.

Data analysis

Data were expressed as median (interquartile range, iqr). We could not use standardised scores for psychomotor development, because PWS infants scored generally below the lowest range, in particular for the subscale of motor development. We therefore expressed these results as developmental age divided by chronological age, multiplied by 100, which reflects the percentage of expected development for that age. Differences between infants with and without OSAS were tested using independent sample t-tests. Multiple regression analysis was used to calculate the associations between anthropometric parameters and psychomotor development. One-way ANOVA was used to test differences in respiratory parameters and psychomotor development between children with paternal deletion and those with uniparental maternal disomy. In order to calculate the magnitude of the difference in development between infants with or without OSAS, a Cohen's d was calculated, using the following formula [16]:

$$\frac{(\bar{\chi}\,\text{OSAS=0} - \bar{\chi}\,\text{OSAS=1})}{\sqrt{\frac{sd^2 * (n-1)_{OSAS=0} + sd^2 * (n-1)_{OSAS=1}}{(n-1)_{OSAS=0} + (n-1)_{OSAS=1}}}}$$

Cohen's d of 0.20 was considered a minor, 0.50 a medium, and 0.80 a major difference. Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS Version 11).

5.4 Results

General Clinical Characteristics

Complete BSID II tests and complete polysomnographies (PSGs) were performed in 22 infants (15 boys) with PWS, who had a median (iqr) age of 1.8 (1.1 to 3.4) years (Table 1). Most infants were not obese, the median BMI of the total group being -0.5 SDS (-1.3 to +1.6). Two of 22 infants (9%) had a BMI above +2 SDS. All infants had a genetically confirmed diagnosis of PWS. Nine patients had a paternal deletion, 10 had a uniparental maternal disomy. In 3 patients PWS was confirmed by methylation test, but the underlying genetic defect was not yet specified.

Psychomotor development and sleep-related breathing disorders (SRBD)

Median mental development in the total group was 73.1% of expected mental development for age and motor development was 55.2% of expected motor development. Both were significantly below normal (Table 1). All infants had sleep-related breathing disorders (SRBD). Median (iqr) AHI was 6.1 (4.2 to 13.3) /h. Most apneas were identified as central apneas. Multiple regression analysis showed no association between mental or motor development and the severity of sleep-related breathing disorders. We did not find any difference between children with paternal deletion and maternal disomy with respect to psychomotor development or respiratory parameters.

Table 1: Clinical characteristics, psychomotor development and respiratory parameters in infants with PWS

	Median	iqr
n	22	
Sex (m/f)	15/7	
Age (yrs)	1.8	1.1 to 3.4
BMI (kg/m^2)	16.1	14.7 to 18.1
BMI (SDS)	-0.5	-1.3 to 1.6
Mental developmental ratio (%)	73.1	64.3 to 79.6
Motor developmental ratio (%)	55.2	46.5 to 63.1
Apnea hypopnea index	6.1	4.2 to 13.3
Central Apnea Index	5.2	2.6 to 7.9
Obstructive apnea index	0.0	0.0 to 0.8
Hypopnea index	0.4	0.0 to 2.7
Duration longest apnea (sec)	14.0	12.8 to 20.3

Age mentioned in Table corresponds with age at time of PSG. Median age during evaluation for psychomotor development was 1.6 (1.2 to 3.3) years.
Respiratory parameters are listed as indices (apneas/hour).

Table 2: Mental and motor development in infants with and without OSAS

	OSAS		No OSAS	
	Median	iqr	Median	iqr
N	4		18	
Age (yrs)	2.6	1.3 to 3.5	1.6	1.2 to 2.7
Sex	4 boys		11 boys/ 7 girls	
BMI (kg/m^2)	17.0	16.5 to 19.7	15.9	14.6 to 18.1
BMI (SDS)	1.4	0.1 to 1.6	0.6	-1.0 to 1.2
Mental development (%)	65.5*	60.0 to 70.3	74.9	64.8 to 81.3
Motor development (%)	50.1	45.2 to 61.8	56.2	47.9 to 63.3

Mental and motor development in % is calculated by dividing developmental age, assessed by BSID II by chronological age and multiplying by 100, which represents the percentage of expected development for age. Mental development is significantly decreased in infants with OSAS compared to infants without OSAS. OSAS is defined as obstructive sleep apnea index over 1/h. * $p<0.05$

Infants with obstructive sleep apnea syndrome (OSAS)

Four infants had obstructive sleep apnea syndrome (OSAS), defined as an obstructive sleep apnea index of more than 1/hour. Motor development was not different between infants with and without OSAS. Mental development was however decreased in infants with OSAS (median (iqr) 65.5% (60.0 to 70.3) of normal) compared to those without OSAS; 74.9% (64.8 to 81.3) (p=0.03) (Figure 1, Table 2). The magnitude of the difference was 0.74 (Cohen's d), indicating a substantial difference. Infants with OSAS tended to be older and tended to have a higher BMI (median 1.4 SDS) than infants without OSAS (median BMI 0.6 SDS), although this did not reach statistical significance. Only one of the infants with OSAS was considered overweight, defined as a BMI above +2 SDS.

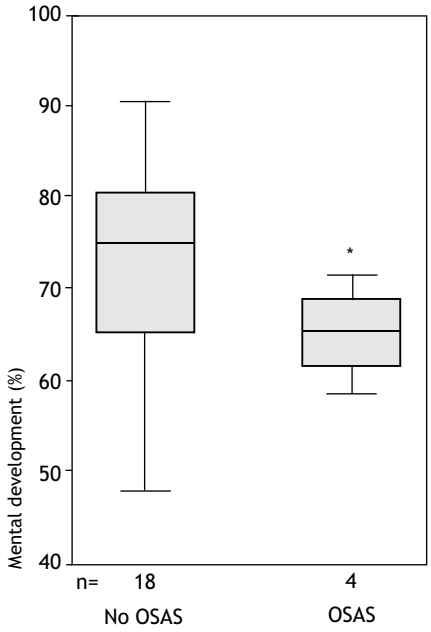

Figure 1. Mental development in infants with or without obstructive sleep apnea syndrome. The Y axis represents mental development expressed as % of expected development for age in children with (n=4) and without (n=18) OSAS. Mental development is significantly lower in infants with OSAS compared to those without. (*p-value: 0.03, independent t-test for observations with unequal variances). Magnitude of the difference (Cohen's d) is 0.74, indicating a medium to large difference.

5.5 Discussion

In PWS infants, mental and motor development were both delayed compared to normal development for age. Sleep-related breathing disorders were common, in particular central sleep apnea. No associations between psychomotor development and any of the respiratory abnormalities were found. In the 4 infants with OSAS, we found a significantly lower mental development compared to infants without OSAS. Infants with OSAS tended to be older and tended to have a higher BMI than the remaining 18 children without OSAS. There was no significant difference in motor development between both groups.

Data concerning associations between psychomotor development and sleep-related breathing disorders in PWS infants are limited. In healthy infants, it was shown that the snoring-related arousal index was associated with the mental developmental index, measured by BSID II [10]. These data suggested that even mild symptoms of sleep-disordered breathing were associated with lower mental development scores in healthy children. In a study by Greenfeld et al, 5 of 29 normal children with OSAS, due to adenotonsillar hypertrophy, had a developmental delay, as subjectively reported by parents. After adenotonsillectomy, the developmental delay resolved in 3 of them [17].

To our knowledge, so far only one author has reported associations between neurobehavioural abnormalities and sleep-related breathing disorders in patients with PWS. In 13 patients (3 infants), aged 1.5 to 28 years, OSAS was prevalent and associated with a higher BMI, and some behavioural abnormalities. Unfortunately, they did not present data on the psychomotor development of the 3 infants [18].

In children, enlargement of adenoid or tonsils, in association with increased upper airway collapsibility, is by far the most common cause of OSAS [19]. In addition, obesity may cause obstructive sleep-related breathing disorders, as has also been found in adults [20]. In children and adults with PWS, both obesity and a small nasopharynx are thought to cause OSAS [3].

We used BMI SDS to define obesity in PWS infants. However, BMI underestimates the actual degree of obesity in individual with PWS, because of their abnormal body composition. As age-related reference data for body fat percentage, measured by dual energy X-ray absorptiometry (DXA) were not available for infants, we did not report DXA scans in the PWS infants.

Our results indicate that OSAS should be treated, by adenoidectomy and/or tonsillectomy when adenoid and/or tonsils are enlarged. In order to optimize

mental development in PWS infants, it is also important to introduce weight control, particularly when obesity is already present. If weight control or adenotonsillectomy does not relieve symptoms of OSAS, the use of nasal continuous positive airway pressure might be considered [21].

The mechanism underlying the negative association between OSAS and mental development is unknown. Beebe and Gozal proposed a model [8], explaining the relation between OSAS, and lower cognition and neurobehavioural abnormalities. According to this model, OSAS-related sleep disruption and intermittent hypoxia and hypercapnia may impair the restorative features of sleep and the cellular or chemical homeostasis in the brain, resulting in a prefrontal cortical dysfunction. The latter would lead to a dysfunction of the cognitive executive system, which is responsible for developing a future-oriented, organized and flexible approach to problem situations. This would result into adverse daytime effects, such as behavioural problems, in particular related to Attention Deficit/Hyperactivity Disorder, and difficulties with problem solving [22,23].

In our study, the infants with OSAS tended to be older and had a higher BMI than infants without OSAS. It cannot be excluded that the higher BMI in infants with OSAS also impaired their motor development. Motor development tended to be more impaired in infants with OSAS, although this did not reach statistical significance.

Remarkable is that the central apneas during sleep in infants with PWS are by far more common than obstructive sleep apneas. However, we found no associations between mental or motor development and central respiratory events during sleep. These results indicate that, although central respiratory abnormalities are more common in PWS, the obstructive events have more severe implications with regard to mental development in infants. Central apneas might reflect the hypothalamic dysfunction in PWS [24]. In addition, it has previously been reported that the Neurally differentiated embryonal carcinoma-cell derived factor (Necdin) gene, one of the protein-coding genes that are deficient in PWS, might be involved in the central SRBD in PWS [25].

To our knowledge, this is the first study in infants with PWS evaluating the association between psychomotor development and sleep-related breathing disorders. Our results indicate that in PWS infants, as recently suggested for the normal population, mental development might be delayed in the presence of obstructive sleep apnea, suggesting that early treatment of OSAS in infancy should be considered.

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Chapter 6

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Cognition and behaviour in pre-pubertal children with Prader-Willi syndrome and associations with sleep-related breathing disorders

6.1 Abstract

Background: Prader-Willi Syndrome (PWS) is a neurogenetic disorder characterised by hypotonia, hypogonadism, obesity and short stature. Neurobehavioural abnormalities, cognitive impairment and sleep-related breathing disorders (SRBD) are common. In the general population consistent relations have been found between neurobehavioural and cognitive abnormalities and SRBD.

Objective: To investigate cognition and behaviour and their associations with sleep-disordered breathing in pre-pubertal PWS children.

Design/patients: For this study, 31 pre-pubertal PWS children, aged 4.0 to 13.2 years, were evaluated (5 with paternal deletion, 14 with maternal disomy, 4 with an imprinting center mutation, and in 8 the defect was not specified). Cognition was assessed by Wechsler Intelligence Scale subtests appropriate for age, and neurobehavioural abnormalities with parent-questionnaires. Polysomnography (PSG) was performed. Cognition, behaviour and associations with SRBD were evaluated.

Results: All cognitive subtests were significantly below 0 SDS, with the lowest median (interquartile range, iqr) scores for one of the performance subtests (Block design) of -2.7 SDS (-3.0 to -0.3). In 60% of children, verbal subtests were less affected than performance subtests. Parents reported problem behaviour on subscales related to "emotions/behaviour not adapted to the social situation" and "insensitivity to social information". All children had a sleep-related breathing disorder, with a median Apnea Hypopnea Index of 4.1/hour (2.6 to 7.9). One of the performance subtestscores was significantly higher in children with better sleep efficiency, and daytime sleepiness was associated with more autistic-like social impairment. In contrast to our expectations, behavioural abnormalities (particularly social relating behaviour) were worse in children with better sleep-related breathing.

Conclusion: In pre-pubertal PWS children, cognition is greatly impaired. Neuro-behavioural abnormalities are common, particularly autistic-like social impairment. Sleep efficiency index was associated with better performance on the Picture Arrangements/Completion subtests, and neurobehavioural abnormalities were associated with daytime sleepiness. In contrast, we could not confirm a positive association of neurobehavioural abnormalities with sleep-related breathing disorders in PWS.

6.2 Introduction

Prader-Willi Syndrome (PWS) is characterised by a number of signs and symptoms, including muscular hypotonia, hypogonadism, psychomotor delay, short stature, and obesity that may become extreme after the age of 2-4 years. Psychomotor delay, neurobehavioural abnormalities and cognitive impairment are common [1,2]. Sleep-related breathing disorders (SRBD) have also been reported [3-5]. The underlying cause of the syndrome is a paternal deletion or a uniparental maternal disomy (UPD) of 15q11-13 [6]. In 1-5%, PWS is the result of an imprinting-center mutation [7], which causes genes in the paternally inherited chromosome 15q11-13 to be silenced. Genetic subtypes have been associated with different behavioural and cognitive phenotypes [2,8].

In healthy children, increasing attention has been drawn to the association between SRBD and neurobehavioural abnormalities, in particular symptoms of Attention-Deficit/Hyperactivity Disorder (ADHD). Associations between SRBD and cognition, school performance and psychiatric and behavioural co-morbidities are consistently reported, and seem to be at least partial reversible [9-11].

In subjects with PWS, there is a lack of data evaluating these associations. Only one report was recently published, describing, in 13 PWS subjects aged 1.5 to 28 years, that SRBD, in particular obstructive sleep apnea syndrome (OSAS), was associated with higher body mass index (BMI), daytime inactivity, sleepiness, autistic-related behaviour, and impulsivity. In this group, 7 of 11 subjects had a BMI >2 standard deviation score (SDS). Nowadays rapid diagnostic testing for PWS is available, leading to early diagnosis in infancy, and optimal management with regard to diet, exercise and education. As a result, the majority of young PWS children are nowadays not severely obese during childhood. In this study we examined cognition by age-appropriate Wechsler subscales, and neurobehavioural abnormalities by parent questionnaires. In addition, we investigated if there was an association between cognition or neurobehavioural abnormalities and SRBD in a homogeneous group of 31 not severely overweight, young, pre-pubertal PWS children.

6.3 Patients and Methods

Patients

In April 2002, a randomised, multicenter, controlled growth hormone (GH) trial in children with genetically confirmed diagnosis of PWS was started, investigating the effects of GH treatment versus no GH on growth, body composition, activity level and psychosocial development. Children were referred to us by their paediatricians. In April 2003, we started a polysomnography (PSG) study in addition to the original protocol. Inclusion criteria for the PSG study were: (1) pre-pubertal at PSG, and (2) no upper respiratory tract infection (URTI). For the current analysis, 31 pre-pubertal children were included. All children were healthy at the time of these studies. Two of them received thyroxin replacement therapy to correct low levels of free T4. All were evaluated before enrolling in GH therapy.

The study protocol was approved of the Medical Ethical Committee of Erasmus Medical Center, Rotterdam, The Netherlands. Written informed consent was obtained from the parents, and from children over 12 years.

Methods

Anthropometry

Height was assessed by a Harpenden stadiometer and weight was assessed on an accurate scale. BMI (kg/m^2) was calculated. Height and BMI were converted into SDS, according to Dutch references for age [12]. Growth Analyser Version 3.0 software was used to calculate BMI, height and BMI SDS (www.growthanalyser.org).

Intelligence

To assess intelligence, a short form of four subtests: Vocabulary, Similarities (verbal IQ subtests) and Block design, Picture arrangement (performance IQ subtests) of the Wechsler Intelligence Scale for Children-Revised, Dutch version (WISC-R), was used in children over 7 years of age or when appropriate [13]. A short form of four subtests: Vocabulary, Similarities (verbal IQ subtests) and Block design, Picture completion (performance IQ subtests) of the Wechsler Preschool and Primary Scale of Intelligence – Revised, Dutch version (WPPSI-R), was used for children with age below 7 years or when appropriate [14,15]. All cognitive tests were conducted by a psychologist (M.W.), experienced also in testing PWS children. All children had a PSG prior to start of GH treatment. All cognitive tests were performed, prior to start of GH, and not more than 6 months before or after PSG. WPPSI was assessed in 17 children and WISC in

10 children. Median age at WPPSI assessment was 6.5 years (5.8 to 6.8) and median age at completion of WISC was 9.6 years (8.5 to 11.9). Four children were too young to be tested with Wechsler scales. These children were excluded from the present analysis.

Behaviour

Developmental Behaviour Checklist

The Developmental Behaviour Checklist (DBC) is a 96-items checklist for all levels of mental disabilities, completed by parents or caregivers to assess emotional or behavioural problems over the last six months in children aged 4 to 18 years. We used the revised scale structure as developed by Dekker et al, containing 5 interpretable and relevant subscales: Disruptive/Antisocial, Self-Absorbed, Communication Disturbance, Anxiety and Social Relating. A total problem behaviour score was calculated by combining all subscale scores. For the DBC, we used reference data for Dutch children with mental disability, who have an IQ between 59 and 70. We considered this reference population to resemble most of our PWS children [16].

VISK

VISK is a Dutch Questionnaire to evaluate social behaviour in children, completed by parents or caregivers. This questionnaire contains 49 items, and 6 subscales: emotions/behaviour not adapted to the social situation, withdrawal, dysorientation with respect to place, time, persons and activity, insensitivity to social information, stereotypic behaviour, and resistance to change. This test is validated, standardised and widely used in The Netherlands. Dutch reference data are available for children with different psychiatric disorders and mental disability. We used Dutch reference data for children with mental disability [17]. We expressed data in SD scores. Both questionnaires were filled out, prior to start of GH, and not more than 6 months before or after PSG.

Polysomnography

Children were admitted to the sleep center at 5.00 p.m., accompanied by one parent. Children underwent complete overnight polysomnography (PSG). 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 strain gauges and oxygen

saturation (SaO_2) by pulse oximetry. All PSG studies were evaluated independently by two persons, both certified in PSG analysis. In case of major discrepancies between both assessments a third expert opinion was available, but this was never required. The polygraphic records were scored according to standard criteria of Rechtschaffen and Kales [18]. A period of apnea or hypopnea was defined as more than 90% (apnea) or 50-80% (hypopnea) reduction of airflow for 3 breaths or longer. For hypopneas, the additional criterion was a reduction of SaO_2 of 4% or more. Periods of apnea and hypopnea were counted over the period of sleep during the night and calculated as mean per hour of sleep, apnea hypopnea index (AHI). An AHI above 1/hour is considered pathological [19]. Apneas were considered obstructive when absence of airflow occurred without a decrease in respiratory effort and central, when thoracic movements were absent. Abnormal SaO_2 was defined as SaO_2 below 92% or more than 4% below baseline values.

Sleep efficiency and daytime sleepiness

Sleep efficiency index (SEI) was calculated as total sleep time/time in bed, as measured during PSG. To assess daytime sleepiness, 2 questions of the Developmental Behaviour Checklist were used, as proposed by O'Donoghue et al [20]: question 48 ("moves slowly, underactive, does little"), was used to score inactivity and question 69 ("sleeps too much") to obtain a measure for excessive daytime sleepiness.

Data analysis

Data were expressed as median, and interquartile range (iqr). To compare results of subtests of Wechsler scales (WPPSI-R and WISC-R), values were expressed as SDS. In order to evaluate the difference between performance and verbal components, we used a plot of the difference between verbal and performance tests within a child against the mean of both subtests according to the method of Bland and Altman. If the difference between verbal and performance subtests was between -2*standard error and +2*standard error, they were considered equally impaired [21,22]. Multiple regression was used to adjust raw data, after adjustment for age, sex and different tests (WISC-R or WPPSI-R). All statistical testing was fixed at 0.05 level (2-sided). Binomial tests were used to compare SDscores for cognitive and behavioural tests with reference data. Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS, Version 11).

6.4 Results

General Clinical Characteristics

Clinical characteristics are shown in Table 1. Diagnosis of PWS was confirmed by positive methylation tests in all children. Five children had a paternal deletion, 14 had uniparental maternal disomy, and 4 had an imprinting center mutation. In 8 children the underlying defect was not yet specified. Five of 31 children were considered to be overweight, defined as BMI over +2 SDS.

Table 1. Baseline clinical characteristics

	Median	iqr
N	31	
Sex (m/f)	14/17	
Age (yrs)	6.4	6.0 to 9.1
Height (SDS)	-2.4	-3.2 to -1.7
BMI (kg/m^2)	18.2	17.0 to 19.7
BMI (SDS)	1.2	0.6 to 1.7
Apnea hypopnea index	4.1	2.6 to 7.9
Central apnea index	2.2	1.3 to 3.0
Obstructive apnea index	0.0	0.0 to 0.5
Hypopnea index	1.1	0.1 to 2.9
Duration longest apnea (sec)	17.0	13.0 to 29.0

Cognition

Data of four subtests of WPPSI and WISC, expressed as SDS compared to normal reference population, are listed in Table 2 and Figure 1a. The median scores on subtests were -1.7 SDS (-2.0 to -0.3) for Vocabulary, -1.7 SDS (-2.7 to -0.3) for Similarities, -2.7 SDS (-3.0 to -1.7) for Block Design, and -1.7 SDS (-2.8 to -0.2) for Picture Completion and Picture Arrangement on WPPSI and WISC, respectively. The median of all subtest-

scores was significantly below the mean of the normal reference population (p<0.001, p<0.005 p<0.001, p<0.001). For individual children (Figure 1b), we found that the psychological profile did not show a consistent impairment on all subtests. A subgroup of children (60% of children) had higher verbal than performance scores, and the opposite was true for other children (24% of children). Only a minority of children had a verbal and performance score within the same range (16% of children).

Table 2. Cognition and behaviour in pre-pubertal children with PWS

	Median	iqr
Cognitive subtests (SDS)		
Vocabulary	-1.7[3]	-2.0 to -0.3
Similarities	-1.7[2]	-2.7 to -0.3
Block design	-2.7[3]	-3.0 to -1.7
Picture completion/Picture arrangement	-1.7[3]	-2.8 to -0.2
Developmental behaviour checklist (SDS)		
Disruptive/Antisocial	-0.5	-0.8 to 0.1
Self-absorbed	-0.7	-0.9 to 0.2
Communication disturbance	-0.3	-0.6 to 0.4
Anxiety	-0.5[2]	-0.9 to -0.2
Social relating	0.6	-0.5 to 1.0
Total score	-0.7	-0.9 to 0.3
VISK (SDS)		
Not adapted	0.5[1]	-0.1 to 1.1
Withdrawal	-0.3	-0.8 to 0.1
Dysorientation	-0.1	-0.4 to 0.1
Insensitive to social information	0.6[1]	-0.2 to 0.8
Stereotypic behaviour	-0.3	-0.6 to 0.7
Resistance to change	-0.2	-0.8 to 0.4
Total score	-0.3	-0.8 to 0.4

Subtestscores of cognition were compared to healthy references. Scores of subtests of DBC and VISK were expressed as SD scores compared to children with mental delay. Higher SD scores indicate higher level of problematic behaviour.

[1] p<0.05, [2] p<0.005, [3] p<0.001.

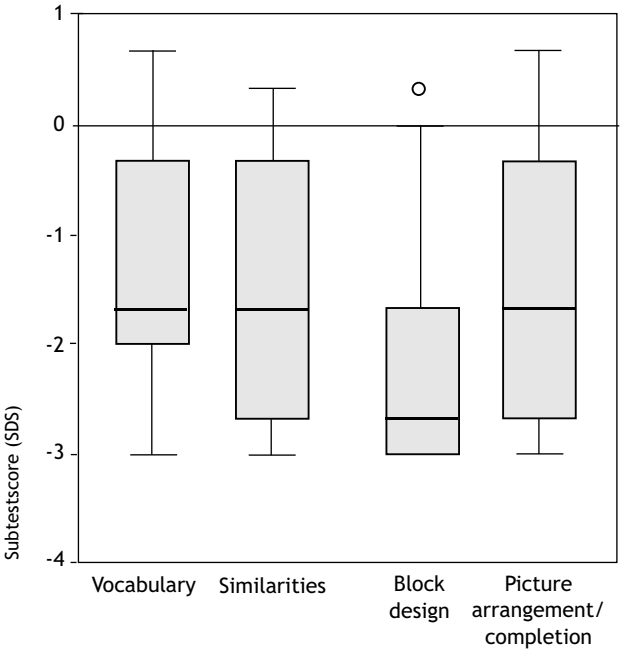

Figure 1a. Subtestscores expressed as SD scores for four subtests of WPPSI and WISC. Line represents 0 SDS (=normal subtestscore)

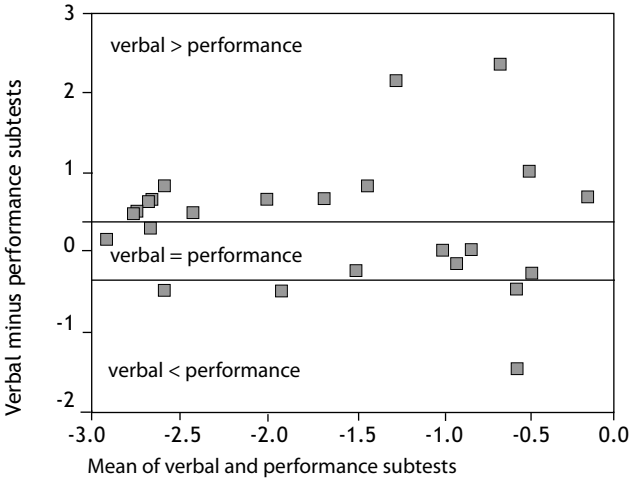

Figure 1b. Disbalance between verbal and performance intelligence profile in PWS children. Verbal and performance subtests of WPPSI and WISC-R are expressed as SD scores compared to a Dutch healthy reference population. If the ratio is higher than 2*SE, verbal subtests are higher than performance subtests and if it is less than -2*SE, performance tests are higher than verbal tests.

Behaviour

In Table 2, subtest-scores of both behaviour questionnaires are presented. In these tests, a higher SD score indicates more severe problem behaviour. Compared to these reference data, our PWS children had less anxiety-related problems on DBC ($p<0.005$), and more behavioural problems on 2 of the subscales of VISK, indicating "emotions/ behaviour not adapted to the social situation" ($p<0.05$), and "insensitivity to social information" ($p<0.05$).

Sleepiness

Inactivity was reported "never" by 8/24 parents, "sometimes" by 10/24 parents and "often" by 6/24 parents. Sleepiness was reported "never" by 19/24 parents, "sometimes" by 4/24 parents, and "often" by 1/24 parents. The median (iqr) sleep efficiency index (SEI) during PSG for all children was 79% (75 to 86).

Associations of cognition and behaviour with sleep-related breathing

All children had sleep-related breathing disorder, defined as an AHI over 1/hour (Table 1). Median (iqr) AHI of the total group was 4.1 (2.6 to 7.9). Most apneas were of central origin, with a median central apnea index of 2.1 (1.3 to 23.0). Obstructive apneas were uncommon, with median obstructive apnea index of 0.0 (0.0 to 0.5). Median duration of longest apnea was 17 sec (13.0 to 29.0). We found no difference in SRBD between children with paternal deletion, maternal disomy or imprinting center mutation.

In Table 3, associations between cognition and SRBD, after adjustment for sex, age, and different tests are summarized. Sleep efficiency index (SEI), measured during PSG, was significantly associated with one of the performance subtests: Picture Completion (of WPPSI-R) and Picture Arrangement (of WISC-R) ($\beta=0.78$, $p<0.001$). None of the respiratory parameters did significantly explain the verbal or other performance subtests of the Wechsler scales.

Associations between behaviour and sleep-related breathing disorders are listed in Table 4. Sleepiness, was consistently associated with problem behaviour, related with subtests of the DBC on 'communication disturbance' ($\beta=0.41$, $p<0.05$), 'anxiety' ($\beta= 0.49$, $p<0.05$), and 'social relating' ($\beta=0.45$, $p<0.05$), and total problem behaviour score ($\beta=0.48$, $p<0.05$). In VISK, two subscales were significantly related to sleepiness, i.e. 'stereotypic behaviour' ($\beta=0.53$, $p<0.05$), and 'anxiety to change' ($\beta=0.53$, $p<0.05$). Surprisingly, PSG measures, in particular AHI, were negatively associated with most subscales of behavioural questionnaires, reaching statistical significance in "total problem behaviour score" ($\beta=-0.41$, $p=0.05$), and "social relating score"

(ß=-0.47, p<0.05), indicating that children with higher apnea hypopnea index, had less problem behaviour. In addition, duration of longest apnea explained variability in problem behaviour negatively, indicating that if apneas had a longer duration, problem behaviour was less severe, reaching statistical significance for subscale of 'communication disturbance' (ß=-0.45, p<0.05).

Figure 2. Bars represent the problem behaviour score for children with PWS, compared to reference data from children with mental delay. Data are expressed as SD scores.
* Represents problem behaviour with a median significantly above or below the reference population.

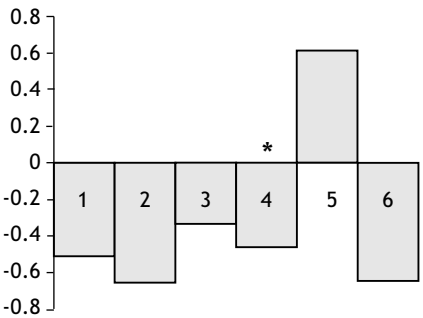

Figure 2a.
Developmental Behaviour Checklist (DBC)
1. Disruptive / Antisocial
2. Self-absorbed
3. Communication Disturbance
4. Anxiety
5. Social Relating
6. Total score

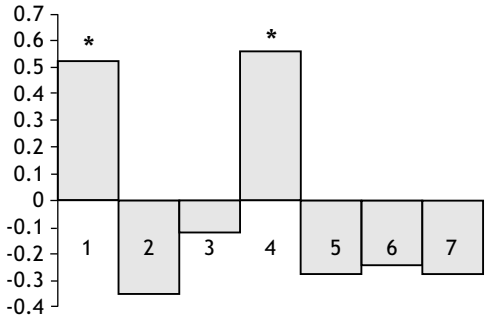

Figure 2b.
Questionnaire for social behaviour in children (VISK)
1. Not Adapted
2. Withdrawal
3. Dysorientation
4. Insensitive to social situations
5. Stereotyping Behaviour
6. Resistance to change
7. Total social problem behaviour

Table 3. Associations between cognition and sleep-related breathing disorders

	AHI		CAI		OAI		HI		Longest Apnea		SEI		Sleepiness	
	ß	p	ß	p	ß	p	ß	p	ß	P	ß	p	ß	p
Verbal subtests														
Vocabulary	**-0.05**	*0.85*	**-0.12**	*0.64*	**0.06**	*0.84*	**-0.02**	*0.93*	**0.07**	*0.81*	**0.31**	*0.31*	**-0.43**	*0.14*
Similarities	**-0.21**	*0.39*	**-0.20**	*0.42*	**-0.08**	*0.79*	**-0.19**	*0.44*	**0.03**	*0.93*	**0.32**	*0.27*	**-0.21**	*0.46*
Performance subtests														
Block Design	**-0.11**	*0.68*	**0.19**	*0.45*	**0.16**	*0.59*	**-0.34**	*0.17*	**-0.23**	*0.44*	**0.27**	*0.38*	**-0.05**	*0.88*
Picture Completion/Arrangement	**-0.26**	*0.25*	**-0.23**	*0.32*	**-0.23**	*0.39*	**-0.21**	*0.37*	**-0.09**	*0.74*	**0.78**	*0.00*	**0.33**	*0.22*

AHI=Apnea Hypopnea Index, CAI=Central Apnea Index, OAI=Obstructive Apnea Index, HI=Hypopnea Index. SEI=Sleep Efficiency Index. Data are corrected for sex, age and type of test.

Table 4. Associations between behaviour (DBC an VISK) and sleep-related breathing disorders

	AHI		CAI		OAI		HI		Longest Apnea		SEI		Sleepiness	
	β	p	β	p	β	p	β	p	β	p	β	p	β	p
DBC														
Disruptive/Antisocial	**-0.39**	*0.07*	**-0.33**	*0.16*	**-0.22**	*0.32*	**-0.37**	*0.09*	**-0.37**	*0.13*	**-0.01**	*0.97*	**0.42**	0.06
Self-Absorbed	**-0.34**	*0.11*	**-0.29**	*0.21*	**-0.26**	*0.24*	**-0.28**	*0.20*	**-0.39**	*0.10*	**-0.16**	*0.51*	**0.38**	*0.08*
Communic. Disturbance	**-0.24**	*0.24*	**-0.13**	*0.56*	**-0.18**	*0.37*	**-0.23**	*0.25*	**-0.45**	*0.04*	**-0.03**	*0.91*	**0.41**	*0.04*
Anxiety	**-0.39**	*0.06*	**-0.37**	*0.10*	**-0.28**	*0.19*	**-0.32**	*0.14*	**-0.38**	*0.10*	**0.16**	*0.48*	**0.49**	*0.02*
Social Relating	**-0.47**	*0.03*	**-0.44**	*0.06*	**-0.36**	*0.10*	**-0.36**	*0.10*	**-0.13**	*0.60*	**0.31**	*0.19*	**0.45**	*0.04*
Total score	**-0.41**	*0.05*	**-0.35**	*0.13*	**-0.29**	*0.20*	**-0.36**	*0.10*	**-0.42**	*0.08*	**0.00**	*0.99*	**0.48**	0.03
VISK														
Not adapted	**-0.42**	*0.06*	**-0.29**	*0.23*	**-0.02**	*0.95*	**-0.50**	*0.03*	**-0.38**	*0.14*	**-0.14**	*0.59*	**0.36**	*0.13*
Withdrawal	**-0.20**	*0.30*	**-0.31**	*0.12*	**-0.11**	*0.60*	**-0.09**	*0.65*	**-0.16**	*0.47*	**0.04**	*0.87*	**0.17**	*0.40*
Orientation problems	**-0.05**	*0.82*	**-0.05**	*0.82*	**-0.06**	*0.82*	**-0.03**	*0.90*	**-0.29**	*0.23*	**-0.22**	*0.36*	**0.11**	*0.63*
Insensitive to social situation	**-0.39**	*0.08*	**-0.28**	*0.23*	**-0.12**	*0.65*	**-0.42**	*0.06*	**-0.37**	*0.14*	**-0.33**	*0.19*	**0.07**	*0.76*
Stereotyping behaviour	**-0.28**	*0.22*	**-0.20**	*0.41*	**0.00**	*0.99*	**-0.33**	*0.15*	**-0.40**	*0.12*	**0.11**	*0.66*	**0.53**	*0.02*
Anxiety to change	**-0.40**	*0.10*	**-0.40**	*0.08*	**-0.25**	*0.33*	**-0.26**	*0.27*	**-0.20**	*0.43*	**-0.07**	*0.78*	**0.53**	*0.02*
Total score	**-0.40**	*0.08*	**-0.34**	*0.14*	**-0.10**	*0.70*	**-0.40**	*0.08*	**-0.42**	*0.10*	**-0.14**	*0.60*	**0.38**	*0.11*

AHI=Apnea Hypopnea Index, CAI=Central Apnea Index, OAI=Obstructive Apnea Index, HI=Hypopnea Index. SEI=Sleep Efficiency Index. Data are corrected for sex, age and type of test.

6.5 Discussion

This study shows that pre-pubertal not overweight PWS children, have impaired cognitive abilities with an unequal distribution. Sixty percent of the children had better scores on verbal than on performance subtests. In addition PWS children had neurobehavioural abnormalities, particularly autism-related social behaviour, and sleep-related breathing disorders which were mainly of central origin. Sleep efficiency index was associated with better performance on the subscales of Picture Completion/Arrangement, and neurobehavioural abnormalities were associated with daytime sleepiness. In contrast, we could not find associations between cognition or neurobehavioural abnormalities and SRBD, as previously described for the general population. Surprisingly, a negative association was found between social relating problem behaviour and total problem behaviour and apnea hypopnea index, indicating that children with more apneas had less behavioural problems.

Our results indicate overall cognitive impairment, although not equally divided between verbal and performance subtests. Sixty percent of children did better on verbal than on performance subtests. In our study group, UPD was relatively common, which might explain this finding, because a significantly higher verbal IQ in PWS subjects with UPD compared to deletions has been reported [2]. Our sample does not reflect the general population of PWS subjects, with respect to the distribution of the underlying genetic defect. However, an increase in UPD, relative to paternal deletion has been reported recently in young PWS children, and has been attributed to increasing maternal age at conception [23]. Better sleep efficiency was associated with a better performance on "picture completion/picture arrangement" subscales, which might be related to executive functioning, as has been proposed by Beebe and Gozal [9]. According to this model, sleep disruption, together with intermittent hypoxia and hypercapnia, elicited by SRBD, induce disruption of the restorative features of sleep and of cellular and chemical homeostasis which in turn results in frontal cortical dysfunction. Dysfunction of the frontal cortex is considered to impair the cognitive executive functioning (indicating the ability to develop an approach to problem situations), which may contribute to the association of performance cognitive subtests and sleep efficiency.

As PWS subjects might have an increased need for nocturnal sleep [24], management strategies to reduce behavioural problems at bedtime may be indicated to improve sleep efficiency [3]. Future research is needed to evaluate whether improvement of sleep efficiency also results in improvement of performance intelligence. We did not

find an association between cognitive parameters and BMI (data not shown) or SRBD, but this might be due to the fact that obstructive sleep apnea and overweight were rather uncommon in our study-group.

We found in our PWS children, compared to reference data for children with mental disability, less anxiety-related problem behaviour and more behavioural problems particularly more "emotions/behaviour not adapted to the social situation" and "insensitivity to social information", which reflect more autistic-like social impairment. Autistic like social behaviour is more common in UPD [25], and the high prevalence of these abnormalities in our study group may be due to the high prevalence of UPD in this group. It is remarkable that, compared to a reference population with mental disability, disruptive/antisocial behaviour (including "impulsiveness" and "temper tantrums") was not commonly reported. Notably, children with more severe behavioural abnormalities had less severe SRBD. In addition, if apneas had a longer duration, problem behaviour in particular on the subscale "communication disturbance', tended to be less severe.

These findings in PWS children are difficult to understand or explain, and not in line with the general pediatric population, in which associations between SRBD (mostly obstructive apnea) measured with PSG and problem behaviour, particularly ADHD, are reported [26].

We cannot explain the inverse relationship between SRBD and behavioural abnormalities. It might be that parents of relatively well functioning PWS children, reported more problem behaviour, because their child attend normal school and they compared their child to healthy peers, whereas children with more severe mental disabilities were compared to other children with mental disability, which may have resulted in underestimation of problem behaviour in more severely affected children.

Another explanation for the absence of a positive association between SRBD and behavioural abnormalities, might be that in children with PWS, neurobehavioural abnormalities, cognitive impairment and SRBD may have different genetic causes. SRBD are proposed to be caused by absence of the Necdin gene in PWS, via abnormal neuronal activity within the respiratory rythm-generating center, the pre-Bötzinger complex, as was shown by Ren et al in Necdin-deficient mice [27]. One of the proposed genes involved in behavioural abnormalities, particularly autistic-like behaviour is the ubiquitin protein ligase gene, UBE3A [8]. UBE3A is maternally expressed in a few specific areas, including the hippocampus and the cerebellar Purkinje cells [28], which both have been implicated in the neuropathology of autism [8]. UBE3A is located on

chromosome 15 and paternally imprinted. In subjects with a UPD of chromosome 15, UBE3A is over-expressed. In particular, increased autistic-like social impairments are consistently more common in UPD cases compared to children with deletions [8]. Also in our study-group, autistic-like social impairment was the most common reported behavioural abnormality and the majority of our PWS children had UPD, which does not reflect the general population of PWS subjects. In addition, our sample size was not large enough to study differences in behavioural phenotypes between the genetic subtypes.

Our results are in contrast with the only previous report in PWS subjects, aged 1.5 to 28 years, including 6 children below the age of 12. In this report autistic behaviour was associated with low sleep efficiency and oxygen desaturations and impulsivity with SRBD. One possible explanation for the difference with our results might be that in these subjects, obesity was more common, which might have caused obstructive sleep apnea, with drops in oxygen saturation, resulting in neurobehavioural abnormalities as found in children with OSAS in the general population.

In conclusion, this is the first study evaluating cognition, behaviour and their associations with sleep-related breathing disorders in a large, homogeneous group of pre-pubertal PWS children. Our results show that PWS children have impaired cognitive abilities, not equally divided between verbal and performance subtests. Their problem behaviour is particularly related to autistic-like social impairment, i.e. “emotions/behaviour not adapted to the social situation” and “insensitivity to social information”. Sleep efficiency index was associated with better performance on the “Picture completion/arrangement” subtests, whereas neurobehavioural abnormalities were associated with daytime sleepiness. Future studies are required to evaluate whether improvement of nocturnal sleep and daytime sleepiness may improve cognitive and behavioural functioning.

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Chapter 7

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Thyroid hormone levels in children with Prader-Willi syndrome before and during growth hormone treatment

7.1 Abstract

Background: Prader-Willi Syndrome (PWS) is a neurogenetic disorder characterised by muscular hypotonia, psychomotor delay, obesity, and short stature. Several endocrine abnormalities have been described, including growth hormone (GH) deficiency, and hypogonadotropic hypogonadism. Published data on thyroid hormone levels in PWS children are very limited.

Objective: To evaluate thyroid function in children with PWS, before and during GH treatment.

Design/Patients: At baseline, serum levels of T4, free T4 (fT4), T3, reverse T3 (rT3) and TSH were assessed in 75 PWS children. After one year, assessments were repeated in 57 of them. These children participated in a randomised study with two groups: GH group (n=34) treated with 1 mg GH/m^2/day and control group (n=23) as controls.

Results: Median age (interquartile range, iqr) of the total group at baseline was 4.7 years (2.7 to 7.6). Median (iqr) TSH level was -0.1 SDS (-0.5 to 0.5), T4 level, -0.6 SDS (-1.7 to 0.0), and fT4 level, -0.8 SDS (-1.3 to -0.3), the latter two being significantly lower than 0 SDS. T3 level, 0.3 SDS (-0.3 to 0.9), was significantly higher than 0 SDS. After one year of GH treatment, fT4 decreased significantly from -0.8 SDS (-1.5 to -0.2) to -1.4 SDS (-1.6 to -0.7), compared with no change in untreated PWS children. T3 did however not change, 0.3 SDS (-0.1 to 0.8).

Conclusions: We found normal fT4 levels in most PWS children. During GH treatment, fT4 decreased significantly to low-normal levels. TSH levels remained completely normal. T3 levels were relatively high or normal, both before and during GH treatment, indicating that PWS children have increased T4 to T3 conversion.

7.2 Introduction

Prader-Willi Syndrome (PWS) is a genetic disorder, in the majority of subjects caused by either a microdeletion on the paternally derived chromosome 15q11-13 or a uniparental maternal disomy affecting the same region [1]. In approximately 1-5%, PWS is the result of an imprinting center mutation [2], which results in silencing of genes that are normally active only in the paternally inherited chromosome 15q11-13.

PWS is characterised by a number of signs and symptoms, including muscular hypotonia, psychomotor delay, short stature and obesity, which may become extreme after the age of 2-4 years. Temperature instability and sleep-related breathing disorders contribute to the clinical characteristics of the PWS. Most of the characteristic features of PWS are thought to be the result of hypothalamic dysfunction [3].

Several endocrine abnormalities have been described, including growth hormone (GH) deficiency and hypogonadotropic hypogonadism [4]. Studies concerning thyroid hormone levels in PWS children are very limited. Congenital hypothyroidism has been reported in one girl with PWS [5].

In general, it has been suggested that GH therapy of non-PWS GH-deficient patients could unmask an underlying TSH deficiency, leading to deficiencies in T4 and T3 [6, 7]. In addition, GH might increase peripheral conversion of T4 to T3 [8-10]. Myers et al found that 3/35 (9%) of PWS children required thyroxin replacement while on GH [11]. This was also reported in other studies [12,13].

Prior to the study we hypothesized that a certain percentage of PWS children would have hypothyroidism (approximately 20%) of central origin and that GH treatment could unmask an underlying TSH deficiency. For that reason, one of our objectives was to evaluate thyroid hormone levels at baseline and during GH treatment. We assessed levels of T4, free T4 (fT4), T3, reverse T3 (rT3) and TSH and their relations, in a group of PWS children in a randomised GH trial, at start and after 1 year.

7.3 Patients and Methods

Patients

Seventy-nine children were enrolled in a large randomised controlled trial to investigate the effects of GH treatment on growth, body composition, activity level and psychosocial development. Participants fulfilled the following inclusion criteria:

(1) genetically confirmed diagnosis of PWS; (2) age between 6 months and 16 years; (3) bone age less than 14 years (girls) or 16 years (boys). Patients with non-cooperative behaviour or patients receiving medication to reduce fat were excluded. There was no familiarity for thyroid disease, and all children were naïve to GH treatment at the start of study. They were included irrespective of their growth hormone status. Caloric intake and activity level of all participants were standardised, and recommendations were given. Compliance to diet and exercise was evaluated by the research nurse, in close collaboration with the dietician and, if indicated, the physiotherapist. After stratification for age and body mass index (BMI), children with an age below 12 years (girls) or 14 years (boys) were randomised, into either the GH treatment group or the control group. Children with an age over 12 years (girls) or 14 years (boys) were all treated with GH.

Seventy-five children were eligible for baseline analysis. Fifty-seven were analysed after 1 year of study, divided into two groups: GH group (n=34) receiving Genotropin® (somatropin) GH 1 mg/m^2/day s.c. (Pfizer)(during the first 4 weeks children received 0.5 mg/m^2/day, to avoid fluid retention) and control group (n=23) as controls. Eighteen patients did not yet reach 1 year of study or we could not obtain a blood sample due to difficult venapuncture. In 4 children, thyroxin treatment was started to correct low levels of fT4, prior to study. They are described separately.

The study protocol was approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam, The Netherlands. Written informed consent was obtained from the parents and children over 12 years of age.

Methods

Collection of blood

Blood samples were collected in the morning, after overnight fasting for assessment of T4, fT4, T3, rT3, and TSH. After centrifugation samples were immediately frozen at -20° until assayed.

Assays

Thyroid hormone levels

Plasma levels of rT3 were measured by established radioimmunoassay procedures, as previously described [14,15]. Serum TSH, fT4, T4 and T3 levels were measured by Vitros Eci technology (Ortho-Clinical Diagnostics, Amersham, UK). The within-assay coefficient of variation (CV) of Vitros was 3-7% and the between-assay CV 5-10%. Thyroid hormone standard deviation scores (SDSs) were calculated with data from

a control group comprising 500 healthy Dutch children (age 1.4-18 years) from the Rotterdam region, The Netherlands, participating in a study to assess reference values. They agreed to a blood test to assess biochemical parameters. The Medical Ethics Committee of the Erasmus Medical Center Rotterdam approved this study, and written informed consent was obtained from the parents or guardians.

IGF-I and IGFBP-3 levels

IGF-I was measured using an immunometric technique on an Advantage Chemiluminescense System (Nicholls Institute Diagnostics, San Juan Capistrano, USA). Serum IGFBP-3 levels were measured using a specific RIA [16] in one laboratory. The intra-assay CV was 4% and the inter-assay CV was 6%. Because of age- and sex-dependency, IGF-I and IGFBP-3 levels were transformed into SDS [16].

Anthropometry

At baseline and after 1 year, standing height (or supine length, when appropriate) was measured with a Harpenden stadiometer. Weight was assessed on an accurate scale, BMI was calculated, and head circumference was measured. Height, BMI, and head circumference were expressed as SD scores adjusting for sex and age according to Dutch reference data for children [17,18]. BMI, height SDS, BMI SDS, and head circumference SDS were calculated with Growthanalyser, Version 3.0. (www.growthanalyser.org).

Statistical analysis

Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS Version 11.0, Chicago, IL). Most of our data were not Gaussian distributed. After log- or square root transformation of skewed data, data were still not normally distributed. We therefore expressed data as median (interquartile range, iqr) and we used non-parametric tests if indicated. Differences between groups at baseline and between changes over time were evaluated using independent sample t-test for anthropometric data, and Mann Whitney U tests on standardised thyroid hormone levels. Changes within subjects were calculated with paired t-test or Wilcoxon signed rank test, respectively. Posthoc Bonferroni correction was used to adjust for multiple testing. To compare the data, expressed as SDS, with those of healthy references (0 SDS), we used binomial test to exclude the influence of outliers. Correlations were calculated using Spearman's correlation coefficient. One-way ANOVA was used to analyse variation in thyroid hormone levels for different genetic defects. P-value <0.05 was considered as statistically significant.

7.4 Results

Clinical characteristics at baseline

Baseline clinical characteristics are summarized in Table 1. The baseline group had a median (iqr) age of 4.7 years (2.7 to 7.6). Median height SDS and head circumference SDS were significantly lower in the patients than in the normal population. BMI was higher in our patients than in the normal population. Twenty-one percent of our patients were considered overweight, defined as BMI > +2 SDS. Five of 79 (6%) children were underweight defined as BMI < -2 SDS. All children had genetically confirmed diagnosis of PWS by positive methylation test. Twenty-six children had a paternal deletion, 24 had a uniparental maternal disomy and 7 had an imprinting center mutation. In one patient, the absence of 15q11-13 was caused by a Robertsonian translocation. In the remaining 17 patients the underlying genetic defect was not yet specified.

Thyroid hormone levels at baseline

Baseline thyroid hormone levels are listed in Table 1. Free T4 was -0.8 SDS (-1.3 to -0.3), which was significantly lower than 0 SDS. Only one patient of 75 (1.4%) had a fT4 level below –2 SDS. In addition, 4 patients were described separately because they received thyroxin treatment to correct low fT4 levels, prior to study. As a result 5/79 (6.3%) had fT4 levels below -2 SDS, thus most children had fT4 levels well within the normal range. Interestingly, median T3 levels were significantly higher than 0 SDS. None of our 75 patients had a T3 level below -2 SDS, and 5.5% had a T3 above +2 SDS. Median rT3 and TSH levels were -0.3 SDS (-0.9 to 0.4) and -0.1 SDS (-0.5 to 0.5), which was not significantly lower than 0 SDS. We did not find any difference in thyroid hormone levels between the different genetic defects, or between boys and girls (data not shown).

Children with low fT4 levels

Four patients were excluded from group analysis, because they received thyroxin replacement therapy, prior to start of GH study. At start of replacement therapy they had a mean age of 3.5 years, and a mean BMI of +1.0 SDS. Free T4 levels, prior to start of thyroxin replacement, were low (ranging from 7.2 to 8.4 pmol/l, normal range: 11-25 pmol/l). TSH levels were completely normal, ranging from 1.52 to 3.48 mU/l. In 2 of them, T3 levels were available, which were within the normal range in both patients (2.1 and 2.2 nmol/l, normal range: 1.43-2.51 nmol/l). One patient of the baseline group (n=75) had a fT4 level below –2 SDS, but T3 level (-0.38 SDS) and TSH

level (-0.32 SDS) were completely normal. Thus, 5/79 (6.3%) patients had a fT4 below -2 SDS at baseline, without abnormalities in TSH and T3 levels.

Table I: Clinical characteristics and thyroid hormone levels before GH treatment

		Median	iqr	p-value
n		75		
Sex	(m/f)	39/36		
Age	(yrs)	4.7	(2.7 to 7.6)	
Height	(SDS)	-2.2	(-3.0 to -1.2)	<0.001
Head circ	(SDS)	-0.8	(-1.6 to -0.3)	<0.001
BMI	(kg/m^2)	17.7	(15.9 to 20.3)	
	(SDS)	1.1	(-0.2 to 1.9)	<0.001
IGF-I	(ng/ml)	53.0	(34.0 to 87.0)	
	(SDS)	-2.0	(-2.7 to -1.3)	<0.001
IGBFBP-3	(ng/ml)	1.2	(0.8 to 1.4)	
	(SDS)	-2.2	(-3.1 to -1.6)	<0.001
T4	(nmol/L)	98.0	(85.3 to 112.8)	
	(SDS)	-0.6	(-1.7 to 0.0)	<0.001
fT4	(pmol/L)	16.2	(14.3 to 17.8)	
	(SDS)	-0.8	(-1.3 to -0.3)	<0.001
T3	(nmol/L)	2.6	(2.3 to 3.0)	
	(SDS)	0.3	(-0.3 to 0.9)	0.02
rT3	(nmol/L)	0.3	(0.3 to 0.4)	
	(SDS)	-0.3	(-0.9 to 0.4)	0.10
TSH	(mU/L)	2.0	(1.6 to 2.7)	
	(SDS)	-0.1	(-0.5 to 0.5)	0.64

Baseline levels of height, BMI and head circumference (n=75), TSH (n=75), T3, fT4 (n=73), T4 (n=72) and rT3 (n=64), IGF-I (n=53), IGFBP-3 (n=52).
p-value represents p-value compared to normal (0 SDS).

Correlations at baseline

T4 SDS and rT3 SDS were positively correlated with age (r=0.68; p<0.001 and r=0.67; p<0.001, respectively) and with BMI SDS (r=0.40; p<0.005 and r=0.44; p<0.001, respectively). As age and BMI SDS were highly correlated, the associations were no

longer significant after correction for BMI or age respectively. Free T4 SDS did neither correlate with age nor with BMI SDS. Free T4 levels were not associated with IGF-I levels (r=0.04, p=0.77). We did not find associations between thyroid hormone levels and height SDS. Interestingly, T3 SDS did significantly correlate with head circumference SDS (r=0.35; p<0.005).

Effects of GH treatment

Table 2 shows the clinical parameters of children, before and after 1 year of follow-up. Although children in the GH group were older (p=0.02), height, BMI and head circumference, expressed as SD scores, were similar in both groups at baseline. During GH treatment, height SDS increased significantly from -2.1 (-2.9 to -1.3) to -1.1 (-1.5 to -0.5) (p<0.005). In controls, height SDS remained unchanged. BMI SDS decreased in GH-treated children from 1.2 SDS (0.0 to 2.3) to 0.8 SDS (-0.4 to 1.7) (p<0.05 compared with change in controls). Head circumference had significantly increased in GH-treated children, compared with no change in controls.

Table 2: Clinical parameters before and during 1 year follow-up

	GH group				Control group			
	Baseline		12 months		Baseline		12 months	
n	34		34		23		23	
Sex (m/f)	17/17		17/17		13/10		13/10	
Age (yrs)	6.3[1]	(3.4 to11.5)	7.3	(4.4 to 12.4)	3.6[1]	(1.6 to 5.2)	4.5	(2.6 to 6.1)
Height (SDS)	-2.1	(-2.9 to -1.3)	-1.1[24]	(-1.5 to -0.5)	-2.2	(-3.3 to -1.4)	-2.3[4]	(-3.5 to -1.8)
BMI (kg/m^2)	17.8	(16.0 to 24.0)	17.7	(15.7 to 22.5)	17.2	(15.9 to19.5)	17.9	(16.4 to 19.8)
BMI (SDS)	1.2	(0.0 to 2.3)	0.8[3]	(-0.4 to 1.7)	1.1	(-0.7 to 1.6)	1.3[3]	(0.1 to 1.6)
Head circ (SDS)	-0.8	(-1.5 to -0.2)	-0.1[23]	(-1.1 to 0.4)	-1.1	(-1.7 to -0.4)	-0.8[3]	(-1.2 to -0.1)
IGF-I (SDS)	-2.1	(-2.8 to -1.3)	2.2[24]	(1.4 to 2.8)	-1.6	(-2.6 to -0.8)	-2.6[4]	(-3.9 to -0.9)
IGFBP-3 (SDS)	-2.3	(-3.1 to -1.9)	0.3[24]	(-0.6 to 0.7)	-2.2	(-3.1 to -1.5)	-2.4[4]	(-3.6 to -1.8)

Data are expressed as median (interquartile range)

[1] p<0.05 Significantly different GH group vs. control group at baseline, after correction for multiple testing

[2] p<0.005 Significantly different 12 months vs. baseline, after correction for multiple testing

[3] p<0.05 [4] p <0.005 Change during follow-up GH group vs. control group, after correction for multiple testing

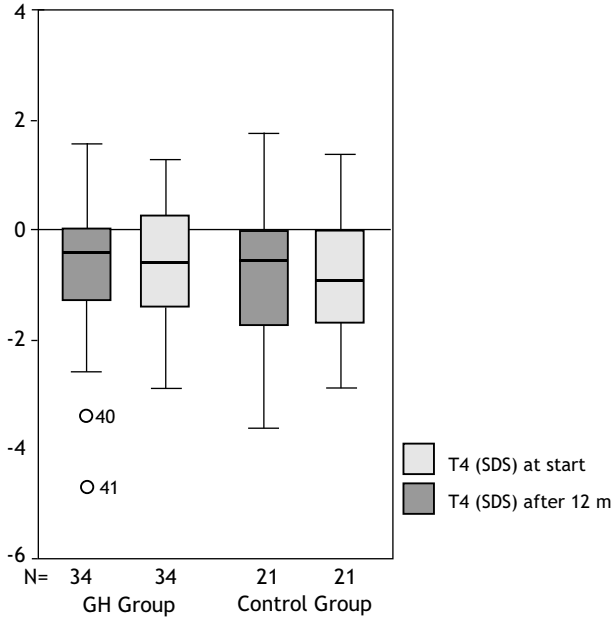
Table 3: Thyroid hormone levels at start and after 12 months of follow-up

	GH group				Control group			
	Baseline		12 months		Baseline		12 months	
T4 (nmol/l)	96.5	(84.5 to 110.3)	93.5	(82.8 to 102.3)	101.0	(87.0 to 118.0)	102.0	(93.0 to 107.0)
(SDS)	-0.4	(-1.3 to 0.1)	-0.6	(-1.5 to 0.3)	-0.6	(-2.1 to 0.1)	-0.9	(-1.8 to 0.0)
fT4 (pmol/l)	16.0	(14.5 to 17.7)	14.1	(13.4 to 16.4)	16.7	(14.6 to 17.8)	15.9	(14.0 to 17.9)
(SDS)	-0.8	(-1.3 to -0.4)	-1.4[1]	(-1.6 to -0.7)	-0.6	(-1.2 to -0.3)	-0.9	(-1.4 to -0.3)
T3 (nmol/l)	2.6	(2.4 to 3.0)	2.6	(2.4 to 2.9)	2.6	(2.3 to 3.1)	2.6	(2.3 to 2.8)
(SDS)	0.4	(-0.2 to 1.1)	0.3	(-0.1 to 0.8)	0.5	(-0.4 to 1.1)	0.1	(-0.4 to 0.5)
rT3(nmol/l)	0.33	(0.28 to 0.37)	0.25	(0.22 to 0.33)	0.36	(0.31 to 0.41)	0.30	(0.26 to 0.34)
(SDS)	0.0	(-0.9 to 0.6)	-0.7[1]	(-1.5 to -0.2)	-0.3	(-0.8 to 0.4)	-0.7	(-1.1 to -0.1)
TSH (mU/l)	1.8	(1.6 to 3.4)	1.8	(1.3 to 2.3)	2.3	(1.8 to 3.0)	2.2	(1.5 to 3.1)
(SDS)	-0.3	(-0.4 to 0.9)	-0.3	(-0.9 to 0.2)	0.1	(-0.3 to 0.6)	0.1	(-0.6 to 0.7)

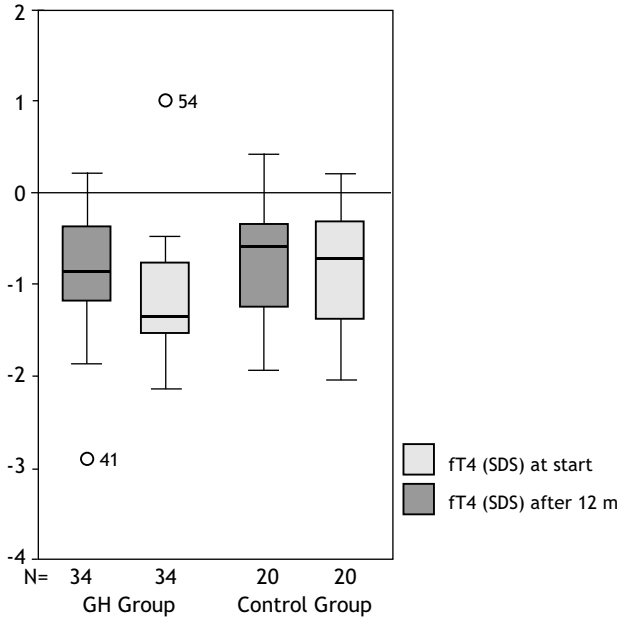
In GH group, rT3 was available in 32 patients at start and after 12 months of follow-up. In control group, fT4, T4, T3 and rT3 were available in 21 children at start of the study. After 12 months of follow-up fT4 and T3 were available in 22 children and rT3 in 19.
Data are expressed as median (interquartile range). At baseline there were no significant differences between both groups.
[1] $p<0.05$ Significantly different 12 months vs. baseline, after correction for multiple testing
No significant change during follow-up GH group vs. control group.

Thyroid hormone levels before and after one year of study are listed in Table 3 and Figure 1. At start of study, thyroid hormone levels were similar in both groups. After 1 year, fT4 levels had significantly decreased in the GH group compared with baseline, from -0.8 SDS (-1.3 to -0.4) to -1.4 (-1.6 to -0.7) ($p<0.05$), and had not changed in controls. In 3/34 GH-treated patients (8.8%) but also in 1/23 control patients (4.5%), fT4 levels were below –2 SDS after 1 year of follow-up. All these patients had, however, T3 levels well within the normal range (range -0.15 to 0.73 SDS). T3 SDS levels did not significantly change during 1 year of follow-up in both groups. Reverse T3 SDS decreased significantly in GH-treated children ($p<0.05$), but not in controls. We found no change in TSH during follow-up. TSH remained well within the normal range. The change in fT4 over 1 year was not significantly correlated with the change in IGF-I (r=-0.21, p=0.29 in the GH group; r=-0.10, p=0.76 in the control group).

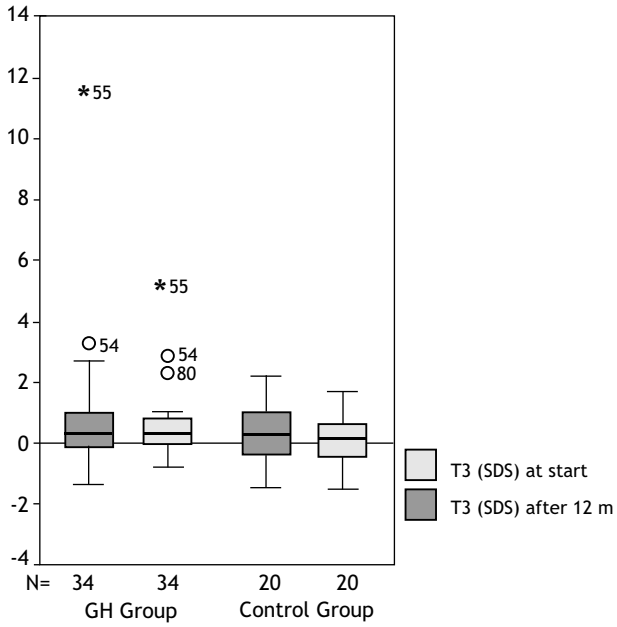
T4 levels



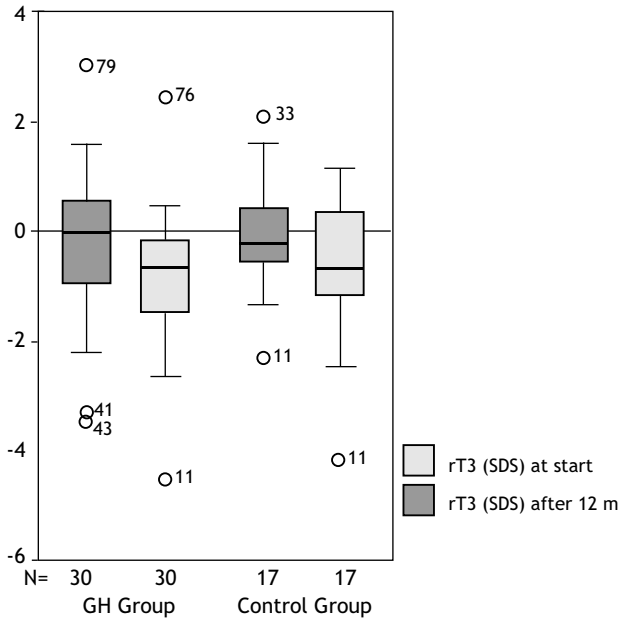
Free T4 levels



T3 levels



rT3 levels



TSH

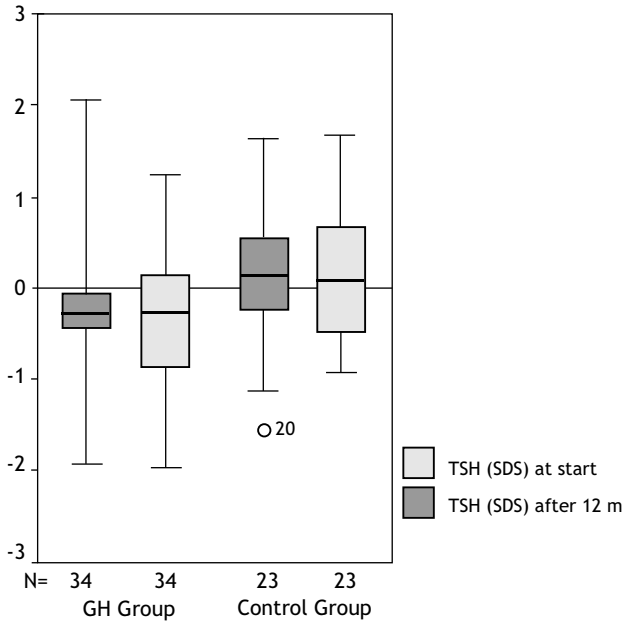


Figure 1 a-e: Figures represent levels of T4, fT4, T3, rT3 and TSH for GH-treated children with Prader-Willi Syndrome and for randomised controls, at start of the study and after 12 months. Lines represent zero SDS. Boxplots represent median and interquartile range.

7.5 Discussion

Our study shows that most PWS children have fT4 levels within the normal range, although significantly lower than 0 SDS. Five of 79 children had fT4 levels below -2 SDS. We found, however, normal T3 levels in all patients, with a median T3 even above the median of the reference population. Sex, height SDS and underlying genetic defect did not explain variations in serum thyroid hormone levels at baseline. T3 SDS correlated positively with head circumference, and T4 and rT3 levels with age and BMI SDS. Thyroid hormone levels did not correlate with IGF-I. After 1 year of study, fT4 levels had significantly decreased in GH-treated children from -0.8 SDS (-1.3 to -0.4) to -1.4 SDS (-1.6 to -0.7) but remained unchanged in controls. In 3/34 GH-treated patients (8.8%), fT4 levels decreased below the -2 SDS, but also in one of the control patients (4.5%). In contrast, T3 levels remained normal in all GH-treated children and controls.

Our finding that the baseline median fT4 level was significantly below 0 SDS might be consistent with the fact that most endocrine abnormalities in PWS are thought to be the result of hypothalamic dysfunction [3]. In line with this, Bray et al [19], showed that 5 obese, adult PWS patients, had a TRH-stimulated TSH response of about 2 SD higher than obese controls, however within the normal range. These findings indicate an alteration in the hypothalamic-pituitary control system for thyroid hormones in PWS.

We did not measure auto-antibodies to thyroglobulin or thyroid peroxidase. Because PWS is primarily a hypothalamic disorder, we expected low fT4 levels as a result of low hypothalamic stimulation. Indeed, we did find low-normal fT4 levels, in combination with completely normal TSH levels. We had no reason to suspect auto-immune abnormalities in the PWS children.

Increased peripheral conversion from fT4 to T3 might compensate for the relatively lower fT4 levels in most PWS patients. Recent in vitro studies showed that cells, transfected with deiodinase type 1 and type 2 expressing plasmids, and incubated with low fT4 concentrations had relatively more deiodinase type 2 activity compared to deiodinase type 1 activity, resulting in an increased fraction of T4 converted to T3 by deiodinase type 2 [20]. Furthermore, in the cortex of rats with low fT4 levels, T4 deiodination increases and D2 mRNA levels elevate, indicating that D2 in the central nervous system is also controlled by T4 status [21]. In addition, leptin was recently suggested to be a potential mediator in the conversion from T4 to T3 via increased deiodinase type 2 mRNA expression, which was shown after intracerebroventricular administration of leptin in rats [22]. Leptin is produced by adipocytes and correlates well with body fat percentage [23]. It has been shown that children with PWS have a relatively high body fat percentage, even when they are not obese [24,25]. They have high leptin levels, which are highly correlated with the increased body fat percentage [26]. Thus, the relatively lower fT4 levels, in combination with normal T3 levels, as shown in most PWS children, might be the result of an increased peripheral conversion by deiodinase type 2, as a result of a combination of high leptin levels and low fT4 levels.

Reports on serum thyroid hormone levels in PWS are very limited, and to our knowledge, T3 levels have not yet been reported in children with PWS [27]. In our study, 4 children were excluded from group analysis, because they received thyroxin treatment, based on low fT4 levels, prior to the study. One child in our study had a baseline fT4 level below -2 SDS, and 3 GH-treated patients and one control patient after one year of study, but all these children had normal T3 and TSH levels.

Although low fT4 levels are considered an important measure in the diagnosis of hypothyroidism, the combination of relatively low fT4 levels within the normal range, with normal or slightly elevated T3 levels and completely normal TSH levels in PWS children might not warrant start of thyroxin treatment.

We found a significant positive correlation between T3 and head circumference. Thyroid hormone plays an essential role in early prenatal brain development [28]. Higher total T4 and fT4 levels were associated with better overall performance on cognitive functioning in healthy, euthyroid men [29]. Brain growth during infancy and early childhood is suggested to be an important determinant of cognitive function [30]. The association between T3 and head circumference, as we found in our patients has, however, never been reported. Future studies are required, to evaluate if there is an association between head circumference, intelligence and thyroid hormone levels, particularly T3, in PWS.

We found no significant correlation between fT4 and T3 and BMI. Several studies reported an elevation of serum T4, T3 and TSH levels in obese versus non-obese healthy children and a decrease of T3 and T4 levels after weight reduction [31-36]. It might be that we found no correlation because most of our patients were not severely obese.

Concerning the effects of GH treatment, we found a decrease in median fT4 in GH-treated children, although levels remained well within the normal range for most children. There was no change in the randomised controls. However, T3 levels in our patients were completely normal, both before and during GH treatment. It has previously been reported that fT4 and TSH levels decrease during GH treatment in GH deficient children and adults [7-10,37-40]. Also in PWS children, thyroxin replacement therapy during GH therapy has been reported [11,12]. Several mechanisms have been proposed. Lippe et al demonstrated an inhibition of the TSH response to TRH during GH treatment and postulated that this might be mediated by somatostatin secretion in response to pulse doses of GH [7]. Others found a decrease of fT4 without changes in TSH, indicating an increased peripheral conversion from fT4 to T3 [10,37]. This effect might be mediated by either GH itself or via IGF-I, or both [10,40]. Jorgensen et al showed in GH deficient adults treated with GH a significant blunting of serum TSH levels and a stimulated peripheral conversion from T4 to T3 [9,38,39]. In our study, we found no change in TSH SDS, whereas the median T3 level was above 0 SDS after 1 year of GH, which might indicate an increased peripheral conversion by outer ring deiodination [41]. The response to GH treatment in terms of growth in our patients was impressive, as in patients with isolated idiopathic GH deficiency, who had during GH

a decrease in fT4 within the normal range [8]. Such a decrease during GH treatment, with normal T3 and TSH levels, might therefore not warrant substitution therapy.

Reverse T3 levels were normal before start of GH treatment, but decreased in GH-treated, and not in non-GH-treated PWS patients after one year of follow-up. Portes et al [10] have observed a similar decrease of rT3, in association with an increase of T3 levels and unchanged TSH values, during long-term GH therapy in non-PWS GH deficient children, but its significance remains to be established.

In conclusion, we found normal fT4 levels in most children with PWS. Five of 79 children had fT4 levels below -2 SDS. After 1 year of GH treatment, fT4 levels decreased significantly, but remained normal in most children. In contrast, baseline levels of T3 were normal or relatively high in all PWS children and remained so during GH treatment, indicating that in PWS children, peripheral conversion from T4 to T3 increases when fT4 levels become lower. To improve our knowledge, we recommend regular monitoring of fT4, T3 and TSH levels in PWS children, particularly during GH treatment.

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Chapter 8

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Adiponectin levels in pre-pubertal children with Prader-Willi Syndrome before and during growth hormone therapy

8.1 Abstract

Background: Children with Prader-Willi Syndrome (PWS) may have obesity and an abnormal body composition with a high body fat percentage, even if they have a normal body weight. Adiponectin has been inversely related to obesity and insulin resistance.

Objective: To evaluate in pre-pubertal PWS children (1) adiponectin levels, body composition, carbohydrate metabolism, and triglyceride levels, (2) associations between adiponectin and body composition, carbohydrate metabolism and triglycerides, and (3) effects of growth hormone (GH) treatment on these outcome measures.

Design/Patients: 20 pre-pubertal PWS children were randomised into a GH treatment group (n=10, 1 mg/m^2/day) and a non-GH-treated control group (n=10).
At baseline, after 1 and 2 years of GH treatment, fasting levels of adiponectin, glucose, insulin, and triglycerides were assessed. Body composition and fat distribution were measured by Dual Energy X-ray Absorptiometry.

Results: PWS children had significantly higher median (interquartile range) adiponectin levels; 17.1 mg/l (13.9 to 23.2), than healthy sex- and age-matched controls; 11.8 mg/l (9.7 to 12.5) ($p<0.005$). Body fat percentage was significantly higher than 0 SDS; 1.8 SDS (1.5 to 2.1) ($p<0.001$). Adiponectin levels were inversely related to triglyceride levels ($r=-0.52$, $p=0.03$). There was a tendency to an inverse relation with body fat percentage and BMI, but no correlation with fasting insulin or glucose levels, the insulin/glucose ratio or HOMA-index. During GH treatment, adiponectin levels increased significantly and did not change in randomised controls.

Conclusion: Adiponectin levels were increased, and inversely associated with triglyceride levels, in pre-pubertal, not overweight PWS children, although they had a relatively high body fat percentage. During GH treatment, adiponectin levels further increased, whereas no change was found in the controls, which is reassuring with respect to the development of insulin resistance during GH treatment.

8.2 Introduction

Prader-Willi Syndrome (PWS) is a genetic disorder, in the majority of subjects caused by either a microdeletion on the paternally derived chromosome 15q11-13 or a uniparental maternal disomy affecting the same region. In approximately 1-5%, PWS is the result of an imprinting center mutation, which results in silencing of genes that are normally active only in the paternally inherited chromosome 15q11-13 [1, 2]. PWS is characterised by a number of signs and symptoms, including hypotonia, psychomotor delay, short stature and obesity.

Children with PWS have an abnormal body composition, even when they have a normal body weight, with a relatively high body fat percentage and a low lean body mass (LBM) [3,4]. As a group, euglycemic PWS individuals have lower fasting insulin, and higher insulin sensitivity than a weight comparable group [5-9]. Suggested mechanisms for the higher insulin sensitivity are GH deficiency [10] and an abnormal body fat distribution compared to simple obesity, as described in PWS female adults [11]. In these PWS women, insulin sensitivity was higher and hepatic insulin extraction was increased compared to women with simple obesity. Nevertheless, a subgroup of PWS individuals does develop insulin resistance and the absolute prevalence of NIDDM in PWS subjects over the age of 20 years is high [12]. A familial component in insulin resistance, as in non-PWS subjects, might also contribute in PWS subjects [12].

Adiponectin is an anti-inflammatory adipocytokine that has been inversely related to adiposity and insulin resistance [13-16], and is thought to be protective with regard to diabetes mellitus and cardiovascular disease [17]. Adiponectin levels increase during weight-loss [18]. Adiponectin levels in obese adults with PWS are high, compared to simple obesity [19-22] and low [19, 22] or similar [21] compared to lean adult controls. So far, no published data concerning adiponectin levels in pre-pubertal, not severely overweight PWS children compared to normal-weight healthy controls are available.

Because body fat, more than body mass index per se is reported to be inversely related to adiponectin [23], we hypothesized that adiponectin levels would be low in PWS children compared to healthy sex- and age matched children, due to their expected relatively high body fat percentage, although the counteraction of their low insulin levels might result in normal adiponectin levels. GH treatment might have a dual effect on adiponectin levels. GH is known to decrease body fat percentage which would increase adiponectin levels. On the other hand, GH treatment has also been associated with lower insulin sensitivity [24]. We hypothesized that the effects of GH on insulin sensitivity would be superior, and therefore that adiponectin levels would decrease.

The aim of the present study was to evaluate (1) serum adiponectin levels, measures of body composition and body fat distribution, carbohydrate metabolism and triglyceride levels, (2) the associations between these parameters and (3) the effects of GH treatment on these parameters, compared with randomised controls in pre-pubertal, generally not overweight PWS children.

8.3 Patients and Methods

Patients

Twenty PWS children were evaluated for the present study. These children were all enrolled in a randomised controlled trial to investigate the effects of GH treatment on growth, body composition, activity level and psychosocial development. They fulfilled the following inclusion criteria: (1) genetically confirmed diagnosis of PWS; (2) age between 4 and 9 years at start of study; (3) pre-pubertal, defined as Tanner breast stage 1 for girls, and testicular volume less than 4 ml for boys [25], both before and during the study. Most children were regularly seen by a dietician and a physiotherapist. The caloric intake and activity level of all children were standardised at 3 months prior to study, and recommendations were given. Compliance to diet and exercise was evaluated by the research nurse, in close collaboration with the dietician and, if indicated, the physiotherapist. All children were naïve to GH treatment at start of study. They were included irrespective of their GH status. After stratification for age and BMI (<2 standard deviation score (SDS) vs. >2 SDS), children were randomised, into either the GH group or the control group. For 2 years, children of the GH group (n=10) were treated with Genotropin® (somatropin) /1 mg/m^2/d s.c. (Pfizer) (during the first 4 weeks they received 0.5 mg/m^2/day, to avoid fluid retention) and children of the control group (n=10) were not treated with GH. After 2 years, the control group also started GH treatment. At start of study, reimbursement for GH treatment in Dutch PWS children had not yet been approved. As there was a lack of controlled data with respect to GH treatment in PWS, a controlled study was considered ethical by the Dutch Advisory Board on GH in children and the Medical Ethics Committee, and the study protocol was approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam, The Netherlands. Written informed consent was obtained from the parents.

Methods

Anthropometry

At baseline and after 1 and 2 years of GH treatment or follow-up in the control group, standing height (or supine length, when appropriate) and weight were measured and BMI was calculated. Height and BMI were expressed as SD scores, adjusting for age and sex, according to Dutch reference data for children [26,27]. We defined overweight as a BMI >+2 SDS. BMI, height SDS, and BMI SDS were calculated with Growthanalyser, Version 3.0. (www.growthanalyser.org)

Body composition

Dual Energy X-ray Absorptiometry (DXA, type Lunar Prodigy, GE Healthcare) was performed in all children and total fat mass, fat percentage, LBM and fat of the trunk region were measured. The trunk region consisted of chest, abdomen and pelvis. The trunk fat to total fat ratio was considered as estimate for fat distribution. Fat mass, fat percentage and LBM were transformed into SD scores adjusting for sex and age, using Dutch reference values for children [28].

Collection of blood

Blood samples were collected in the morning after a 12h overnight fast, immediately centrifuged and stored at -20° until assayed.

Assays

Each assay was performed in the same laboratory. Serum glucose and triglyceride levels were assessed on Abbott Architect Clinical Chemistry Analyzer (Abbott Laboratories, Irving, TX, USA). The intra- and interassay coefficient of variation (CV) were respectively 0.7% and 0.8% for glucose and 0.5% and 0.6% for triglycerides. Serum insulin levels were measured by IRMA (Medgenix, Biosource Europe). Fasting normal range was <15 mU/l (manufacturer's information). The intra-assay CV was 2 to 4.7% and the inter-assay CV was 4.2 to 11.3%. The minimal detectable dose (MDD) was 1 mU/l, and no cross-reactivity with pro-insulin was observed. Assessment of insulin resistance was calculated using the homeostasis model assessment (HOMA) as previously described [29,30]. Serum adiponectin levels were assessed in duplicate by an enzyme-linked immunosorbent assay (ELISA; R&D Systems Inc. Minneapolis. USA). The intra-assay CV was <7 %, and the inter-assay CV was <7 %. The MDD was 0.246 ng/ml, and no significant cross-reactivity or interference was observed. Adiponectin control values were obtained from 40 healthy pre-pubertal children with normal height

and BMI (23 boys, 17 girls), aged 5.0 – 10.2 years, attending the outpatient clinic for a minor surgical procedure. Children suffering from any systemic illness, syndrome or dysmorphic feature were excluded. Normal height and BMI were defined as height and BMI between -2 SDS and +2 SDS, according to Dutch standards. The reference study was approved by the Medical Ethics Committee of Erasmus Medical Center, Rotterdam, The Netherlands and written informed consent was obtained from the parents. Serum insulin like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) were measured using a specific RIA in one laboratory [31]. The intra-assay CV was 4% and the inter-assay CV was 6%. The MDD for IGF-I was 6.0 ng/mL and for IGFBP-3 0.002 mg/L, and no significant cross-reactivity or interference was observed. For IGF-I and IGFBP-3, sex- and age-matched references were available obtained from a healthy Dutch population. This reference study was approved by the Medical Ethics Committee of Wilhelmina's Children's Hospital Utrecht, The Netherlands and written informed consent was obtained from the parents. Because of the age- and sex dependency IGF-I and IGFBP-3 levels were transformed into SD scores.

Statistics

Statistical analysis was performed by the Statistical Package for Social Sciences (SPSS), Version 11.0. Most of our data were not Gaussian distributed. We therefore expressed data as median (interquartile range, iqr) and we used non-parametric tests. Differences compared with baseline within groups were calculated using Wilcoxon signed rank test. Differences in changes compared with baseline between the GH group and the control group were calculated with Mann Whitney U tests. Afterwards, we used Bonferroni's correction for multiple testing for both inter- and intra-individual testing. Correlations were calculated by partial correlation coefficient, adjusting for age and sex. We compared the adiponectin levels of PWS children with reference data of healthy sex- and age matched controls (n = 40) with Wilcoxon signed rank test.

8.4 Results

Clinical characteristics

Clinical characteristics of the study group are listed in Table 1. All children had a genetically confirmed diagnosis of PWS. Six children had a paternal deletion, 5 had a uniparental maternal disomy, 2 had an imprinting center mutation, and in 7, PWS was confirmed by a positive methylation test, but the underlying genetic defect was

yet unknown. There was no significant difference between age, height or BMI of both groups at start of the study. The median (iqr) BMI at start of study was 0.8 SDS (0.1 to 1.2) in the GH group and 1.1 SDS (0.6 to 1.5) in the control group. Only two children were considered overweight, at start of the study, defined as BMI>+2 SDS (1 patient in the GH group and 1 in the control group). Median (iqr) height SDS increased significantly during the first and the second year of GH treatment from -2.2 (-3.1 to -1.8) to -1.3 (-1.7 to -0.8) ($p<0.05$) to -0.6 (-0.9 to -0.3) ($p<0.05$), but remained low in control group; from -2.8 (-3.4 to 7.8) to -2.8 (-3.5 to -2.0) to -3.0 (-3.5 to -1.8). BMI tended to decrease in the GH group during the first year, resulting in a significantly greater decrease in BMI SDS compared with baseline in the GH group than in the control group after 1 year of study ($p<0.05$).

Table 1: General clinical characteristics in GH Group and Control Group

	GH Group			Control Group		
	Baseline	**1 year**	**2 years**	**Baseline**	**1 year**	**2 years**
N (m/f)	10 (5/5)			10 (3/7)		
Age (yrs)	6.2 (5.1 to 7.1)	7.2 (6.1 to 8.1)	8.2 (7.2 to 9.1)	5.8 (4.9 to 7.8)	6.8 (5.9 to 8.8)	7.8 (6.9 to 9.8)
Height (SDS)	-2.2 (-3.1 to -1.8)	-1.3[1,3] (-1.7 to -0.8)	-0.6[1] (-0.9 to -0.3)	-2.8 (-3.4 to -2.0)	-2.8 (-3.5 to -2.0)	-3.0 (-3.5 to -1.8)
BMI (kg/m^2)	16.9 (15.8 to 17.7)	16.1[2] (15.2 to 17.6)	16.3 (15.8 to 19.0)	17.3 (16.4 to 19.3)	18.5 (17.6 to 19.3)	18.5 (17.5 to 20.6)
BMI (SDS)	0.8 (0.1 to 1.2)	0.2[2] (-0.2 to 0.8)	0.4 (-0.3 to 1.1)	1.1 (0.6 to 1.5)	1.3 (1.0 to 1.6)	1.2 (0.9 to 1.5)

Data are expressed as median (interquartile range)
[1]$p<0.05$, Compared with baseline *)
[2]$p<0.05$, [3]$p<0.01$ Change compared with baseline in GH group vs control group *)
*) Corrected for multiple testing

Adiponectin

Adiponectin levels are presented in Figure 1 and Table 2. At baseline, adiponectin levels were significantly higher in the total group of PWS children; 17.1 mg/l (13.9-23.2), compared to healthy age- and sex matched controls; 11.8 mg/l (9.7-12.5) ($p<0.005$). During GH treatment, median (iqr) adiponectin significantly increased from 15.9 mg/l (13.3 to 23.9) to 24.7 mg/l (15.0 to 25.9) ($p<0.05$) to 24.6 mg/l (15.4 to 28.2) ($p<0.05$) after 1 and 2 years, respectively. In the control group, adiponectin levels did not

change significantly during the study; from 17.1 mg/l (13.1 to 23.1) to 13.4 mg/l (11.6 to 21.4) to 15.8 mg/l (12.5 to 19.2). During 2 years, the increase in adiponectin was significantly greater in the GH group than in the control group ($p<0.05$).

Body composition

Body composition of the study groups is presented in Table 3. Median LBM increased significantly, during 2 years of GH treatment compared with baseline from -2.2 SDS (-2.7 to -2.0) to -1.6 SDS (-1.9 to -1.4) ($p<0.05$) to -1.2 SDS (-1.7 to -1.1) ($p<0.05$). In the control group, LBM decreased compared with baseline from -2.3 SDS (-2.8 to -1.8) to -2.5 SDS (-3.0 to -1.8) to -2.8 SDS (-3.3 to -1.9), ($p<0.05$ after 2 years). Body fat percentage decreased during the first year of GH treatment from 1.7 SDS (1.6 to 2.0) to 1.4 SDS (0.9 to 1.7) ($p<0.05$) and increased during the second year to 1.7 SDS (0.9 to 1.9). In contrast, in the control group, body fat percentage increased significantly over time from 1.8 SDS (1.5 to 2.4) to 2.1 SDS (1.8 to 2.2) to 2.1 SDS (1.9 to 2.4) ($p<0.05$ after 2 years). Trunk fat/total fat ratio tended to decrease compared with baseline during the first year of GH treatment ($p = 0.06$). After 24 months of GH treatment the trunk fat/ total fat ratio was not significantly lower than at baseline ($p=0.12$).

Fasting insulin, glucose and triglyceride levels

Median fasting insulin and glucose levels, the insulin glucose ratio, and the HOMA-index as a measure of insulin resistance (HOMA-IR) were within normal range for the total group (Table 2). The change after 1 and 2 years of study was not significantly different between the GH group and the control group. In the GH group, fasting insulin levels and insulin/glucose ratio increased significantly during the first year ($p<0.05$), but returned to baseline during the second year. In contrast, in the control group, fasting insulin levels, insulin/glucose ratio, and HOMA-IR had significantly increased after 2 years compared with baseline ($p<0.05$). All children of the GH group had insulin and glucose levels, insulin/glucose ratio, and HOMA-IR within the normal range during the entire study. In contrast, 3 children of the control group had fasting insulin levels above the upper limit of normal after 2 years of study (range 19 to 31 mU/l). Fasting triglycerides at start were above normal range in 3/20 (15%) children (2 children of the GH group and 1 of the control group). Of the 2 overweight PWS children at start of study, one had increased serum triglycerides.

Table 2: Fasting glucose, insulin, triglyceride and adiponectin levels in GH Group and Control Group

		Baseline		1 year		2 years	
GH	Adiponectin (mg/l)	15.9	(13.3 to 23.9)	24.7[1,2]	(15.0 to 25.9)	24.6[1,2]	(15.4 to 28.2)
Group	Glucose (mmol/l)	4.8	(4.6 to 5.0)	4.4	(4.2 to 5.0)	4.6	(4.2 to 5.0)
	Insulin (mU/l)	6.0	(3.8 to 10.0)	9.01	(6.5 to 13.5)	7.5	(6.0 to 11.5)
	Insulin glucose ratio	1.3	(0.8 to 2.1)	2.11	(1.5 to 2.6)	1.6	(1.5 to 2.2)
	HOMA-index	0.8	(0.5 to 1.3)	1.2	(0.8 to 1.8)	1.0	(0.7 to 1.5)
	Triglycerides (mmol/l)	0.9	(0.7 to 1.7)	0.8	(0.6 to 1.3)	0.7	(0.6 to 0.8)
	IGF-I (SDS)	-1.7	(-2.2 to -1.2)	2.3[1,3]	(1.6 to 3.0)	2.3[1,3]	(2.1 to 2.9)
	IGFBP-3 (SDS)	-2.0	(-3.0 to -1.3)	0.5[1,3]	(-0.1 to 1.0)	0.6[1,3]	(0.4 to 1.1)
Control	Adiponectin (mg/l)	17.1	(13.1 to 23.1)	13.4	(11.6 to 21.4)	15.8	(12.5 to 19.2)
Group	Glucose (mmol/l)	4.4	(4.3 to 4.7)	4.6	(4.3 to 4.8)	4.7	(4.3 to 4.9)
	Insulin (mU/l)	5.5	(4.8 to 7.3)	6.0	(3.3 to 8.3)	11.01	(6.0 to 24.0)
	Insulin glucose ratio	1.3	(1.0 to 1.6)	1.3	(0.8 to 1.9)	2.31	(1.4 to 2.2)
	HOMA-index	0.7	(0.6 to 0.9)	0.8	(0.4 to 1.0)	1.41	(0.8 to 3.0)
	Triglycerides (mmol/l)	0.7	(0.6 to 1.0)	0.6	(0.5 to 1.0)	1.0	(0.6 to 1.0)
	IGF-I (SDS)	-1.7	(-2.9 to -1.0)	-2.5	(-3.2 to -0.8)	-2.0	(-2.7 to 1.0)
	IGFBP-3 (SDS)	-2.5	(-3.2 to -1.5)	-2.4	(-3.8 to -1.9)	-1.8	(-2.7 to -1.5)

Data are expressed as median (iqr).
[1]$p<0.05$ Compared with baseline *)
[2]$p<0.05$, [3]$p<0.001$ Change compared with baseline in GH Group vs Control Group *)
*) Corrected for multiple testing

IGF-I and IGFBP-3 levels

IGF-I and IGFBP-3 levels were low compared to normal in our PWS children. During 2 years of GH treatment, IGF-I SDS increased significantly from -1.7 (-2.2 to -1.2) to 2.3 (1.6 to 3.0) ($p<0.05$) to 2.3 (2.1 to 2.9) ($p<0.05$) and IGFBP-3 SDS from -2.0 (-3.0 to -1.3) to 0.5 (-0.1 to 1.0) ($p<0.05$) to 0.6 (0.4 to 1.1) ($p<0.05$), but IGF-I and IGFBP-3 remained low in the control group.

Table 3: Body composition in GH Group and Control Group

		Baseline		1 year		2 years	
GH	LBM (SDS)	-2.2	(-2.7 to -2.0)	-1.6[1]	(-1.9 to -1.4)	-1.2[1]	(-1.7 to -1.1)
Group	Fat mass (SDS)	0.8	(0.6 to 1.0)	0.5	(0.2 to 1.0)	0.9	(0.4 to 1.4)
	% fat (SDS)	1.7	(1.6 to 2.0)	1.4[1]	(0.9 to 1.7)	1.7[1]	(0.9 to 1.9)
	Trunk fat/total fat	0.44	(0.34 to 0.47)	0.40	(0.33 to 0.42)	0.41	(0.34 to 0.46)
Control	LBM (SDS)	-2.3	(-2.8 to -1.8)	-2.5	(-3.0 to -1.8)	-2.8[1]	(-3.3 to -1.9)
Group	Fat mass (SDS)	0.8	(0.6 to 1.2)	1.1[1]	(0.9 to 1.2)	1.2[1]	(0.9 to 1.4)
	% fat (SDS)	1.8	(1.5 to 2.4)	2.1	(1.8 to 2.2)	2.1[1]	(1.9 to 2.4)
	Trunk fat/total fat	0.40	(0.35 to 0.46)	0.41	(0.40 to 0.44)	0.41	(0.38 to 0.45)

Data are expressed as median (interquartile range)
[1]$p<0.05$, Compared with baseline, corrected for multiple testing

Table 4: Partial correlation coefficients (r) and p-values between serum adiponectin levels and the various parameters, adjusted for age and sex at baseline.

Parameter	r	p-value
Triglycerides	-0.52	0.03*
Body fat % (SDS)	-0.46	0.07
BMI (SDS)	-0.44	0.08
Trunk fat/Total fat	-0.32	0.21
LBM (SDS)	-0.20	0.45
Glucose	0.02	0.95
Insulin	-0.07	0.78
Insulin glucose ratio	-0.10	0.71
HOMA index	-0.10	0.71

p-value <0.05 is considered statistically significant.

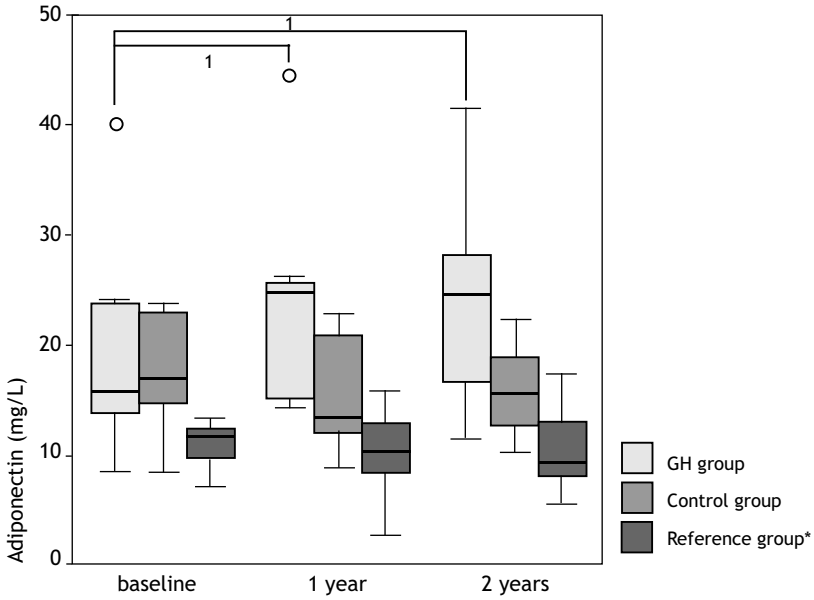

Figure 1: Adiponectin levels in PWS children and controls. Boxplots represent adiponectin levels during study. The lower boundary is the 25th percentile, and the upper boundary the 75th percentile. The line in the box represents the median. Lines are drawn from the smallest to the largest observed value that is not an outlier. Open circles are outliers.

[1] $p < 0.05$ adiponectin levels compared with baseline levels.

Changes in adiponectin levels from baseline are significantly greater in the GH group compared with the randomised PWS control group after 12 ($p<0.001$) and after 24 months ($p<0.001$).

PWS children had significantly higher adiponectin levels than healthy sex- and age matched controls ($p<0.005$).

*Healthy sex-, and age-matched controls.

Associations between adiponectin and body composition, triglyceride levels, and carbohydrate metabolism

Results of correlations between serum adiponectin levels, body composition and body fat distribution, carbohydrate metabolism and circulating triglycerides, after adjustment for age and sex, are listed in Table 4. There was a significant inverse relation between adiponectin and triglyceride levels ($r=-0.52$, $p=0.03$). Although there was a tendency towards inverse relationships between adiponectin and BMI and body fat percentage, these did not reach statistical significance (p-value 0.08 and 0.07, respectively). Notably, no association was found between adiponectin levels and the

HOMA-IR or insulin/glucose ratio (r=-0.10, p=0.71). The increase in adiponectin levels after 2 years of follow-up, was strongly inversely correlated with the change in body fat percentage (r=-0.81, p<0.001), but there was no significant association between the increase in adiponectin levels and the change in triglyceride levels.

8.5 Discussion

Our study demonstrates high serum adiponectin levels in young, pre-pubertal, generally not overweight children with Prader-Willi Syndrome, compared to healthy sex- and age-matched controls. Serum adiponectin levels related inversely with triglyceride levels. There was a tendency to an inverse relation between adiponectin and body fat percentage and BMI, but no significant association between serum adiponectin levels and fasting insulin and glucose levels, insulin/glucose ratio, and HOMA-IR. During GH treatment, serum adiponectin levels increased significantly, compared with no change in the control group. The increase in adiponectin levels was strongly, inversely correlated with the change in body fat percentage. Fasting insulin and glucose levels, as well as insulin/glucose ratio, increased significantly during the first year of GH treatment, but returned to baseline levels during the second year. In the control group, fasting insulin, insulin/glucose ratio and HOMA-IR significantly increased over 2 years compared with baseline.

This is the first report showing that young, pre-pubertal, not severely overweight PWS children have high serum adiponectin levels compared to healthy sex- and age-matched controls. As the reported prevalence of NIDDM in individuals with PWS is high [12], and body fat percentage in PWS is relatively high, we expected relatively low adiponectin levels. In previous studies in obese adults with PWS, serum adiponectin levels were high compared to obese non-PWS controls [19-22], but compared to lean adult controls, adiponectin levels were low [19,22] or similar [21]. Previous studies showed that, in PWS children and adults, insulin sensitivity is high [6,10], which might be due to a relatively low amount of visceral fat compared to obese controls [11]. We postulate that, in addition to a relatively low amount of visceral fat, high adiponectin levels might contribute to the increased insulin sensitivity in PWS children.

We found no significant association between adiponectin levels and BMI and body fat percentage, although there was a tendency towards an inverse relationship. In healthy subjects, a negative association has been described [13], but nowadays it is concluded that low adiponectin levels are more closely related to the degree of insulin resistance and hyperinsulinemia than to the degree of adipositas [16]. Also Hoybye et al reported the absence of a relationship between adiponectin and BMI in adult PWS patients [22]. Our study did not show an association between adiponectin levels and trunk/total fat mass as measured by DXA. This was in contrast to our expectations, because previous studies showed that adiponectin concentrations correlated stronger with central adiposity than with overall adiposity [32-34]. The trunk/total fat mass ratio is however not a validated procedure for measuring visceral adipose tissue, which might explain the absence of the relation. It might also be that, particularly in PWS subjects trunk/total fat is not a good measure for visceral fat. It has been shown in PWS women [11], that they had low visceral fat, as measured by MRI, whereas measures of waist diameter, waist-hip ratio, waist/height ratio, or trunk/limb skinfold were similar to obese controls. This is most likely due to the fact that in PWS subjects particularly the subcutaneous trunk fat, instead of the visceral fat is high. These data strongly ask for more accurate measurements by MRI of visceral fat vs. subcutaneous fat in PWS.

We found a significant inverse association between serum adiponectin and triglyceride levels in young PWS children. This is in line with findings in healthy obese and non-obese adolescents [35]. It might well be that adiponectin modulates insulin sensitivity by diminishing the accumulation of triglycerides in the skeletal muscle tissue, as was found in animal studies [36].

We did not find a significant correlation between baseline adiponectin levels and insulin/glucose ratio, or HOMA index, which is in contrast to previous findings in obese adults with PWS [20]. This discrepancy might be explained by the fact that insulin resistance and overweight were not yet present in our young PWS children. In healthy children, adiponectin correlated with body weight, BMI, waist, fasting glucose and 30 min insulin, but the correlations were positive in boys and negative in girls, indicating that plasma adiponectin is not a simple marker of central fat and insulin sensitivity in children [37]. In our study, we did not find such sex differences (data not shown).

During the first year of GH treatment, adiponectin levels increased, and remained at the same level during the second year. Also body fat percentage, LBM and IGF-I levels changed, particularly during the first year. Based on these findings, the increase of adiponectin levels during GH treatment might reflect the decline in body

fat percentage during GH treatment compared with the control group. The increase in LBM during GH treatment might also be related to the increased adiponectin levels. As skeletal muscle is the primary tissue of insulin-stimulated glucose uptake, disposal and storage [38], the increased LBM might contribute to the increased insulin sensitivity.

Insulin levels were normal at start of the study. During the first year of GH treatment, insulin and glucose levels did increase, but levels returned to baseline after 2 years. The high adiponectin levels in PWS children may contribute to the relatively low insulin levels, since adiponectin is suggested to be protective against insulin resistance and diabetes mellitus [14,15]. The high IGF-I levels after 2 years of treatment, indicate that children were compliant to GH treatment.

In conclusion, adiponectin levels are increased in young, pre-pubertal, not severely overweight PWS children. This is in line with the relatively high insulin sensitivity in PWS. Adiponectin levels are inversely related to serum triglyceride levels. There is a non-significant tendency to an inverse relation with body fat percentage and BMI, and no relation with carbohydrate metabolism. GH treatment resulted into a significant increase in serum adiponectin levels compared with the control group, which is particularly important, because adiponectin is considered protective for cardiovascular disease and insulin resistance. Our results are therefore reassuring with respect to the development of insulin resistance during GH treatment in PWS children.

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Chapter 9

General Discussion

This thesis describes several aspects of a large cohort of generally non-severely obese children with genetically confirmed diagnosis of Prader-Willi Syndrome (PWS). We evaluated several aspects of the clinical picture of PWS, such as anthropometry and body composition, sleep-related breathing, cognition, behaviour, psychomotor development, thyroid hormone levels, adiponectin levels, and the effects of growth hormone (GH) treatment on these aspects. In this chapter, results of this study are compared and discussed in view of current literature. Subsequently, clinical implications and directions for future research are given.

9.1 Growth and body composition and effects of growth hormone treatment

We evaluated height, body composition, and body proportions in 89 pre-pubertal PWS children, prior to and during 2 years of the study. Children were treated with GH 1 mg/m^2/day or followed as randomised controls for 1 year (infants <3.5 years) or 2 years (pre-pubertal children >3.5 years). Height, head circumference, length of foot and tibia, span width, and sitting height were all significantly lower than 0 SDS prior to start of GH treatment. Body mass index (BMI) was higher than 0 SDS, and body composition was abnormal, with a high body fat percentage and low lean body mass (LBM), after correction for age and sex (LBM_{age}) or for height and sex (LBM_{height}).

We found a complete normalisation of median SD scores of height, BMI, and head circumference after 2 years of GH. Body fat percentage, length of foot, tibia, span width, and sitting height improved but did not completely normalise (Figure 1). LBM_{age} improved in GH-treated patients, compared with randomised controls. LBM_{height} did not improve in GH-treated children, but did decrease in randomised controls, indicating that GH is beneficial for LBM in PWS children.

This is the first study, including a control group for 2 years. Previous studies were not controlled for more than one year [1-3]. Those studies reported mostly obese PWS children. Nowadays, rapid diagnostic testing is available, and diagnosis is usually confirmed within the first months of life, whereas extreme obesity can often be prevented by a strictly controlled diet. In our experience, most PWS patients are nowadays not severely overweight during childhood.

To our opinion, the most important metabolic effect of GH is the improvement of LBM. Low LBM in children with PWS most likely reflects low muscle mass. Low muscle mass may be responsible for the clinical hypotonia in young PWS infants and for exercise intolerance in PWS children and adults. Muscle mass is metabolically more active. Low muscle mass might therefore contribute to decreased energy expenditure that

has been reported in PWS [4,5]. It has been shown that LBM progressively decreases with age [6]. It is remarkable that previous controlled GH studies in PWS, described effects of GH on LBM in absolute increase [2,3]. LBM is sex- and age dependent, and should therefore be expressed compared to children with similar age and sex. However, expressing LBM in SDS adjusting for age and sex, does not take into account that LBM may be underestimated in children with short stature. Additionally, during GH, the increased growth velocity may result in an overestimation of the GH effect on LBM. This is of major importance because we are particularly interested in the additional effect of GH beyond the increased growth velocity. This was first pointed out by Eiholzer et al [7]. These authors showed that LBM after correction for height (LBM_{height}), did not significantly increase during GH, indicating that the increase in LBM is growth related, and that the initial deficit of lean mass cannot be compensated for by GH therapy. However, this was a non-controlled study in 12 overweight patients. Our controlled study is the first one to show that: (1) during longitudinal follow-up, LBM_{height} progressively decreases in non-GH-treated PWS subjects; (2) there is no significant effect of GH on LBM_{height}, during 2 years of follow-up compared with baseline, but there is a significant difference in LBM_{height} compared with the untreated control group, because the latter decreased LBM_{height} over 2 years. Thus, our results therefore indicate that, even when taking into account the height dependency of LBM, GH treatment has a beneficial effect on LBM.

We found a strong correlation between BMI and height SDS at baseline. This is in favour of the hypothesis that growth in PWS is the result of 2 opposite mechanisms: a lower growth velocity as a result of GH deficiency, which is counteracted by an increased growth velocity, as found in children with simple obesity [8]. In non-syndromal obesity, GH levels are usually decreased whereas other mechanisms are suggested to promote growth velocity, such as increased insulin levels, and decreased Insuline-like growth factor (IGF) binding protein (BP)-1 levels, resulting in an increase in free IGF-I, and consequently in its biological activity. A third proposed mechanism is via increased leptin levels [9]. In vitro studies, using mice mandibular condyle, demonstrated that leptin administration resulted in proliferation and differentiation of chondrocytes within the growth center. In addition, leptin administration resulted in increased expression of the IGF-I receptor in the growth center, suggesting that the effect of leptin may, at least partially be mediated via local IGF-I [10]. Because insulin levels in PWS children are decreased compared to normal children [11], and circulating IGF-I levels are low due to GH deficiency [11], we postulate that the association between BMI and height SDS is mediated via leptin. It has been shown that leptin in

PWS, as in the normal population, is highly correlated with body fat percentage [12], and in children with PWS, body fat percentage is high [6].

IGF-I levels increased during GH treatment, from levels below the normal range to levels above normal range in most GH-treated PWS children, in some children to extreme levels of 5 SDS. This is in line with previously reported IGF-I levels in PWS children [2,13,14], indicating that they are highly sensitive to GH treatment in terms of IGF-I response. IGFBP-3 also increased, but to levels completely within the normal range, resulting in an increase in the IGF-I/IGFBP-3 ratio. This suggests that free IGF-I, which is believed to represent the biologically active IGF-I [15], had particularly increased during GH. According to the study protocol, IGF-I levels were assessed retrospectively in our study. Based on our findings, however, we do recommend to monitor IGF-I levels in PWS children, and titrate the GH dose in order to maintain IGF-I levels below 3 SDS.

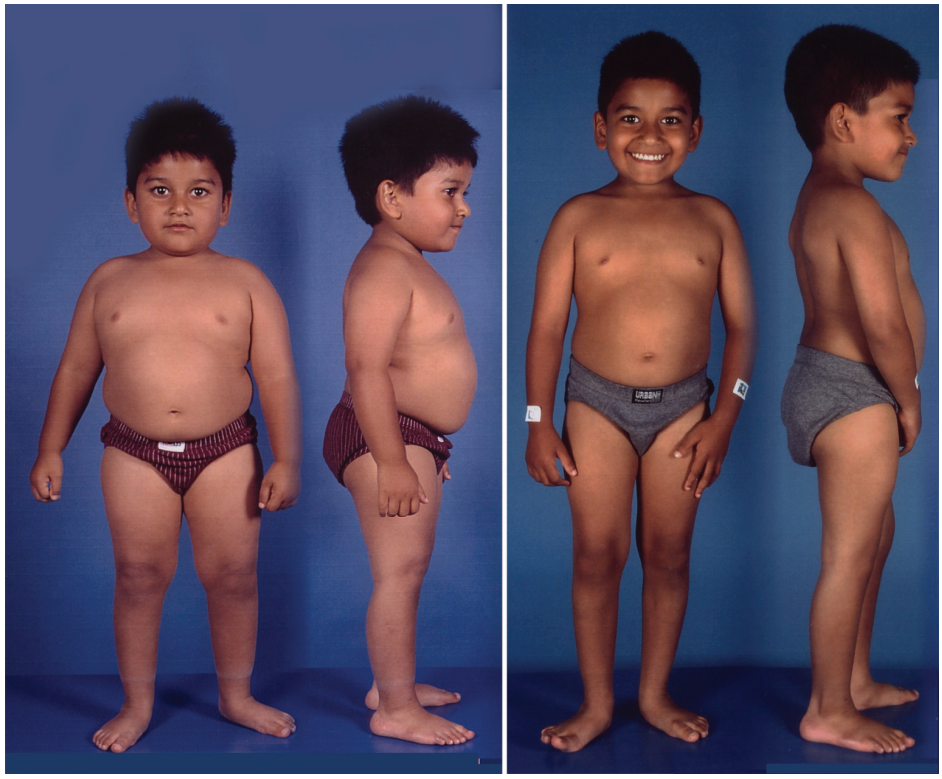

Figure 1: Boy with PWS, before and 1 year after start of GH treatment *(with permission)*.

- GH treatment normalises height, BMI and head circumference
- GH treatment improves, but does not normalise body fat percentage and body proportions
- LBM_{height} did not significantly improve during GH, but did decline in the control group, resulting in an improvement of LBM in GH-treated children compared with randomised controls
- IGF-I levels increased to levels above normal range in most children, indicating that PWS children are highly sensitive to GH
- We recommend monitoring of IGF-I levels in order to titrate the GH dose and maintain IGF-I levels below 3 SDS

9.2 Psychomotor development in PWS infants

In 1992, a diagnostic genetic test has been developed (PW71 methylation test), which provides the possibility of rapid diagnosis of PWS in newborns and infants with hypotonia [16]. This method has been validated for PWS infants and is nowadays the method of choice [17]. Together with the increased awareness of neonatologists and pediatricians with the clinical picture of PWS in infancy, the diagnosis is now usually confirmed early in life. The question was therefore raised whether it might be beneficial to start GH treatment in infancy.

We performed a randomised, controlled GH trial in PWS infants evaluating the effects of both mental and motor development. This study was carried out in collaboration with the Swedish group (Dr. A.C. Lindgren, Dr. B. Böhm), to enlarge the sample size. We used the Bayley Scales of Infant Development, which is a widely used and well-validated method for evaluating psychomotor development. Reference data for healthy infants are available [18].

The PWS infants scored below the low-normal range, particularly for motor development. We therefore expressed mental and motor development as developmental age, and we calculated the percentage of expected development by dividing the developmental age by the chronological age. Our data show that mental development significantly improved after 1 year of GH compared with randomised controls. This improvement was accompanied by a normalisation of head circumference in the GH group, and not in the control group. Our data did not reveal a significant correlation between the change in head circumference and the change in mental development during follow-up, although there was a non-significant tendency to a positive correlation. It might be that the sample size was not large enough to find such an

association. Recently, it has been demonstrated in the general population, that brain growth during infancy and early childhood is more important than growth during foetal life in determining cognitive function [19]. This study in 221 healthy children demonstrated that full scale IQ at the age of 9 years increased by 1.98 points (95% confidence interval, CI 0.34 to 3.62) for each SD increase in head circumference at the age of 9 months and with 2.87 points (95% CI 1.05 to 4.96) for each SD increase in head circumference at the age of 9 years, after adjustment for many confounding factors. In this perspective it might be that early normalisation of head circumference in our PWS infants may be related to the improvement of mental development in the GH-treated infants compared with randomised controls.

Parallel to the increase in mental development, we found a rapid increase in IGF-I levels in our patients from levels below the normal range prior to start of GH to levels above the upper limit of normal during GH, comparable to levels reported by others [13]. As IGF-I receptors have been localized in several areas of the human brain, these may have a neuroregulatory role in the central nervous system [20]. Theoretically, GH treatment might thus affect mental development via IGF-I, either by a direct influence on the central nervous system or GH might induce local IGF-I expression in brain tissue.

A third explanation might be that mental development improved as a consequence of the GH effects on motor development. Early acquisition of abilities such as sitting, standing and walking may independently improve mental development because the child is more capable of exploring and interacting with his environment. Our study indeed demonstrated that also motor development significantly improved during GH treatment. If children were subdivided according to their motor abilities before start of study, the motor improvement of GH-treated children compared with controls was only significant in children with less motor abilities at start of study. It has been suggested that the improvement of motor development is the result of the increased muscle mass during GH treatment, but we could not find such an association. This might be due to a lack of age-, height- and sex adjusted reference data for DXA results in this age group.

An increase in LBM in GH-treated PWS infants has been reported in 2 previous studies, one of which also adjusted for the GH-induced increase in height [13,21], but this study did not include a control group of similar age. Only one controlled study evaluated effects of GH on psychomotor development in PWS infants [13,22]. This study demonstrated an improvement of mobility (the ability to move the body from one position to another), but not stability during GH treatment compared with randomised

controls, only if GH was started before the age of 18 months (13). In addition, an improvement of cognition (as measured with tests) and language (as reported by parents) was found in 7 GH-treated PWS infants compared with 5 randomised controls [22].

Our data suggest that GH should be considered in an early developmental stage in order to optimize the effects of GH on motor development.

- GH improves both mental and motor development in PWS infants, compared with randomised controls
- If GH is started at an early developmental age, infants might benefit with respect to motor development

9.3 Sleep related breathing disorders in children with PWS

The prevalence of sleep-related breathing disorders in children and adults with PWS is difficult to establish based on the literature, due to different patient selection (including adults or children, with or without genetic confirmation of PWS, with a different degree of obesity, or selection, based on symptoms indicating breathing abnormalities). In addition, many different methods for evaluating sleep-related breathing disorders have been used. Pulmonary function tests, ventilatory and arousal response tests during hypercapnic, hyperoxic and hypoxic challenges, and polysomnography (PSG), with many different definitions of apneas, or oxygen desaturation have been reported, which make comparison difficult.

We evaluated the prevalence of sleep-related breathing disorders with PSG prior to start of GH treatment in 53 pre-pubertal children, who were not severely overweight. Only 8 children (15%) had a BMI over +2 SDS and could therefore be considered overweight.

The most striking finding of our study was, that all children had increased central sleep apneas. These apneas were generally not accompanied by oxygen desaturation and the median duration of the longest measured apnea was 15.0 sec. Our finding is in line with recent findings of others and suggests a primary disturbance of the central respiratory control mechanism [23-25]. A hypothalamic origin of sleep-related breathing disorders in PWS was already postulated 20 years ago [26]. A decreased number of oxytocin neurons, the putative satiety neurons in the hypothalamic paraventricular nucleus was reported and was suggested as the basis of the insatiable appetite and obesity in PWS patients [27]. Oxytocin neurons might also be involved

in neural modulation of breathing and might play a role in central sleep apnea in PWS [28]. Mack et al described the projections of oxytocin-containing neurons from the hypothalamic paraventricular nucleus to the phrenic nuclei and to the pre-Bötzingercomplex in the rostral ventrolateral medullary region in rats (Figure 2), which is involved in respiratory rhythm generation [28]. Recently, Ren et al [29] proposed that necdin (neurally differentiated embryonal carcinoma-cell derived factor) deficiency may contribute to the observed respiratory abnormalities in individuals with PWS. Necdin is one of the protein-coding genes which are lacking in PWS [30]. Deficiency of necdin in mice results in neonatal hypoventilation, which is usually fatal [30]. Ren et al demonstrated that this effect can be explained by abnormal neuronal activity within the putative respiratory rhythm-generating center, the pre-Bötzinger complex. The authors suggested that the developing respiratory center is particularly sensitive to the loss of necdin activity and may reflect abnormalities of respiratory rhythm-generating neurons. Interestingly, a previous study investigating Necdin deficient mice, showed hypothalamic and behavioural alterations which resembled human Prader-Willi syndrome, including a decrease of oxytocin-producing neurons in the hypothalamus compared to wild type mice [31].

In contrast to our expectations, obstructive sleep apnea syndrome (OSAS) was relatively uncommon in our PWS patients and for the total group, no correlation between OSAS and BMI was found. However, 50% of the patients with BMI > 2 SDS had OSAS, whereas of the other patients only 8% had OSAS. It seems therefore that OSAS is related to obesity in PWS, as in the general population. Several mechanisms have been proposed for the association between OSAS and BMI. The increased fat deposition around the upper airway may compress the upper airway, which may contribute to increased pharyngeal collapsibility as has been shown in obese adults [32-34]. This is supported by the finding that after weight loss, pharyngeal adipose tissue volume decreased together with a reduction in apneas and hypopneas [33]. OSAS is also associated with increased efforts of accessory respiratory muscles, which are the result of an increased amplitude of out-of-phase respiratory movements of the thorax and the abdomen [34]. Furthermore, chest wall compliance may be decreased, due to increased weight load of the thorax, whereas the obese abdomen displaces the diaphragm, particularly in the supine position, resulting in a small lung volume. This may lead to narrowing of the upper airway, as the pharyngeal cross-sectional area has been described to directly parallel changes in lung volume in normal men [35]. In PWS, as in the general population, increased BMI has been associated with obstructive hypoventilation, decreased oxygen saturation (SaO_2) and increased apnea

hypopnea index (AHI) [36]. Moreover, weight-loss in children and adults with PWS improved OSAS and hypoventilation [36].

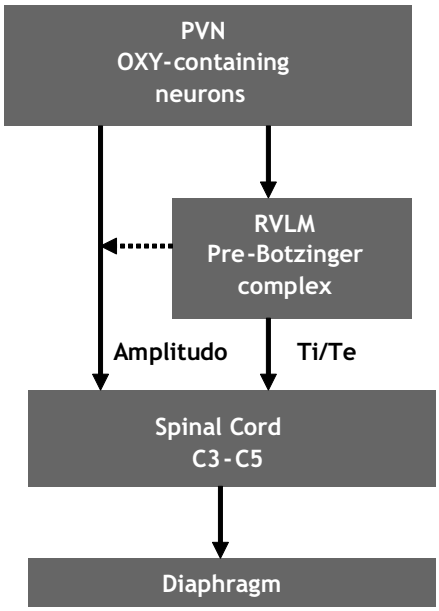

Figure 2: Schematic representation of the neural pathways involved in the oxytocin-related regulation of respiratory output. PVN=paraventricular nucleus, OXY=oxytocin, RVLM=rostral ventrolateral medulla, Ti=inspiratory time, Te=expiratory time. *Adapted from* [28].

In addition to the relation with BMI, other possible mechanisms for OSAS in PWS should be considered. In healthy children, adenotonsillar hypertrophy is the most common cause of OSAS [34,37]. Also in PWS children, with or without obesity, adenotonsillar hypertrophy may contribute to OSAS, and adenotonsillectomy has been shown to improve OSAS in both obese and non-obese PWS children [38]. In addition, PWS children may have a smaller naso- and hypopharynx. Richards et al demonstrated in PWS children, during radiological assessment performed in supine posture, a reduction in the cross-sectional area, compared to normal control subjects, at the oropharyngeal level in 9 out of 14 subjects and at the nasopharyngeal level in 4 out of 14 subjects [39]. The relatively small naso- and oropharynx may contribute to the obstruction caused by adenotonsillar hypertrophy in PWS subjects. Sticky saliva and muscular hypotonia may contribute to OSAS as well. In addition, OSAS has been described in non-PWS patients with severe kyphoscoliosis [40]. In PWS, scoliosis is

common, and may be severe in some cases [41]. Scoliosis may therefore also add to OSAS, in a subgroup of PWS patients.

- Pre-pubertal, non-severely overweight PWS children have sleep-related breathing disorders, mainly of central origin
- Obstructive sleep apnea is rare, but is more common in children with overweight

Does GH treatment induce or aggravate sleep-related breathing disorders?

Since October 2002, several reports of unexpected death in infants and children with PWS during GH treatment were published [42-45], most of them related to a complicated course of a relatively mild respiratory tract infection, sleep apnea, adenoid and/or tonsil hypertrophy, hypoventilation and aspiration, or related to obesity. At first, a causal relationship between GH and unexpected death in PWS was suggested [42,43,46]. Two mechanisms were proposed by which GH could be related to unexpected death during the initial phase of therapy. The incidence of OSAS might increase as a result of hypertrophy of adenoid and/or tonsils mediated via increased serum IGF-I levels due to GH, as it had been reported that sleep apnea, both obstructive and central, occurred more frequently in adults with GH excess (acromegaly) [47]. One of the proposed mechanisms for OSAS in acromegaly was thickening of upper airway soft tissue bulk. A decrease of IGF-I in acromegalic patients was correlated with a decrease in soft tissue swelling of the tongue, as measured with MRI, and it was suggested that improvement of sleep apnea was mediated via a reduction of soft tissue bulk [48]. Although it was proposed that GH treatment resulted in an increase in tonsil size [49], to our knowledge, no controlled, prospective studies on this topic are available. The second explanation proposed an augmentation of volume load, because short-term GH therapy might cause sodium retention with secondary water retention due to prostaglandin-mediated activation of the renine-angiotensin-aldosterone system, which resolves after continuation of GH, and via increased distal nephron sodium and water reabsorption [50]. This has been reported for GH-treated adults.

We therefore performed PSG prior to and after 6 months of GH treatment. Our data did not show any change in sleep-related breathing abnormalities compared with baseline. Importantly, no increment of obstructive apneas was found.

Our study is the largest controlled PSG study in PWS children, and our results are in line with other studies, evaluating the effects of GH on breathing disorders in PWS [3,51-53]. Lindgren et al found improved CO_2-responsiveness, resting ventilation and airway occlusion pressure in 9 children with PWS, aged 7-14 years, after 6-9 months of GH compared with baseline [52]. Myers et al demonstrated that inspiratory and expiratory muscle strength improved in 20 children with PWS, aged 4 to 16 years, after 12 months of GH compared with 10 controls [3]. Haqq et al reported in a double-blind placebo-controlled cross-over study a decrease in AHI after 6 months of GH in 12 PWS children, aged 4.5 to 14.5 years, although the difference compared with controls was not statistically significant. In addition, various components of pulmonary function improved [51]. Very recently, Miller et al reported the AHI on polysomnography after 6 weeks of GH treatment in a mixed group of 25 adults and children. They reported a decrease in most patients, a subset of patients, however, had an increased AHI with more obstructive events after 6 weeks of GH. Most of these patients had upper respiratory tract infections and adenoid/tonsil hypertrophy during the second evaluation [53]. The mechanism by which GH improved sleep apnea and CO_2 responsiveness is yet unknown. A direct effect of GH or IGF-I, via GH and IGF-I receptors has been postulated. Improved weight control and increased respiratory muscle strength during GH treatment may also play a role.

Notably, 5 patients had adenotonsillectomy between the first and the second PSG. It cannot be excluded that this confounds our results, but for obvious safety reasons we could not avoid this.

GH treatment does not aggravate the pre-existing sleep-related breathing disorders in PWS children

Effects of illness on sleep-related breathing disorders

We did not aim to evaluate sleep-related breathing during illness, but we had the opportunity to measure 4 patients with PSG during an episode of URTI. We found a dramatic increase in total AHI in these patients, with a particular increase in obstructive apneas. Results of one of the patients are shown in Figure 3: A 3-year old girl was evaluated with PSG prior to start of GH treatment (AHI was 7.9/h, all central apneas). After 6 months of GH, she had an upper respiratory tract infection and adenoid hypertrophy, and AHI increased to 38.6/h, including many obstructive apneas/hypopneas. GH was continued, adenoidectomy was performed and PSG after

recovery showed an AHI of 3.4/h (all central apneas). Our results are in line with those of Miller et al [53]. In our study, one boy died unexpectedly. He was 3 years old and had GH treatment for 13 months. He responded very well in terms of growth and body composition. PSG was performed before and 6 months after start of GH. His PSGs were nearly normal (AHI 1.7/h and 1.4/h, respectively), and BMI and tonsil size were well within the normal limits. He had a mild URTI and was clinically evaluated by his pediatrician the day prior to his death. At that time he was in a good condition, running around and not generally ill. During the night, he suddenly deteriorated and was found dead in the morning. Autopsy did not reveal the cause of death. Thus, a relatively normal PSG does not exclude a complicated course of relatively mild respiratory tract infection, or even unexpected death.

The consequences of the respiratory muscle weakness itself could also be severe. When the maximum expiratory pressure is reduced to less than 40 cmH_2O, as has been found in 7/35 PWS patients (including both adults and children), patients can no longer breathe deeply or cough effectively [54]. It was postulated that thoracic muscle weakness could result in a decreased ability to clear airway secretions, predisposing patients to pulmonary infections, and airway obstructions. Another contributing factor that warrant alertness in PWS is temperature instability in infants or altered temperature sensitivity in older children and adults, which is one of the supportive findings for the clinical diagnosis of PWS [55]. Many patients will not present with fever during respiratory tract infection, which may result in an underestimation of disease severity.

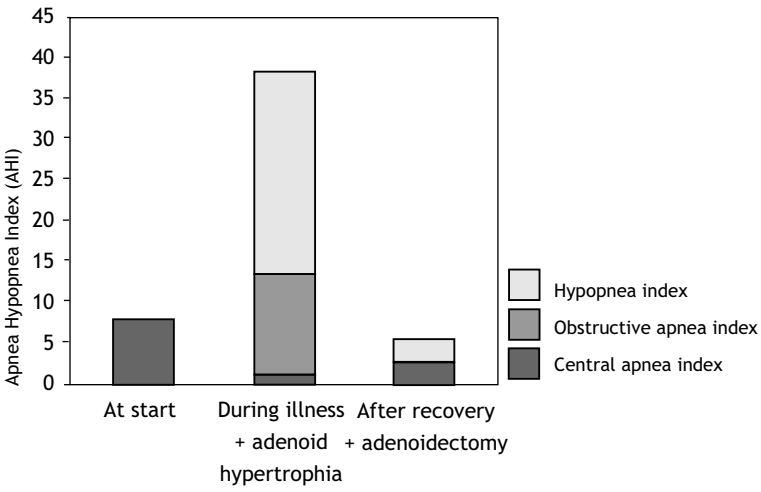

Figure 3: Sleep related breathing during illness and adenoid hypertrophy

- During illness the apnea hypopnea index increased dramatically and obstructive sleep apnea may be present
- Normal PSG during health does not exclude sudden deterioration during mild upper respiratory tract infection

9.4 Sleep-related breathing, psychomotor development, cognition, and neurobehavioural abnormalities

In the general population, associations between sleep-related breathing disorders and cognition, school performance, and psychiatric and behavioural co-morbidities are consistently reported [56-58]. Also in individuals with PWS, OSAS is associated with some behavioural disturbances, such as autistic relating behaviour, and impulsiveness [59]. Beebe and Gozal proposed a model by which cognitive and neurobehavioural abnormalities in sleep-related breathing disorders are caused by sleep disruption, intermittent hypoxia and hypercapnia, leading to disruption of restorative features of sleep and cellular and chemical homeostasis which results in frontal cortical dysfunction. Dysfunction of the frontal cortex is considered to impair the cognitive executive functioning, which is the ability to develop an approach to problem situations, resulting in neurobehavioural abnormalities, such as over-activity and impulsivity [60] (Figure 4).

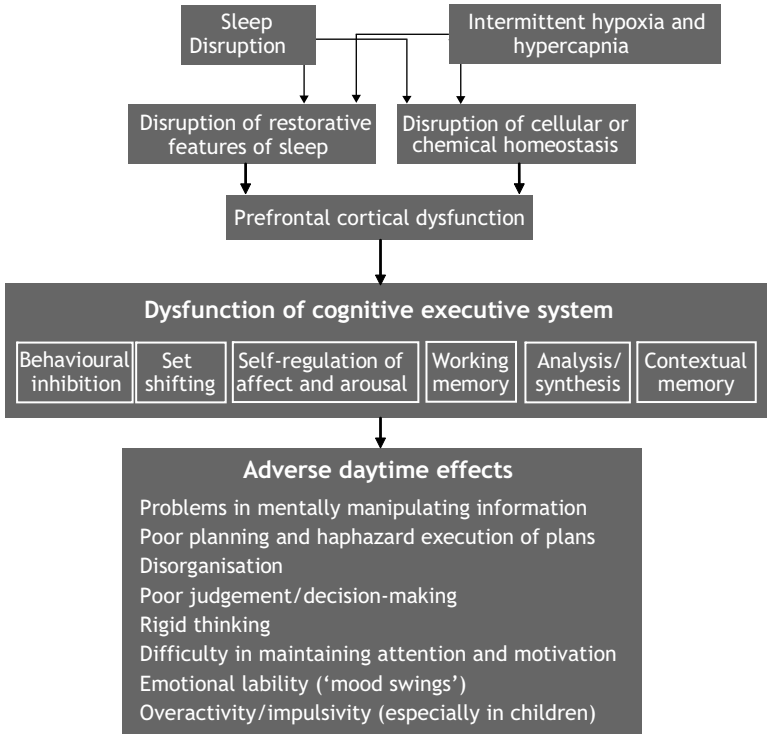

Figure 4: Model proposed by Beebe and Gozal for the association between sleep-related breathing disorders and cognitive and behavioural problems. *Adapted from* [60]

Are sleep-related breathing disorders related with mental or motor development in PWS infants and toddlers?

A recent study in healthy infants indicated that higher snoring-related arousal indices, being a measure for mild obstruction during sleep, were associated with lower scores on tests for mental development [61].

We investigated if there was an association between sleep-related breathing, as measured with PSG, and the delay in psychomotor development, as measured with Bayley Scales of Infant Development in PWS infants and toddlers.

For the total group, we did not find an association between the total AHI and scores of mental or motor developmental tests. However, when we subdivided the group in infants with or without OSAS, we found that children with OSAS had significantly lower mental development. The children with OSAS tended to be older, to have a higher BMI and to have a lower motor development, although those differences did not reach statistical significance.

This is the first study reporting an association between mental development and OSAS in young PWS children. Our results indicate that OSAS in young PWS children should be treated early, in order to prevent further delay in mental development.

- OSAS in young infants and toddlers is associated with lower mental development
- Our results indicate that OSAS in PWS should be treated early in order to prevent further delay in mental development

Are sleep-related breathing disorders related with cognitive deficits and neurobehavioural abnormalities in pre-pubertal PWS children?

We evaluated cognition using a short form of age-appropriate Wechsler intelligence scales, which is validated and widely used for assessment of intelligence in children. Neurobehavioural abnormalities were evaluated using two parent questionnaires for children with mental disabilities: Developmental Behaviour Checklist (DBC) and VISK (Vragenlijst voor Inventarisatie van Sociaal gedrag bij Kinderen). The latter is a widely used Dutch questionnaire to evaluate particularly social behaviour in children. Dutch reference data are available for children with several degrees of mental disabilities for both tests. We related the results on cognition and behaviour to the results of sleep-related breathing as measured with PSG before start of GH treatment.

Our study demonstrated that pre-pubertal PWS children have overall impaired cognitive abilities compared to healthy children. Interestingly, the impairment was not equally distributed between verbal and performance subtests. Sixty percent of children scored significantly better on verbal subtests compared to performance subtests. Although we could not find a relation with the genetic defects, it might be that the relatively high number of patients with uniparental maternal disomy (UPD) in our study group contributes to this finding, because previous studies have shown that verbal IQ is higher in PWS patients with UPD compared to those with deletions [62]. In addition, compared to children with similar mental abilities, PWS children have less anxiety-related behavioural problems and more autism-related social problem behaviour, particularly on the subscales “emotions and behaviour not adapted to the social situation” and “insensitive to social information”. Notably, compared to the mentally disabled reference population, disruptive/antisocial behaviour (including “impulsiveness” and “temper tantrums”) is not commonly reported. The relatively high incidence of autistic like social behaviour might also be due to the relatively high

incidence of UPD in this group. Previous studies showed that UPD is associated with autistic-like social behaviour [63].

We found a positive association between sleep efficiency index, as measured with PSG, and one of the performance subtests (Picture arrangement/completion), after adjustment for age, sex and different type of test. One suggestion is that better sleep efficiency, with less sleep disruption results in better executive functioning, according to the model proposed by Beebe and Gozal [60] (Figure 4). PWS patients may have an increased need for nocturnal sleep [64]. Sleep efficiency might be improved by behavioural management strategies when PWS children are put to bed as has been suggested by Nixon et al [65]. Future studies are needed to evaluate whether improvement of sleep efficiency might contribute to better cognitive functioning in PWS children.

In our study, children with more severe sleep-related breathing abnormalities had less severe behavioural abnormalities. This is neither in line with the general population [66], nor with the only previous report in PWS [59]. In the latter study in a group of 13 PWS patients, aged between 1.5 and 28 years, including 6 children below the age of 12 years, autistic like behaviour was associated with low sleep efficiency and oxygen desaturation and impulsivity with sleep-related breathing disorders. The difference between this study and our results might be related to patient selection. Our study group consisted of pre-pubertal non-severely obese PWS children, in which obesity-related OSAS is uncommon.

In the model proposed by Beebe and Gozal [60] (Figure 4), sleep disruption and intermittent hypoxia and hypercapnia induce the cascade of frontal dysfunction. One explanation why we did not find an association between sleep-related breathing disorders (SRBD) and behaviour is that children with PWS have a higher arousal treshold in response to hypercapnia [25].

We cannot explain why we found an inverse association between sleep-related breathing disorders and neurobehavioural abnormalities. It might be that parents of high functioning PWS children compared their children to healthy peers, whereas parents of lower functioning PWS children compared them to other children with similar mental disability. Most likely, sleep-related breathing and neurobehavioural abnormalities are not causally related in PWS, as both may have a different genetic cause. Sleep-related breathing disorders have been associated with Necdin deficiency [29] and autistic like social behaviour has been associated with the UBE3A gene (Ubiquitin protein ligase gene 3A) [67,68]. UBE3A is maternally expressed in a few specific areas, which have been implicated in the neuropathology of autism, such as the

hippocampus and the cerebellar Purkinje cells [67]. UBE3A is located on chromosome 15 in the PWS/Angelman Syndrome critical region, and paternally imprinted. In patients with maternal UPD, UBE3A is therefore over-expressed. In particular autistic like social impairment is more common in patient with UPD compared to patients with paternal deletion [68], which might be attributed to the overexpression of UBE3A. We therefore suggest that, in contrast to the general population, in PWS SRBD and cognitive/neurobehavioural deficits are not causally related, but both the result of the genetic defect in PWS.

- We found a positive relationship between sleep efficiency and one of the performance subtests, and between behavioural problems and daytime sleepiness, suggesting that improvement of nocturnal sleep and daytime sleepiness may improve cognition and behaviour in PWS children
- We did not find a positive association between sleep-related breathing disorders and neurobehavioural abnormalities

9.5 Thyroid hormone levels

Hypothalamic dysfunction in PWS may result in several endocrine abnormalities including GH deficiency, and hypogonadotropic hyopogonadism [69]. The thyroid hormone axis, however, was not yet extensively investigated in PWS children. Previous studies in small patient groups suggested normal thyroid hormone function or minor alterations in the hypothalamo-pituitary-thyroid axis in adults with PWS, mostly overweight and with a clinical diagnosis of PWS without genetic confirmation [70,71]. During GH treatment in non-PWS GH deficient patients, a decrease of fT4 has been reported. This might be due to either unmasking of an underlying TSH deficiency, or to increased peripheral conversion from fT4 to T3 [72-76]. Our study is the first study to prospectively evaluate thyroid hormone function in a large group of relatively young, non-severely overweight PWS children. Four patients received thyroxin replacement therapy because of low fT4 levels prior to start of study. We found that fT4 was within the normal range in almost all patients, but significantly below 0 SDS. Surprisingly, T3 levels were significantly higher than 0 SDS, suggesting an increased peripheral conversion from T4 to T3. T3 is the more active thyroid hormone and increased conversion might compensate for the relatively lower fT4 levels in most PWS patients.

Recently, an in vitro model was developed in which human deiodinase type 1 and deiodinase type 2 were transiently expressed to compare their different catalytic efficiency for converting T4 to T3. These authors showed that at lower fT4 concentrations, deiodinase type 2 was relatively more active than deiodinase type1, and that higher fT4 levels inhibited the activity of deiodinase type 2, but not type 1 [77]. In the cortex of rats with low fT4 levels, T4 deiodination, and D2 mRNA levels increased. This indicates that also in the central nervous system, the expression of deiodinase type 2 is regulated by T4 status (78). In addition, leptin was recently suggested as a potential mediator in the conversion from T4 to T3 via increased deiodinase type 2 mRNA expression, which was shown after intracerebroventricular administration of leptin in rats [79]. Leptin is an adipocytokine which is highly correlated with body fat percentage [80]. Children with PWS have a relatively high body fat percentage, even when they are not obese [6,81]. They have high leptin levels, correlating with their body fat percentage [12]. Thus, we postulate that the relatively lower fT4 levels, in combination with normal T3 levels, as we found in most PWS children, might result from an increased peripheral conversion by deiodinase type 2, as a result of a combination of high leptin levels and low fT4 levels.

Recently, Butler et al showed in 47, mostly adult PWS subjects, that 2.1% (= 1/47) had hypothyroidism (defined as low fT4, low T4 and normal TSH levels), which is similar to the expected frequency of 2% for the normal population. The authors therefore concluded that there is most likely no general hypothalamic pituitary dysfunction in PWS with respect to the thyroid axis [82]. The incidence of hypothyroidism in our study is higher (6.3% prior to start of study). This may be the result of the lower prevalence of overweight in our study, which included mostly non-obese patients. Obese patients as described by Butler may have upregulation of their thyroid hormone levels, probably reflecting the reset of their central thyrostat at a higher level [83]. This might counteract mild hypothyroidism in obese PWS subjects.

During GH treatment, fT4 decreased significantly compared with baseline, although levels remained within the normal range in most children. T3 levels, however, remained completely normal, indicating an increased peripheral conversion from T4 to T3. PWS children, who needed thyroid replacement therapy during GH therapy had been reported previously [2,3,84], and although thyroid hormone levels are within the normal range in most of our patients, and low fT4 levels are compensated by increased T3 levels, monitoring of thyroid hormone levels in PWS before and during GH should be recommended. It is not yet established whether the combination of low fT4 levels and completely normal T3 levels warrants start with thyroxin replacement therapy.

- Free T4 levels are relatively low in pre-pubertal children with PWS, although for most children, within the normal range. Surprisingly, T3 levels are significantly higher than 0 SDS, indicating a compensation of relatively low fT4 levels by increased peripheral conversion from T4 to T3
- During GH treatment, fT4 further decreased, but remained within the normal range for most patients. It remains to be established whether low-normal levels of fT4 with completely normal, or even elevated levels of T3 warrants thyroxin replacement therapy
- Monitoring of thyroid hormone levels before and during GH is indicated

9.6 Adiponectin levels and cardiovascular risk factors

Adult patients with PWS have a high prevalence of diabetes mellitus type 2, associated with BMI, and with a median age of onset of 20 years [85]. However, as a group, euglycemic PWS patients (some studies including children) have lower fasting insulin levels and a relatively lower amount of visceral fat than weight comparable control subjects [70,86-90]. During GH treatment of non-PWS children, increased insulin resistance has been reported. Adiponectin is highly and specifically expressed in differentiated adipocytes and circulates at high levels in the bloodstream [91]. Adiponectin is an anti-inflammatory adipocytokine, which is inversely related to adiposity, and to insulin resistance [92-95]. Adiponectin is considered protective against diabetes mellitus and insulin resistance [96]. At the time we started the GH study, it was not known whether adiponectin in PWS patients was low (due to their relatively high total body fat percentage) or high, because visceral fat is relatively low, and because insulin sensitivity is relatively high amongst euglycemic PWS children. It was also unclear whether insulin sensitivity would increase during GH treatment (due to the lipolytic effects of GH treatment) or decrease (due to increased insulin resistance during GH).

Our data showed that adiponectin levels were high in pre-pubertal non-severely overweight PWS children compared to normal weight healthy controls, prior to start of study. In addition, adiponectin levels were highly and inversely correlated with triglyceride levels. The latter is in line with findings in healthy obese and non-obese adolescents [97]. Interestingly, in a mouse model with lipo-atrophy and complete absence of white adipose tissue and subsequently of adiponectin, circulating free fatty acids and triglycerides, as well as triglyceride levels in skeletal muscle and liver were elevated. Treatment of these mice with adiponectin increased the expression of

molecules involved in fatty acid transport and combustion in skeletal muscle, which resulted in a decreased triglyceride content in the muscle and lower serum free fatty acids and triglyceride levels [98]. It might well be that adiponectin modulates insulin sensitivity by diminishing the accumulation of triglyceride levels in the muscle and by decreasing serum free fatty acids and triglyceride levels.

During follow-up, adiponectin levels increased in GH-treated children, compared with randomised PWS controls. This might be explained by altered body composition, during GH treatment. Body fat percentage decreased during GH treatment, and adiponectin is considered inversely associated with body fat [99]. LBM, on the other hand, increased and might independently contribute to higher insulin sensitivity, by increasing the insulin-dependent glucose uptake, storage and disposal in the skeletal muscle [100]. In addition, a recent in vitro study showed that GH increases adiponectin gene expression directly in a dose dependent matter through the JAK2-P38 MAP kinase pathway, suggesting a direct effect of GH on adiponectin expression [101]. Another report showed that GH is also the positive regulator of the newly identified adiponectin receptors, suggesting that regulation of the adiponectin pathway might represent an important aspect of GH actions [102] (Figure 5).

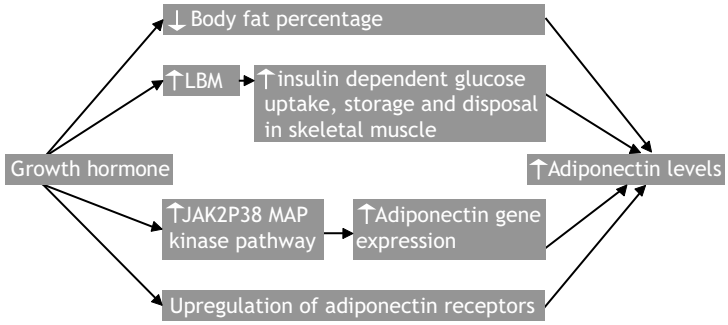

Figure 5: Hypothetical model summarizing possible mechanism for the increased serum adiponectin levels during GH treatment in PWS children

In contrast, in conditions with GH-excess, such as in acromegalic patients, serum adiponectin levels are reduced, and as such probably involved in the induction of insulin resistance, generated by these conditions [103]. The finding of increased adiponectin levels during GH treatment is particularly important, because adiponectin is considered protective against cardiovascular disease and insulin resistance. Our results are therefore reassuring with respect to the development of insulin-resistance during GH in PWS.

- Adiponectin levels are increased in PWS children compared to healthy controls
- During GH treatment, adiponectin levels increased, indicating that GH treatment does not induce insulin resistance in PWS children

9.7 Clinical implications and considerations for future research

GH is beneficial for improving height, body composition, and body proportions in PWS children. In addition, both mental and motor development is better in GH treated children compared with their randomised controls. These data suggest that GH treatment should be considered in PWS children, from 6 months or older. It remains to be established whether early start with GH, influences cognition later in life.

According to the study protocol we evaluated IGF-I levels, after 1 year of GH treatment and we found that PWS children were highly sensitive to GH with respect to IGF-I response. Based on our data, we do recommend to monitor IGF-I levels, during GH treatment, particularly in PWS children, to keep circulating IGF-I within reasonable limits (below 3 SDS).

Our study did not find a negative effect of GH on thyroid hormone levels or adiponectin levels. PWS children may have a higher risk of (subclinical) hypothyroidism. They are at risk of developing insulin resistance if they would become extremely overweight, which is intrinsic to PWS. Therefore monitoring of endocrine and metabolic aspects is of major clinical importance, before and during GH.

Our study shows that most children with PWS have sleep-related breathing disorders and that in young infants, OSAS is associated with lower mental development. This indicates that screening for sleep-related breathing disorders by PSG should be considered in all PWS children. If OSAS is present, particularly in young infants, this should be treated in order to prevent deterioration of mental development. If adenoid and/or tonsils are enlarged, adenoidectomy and/or tonsillectomy should be considered. It is also important to introduce weight control, particularly when obesity is already present in infancy. If OSAS is still present despite adequate weight control and adenotonsillectomy, the use of nasal continuous positive airway pressure might be considered [104].

With regard to safety monitoring, it is particularly important that future research will focus on sleep-related breathing during illness. There is need for guidelines for parents and pediatricians, what to do if a PWS child becomes ill with or without respiratory tract infections. The possible role for home monitoring of sleep-related

breathing (during illness) remains to be established. Meanwhile, parents should be strongly cautioned about the risk of sleep-related breathing disorders during times of upper respiratory tract infections.

No studies are available with respect to final height in PWS children. GH is indicated, only for children with PWS, irrespective their endogeneous GH secretion. The aim is to improve growth velocity and body composition. When epiphysial fusion is complete and final height is reached, GH treatment has to be discontinued. Preliminary studies have now showed that also PWS adults might benefit from GH treatment [105-107], especially with regard to body composition, and psychosocial wellbeing. The optimal duration of treatment or dosage has, however, not yet been established. Further research should focus on the effects of GH treatment during the transition period from reaching final height until approximately 24 years. The effects of GH on weight, body composition, psychosocial functioning, carbohydrate metabolism, circulating lipids, and respiratory function in young adults with PWS needs to be investigated.

9.8 References

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Chapter 10

Summary

This doctoral thesis describes the results of the Dutch multicenter, randomised growth hormone (GH) trial in children with Prader-Willi syndrome (PWS). This study investigated several aspects of PWS, such as anthropometry, psychomotor development, cognition, behaviour, sleep-related breathing disorders (SRBD), thyroid hormone levels, and adiponectin levels and effects of GH on these outcome measures.

Chapter 1

This chapter provides an overview of the prevalence, etiology and clinical picture of the PWS, including several endocrinological aspects associated with PWS. It also provides a summary of previously reported data on the effects of GH treatment in PWS. At the end of this chapter, the aims and outlines of this thesis are presented as well as the study design, and in- and exclusion criteria of the study.

Chapter 2

Children with PWS have impaired growth, and an abnormal body composition, with a high body fat percentage and a low lean body mass (LBM). Previous one-year controlled studies demonstrated that GH treatment resulted in improved growth and body composition in PWS. These studies, however, described relatively small patient groups of -mostly overweight- PWS patients. We performed a randomised controlled GH trial in 91 pre-pubertal PWS children (42 infants, 49 children aged 3-14 years) to evaluate growth, body composition, and body proportions. Infants were randomly assigned to GH treatment (GH group; 1 mg/m^2/day; n=20), or no treatment (control group; n=22) for 1 year. Children >3yrs, were randomly assigned to GH treatment (GH group; 1 mg/m^2/day; n=27) or no treatment (control group; n=22) for 2 years. We performed anthropometric assessments yearly, and body composition (in children >4 years) was measured by Dual Energy X-ray Absorptiometry (DXA). After 1 and 2 years of GH treatment, height, body mass index (BMI) and head circumference completely normalised. Body composition, after adjustment for age and sex, and body proportions improved, but did not completely normalise during GH. LBM, adjusted for height and sex, did not increase in the GH group, but decreased in the control group, resulting in a better outcome for GH-treated children. Serum insulin-like growth factor I (IGF-I) increased to supra-physiological levels in most children. In conclusion, 2 years of GH in PWS children normalises height, BMI and head circumference, and improves body composition, and body proportions. PWS children are highly sensitive to GH, suggesting that monitoring of serum IGF-I is indicated.

Chapter 3

Previous GH studies in PWS have mainly focused on anthropometry and body composition. Data on the effects of GH on psychomotor development in infants with PWS are limited. Therefore we evaluated psychomotor development before and after 1 year of GH treatment in PWS children. Forty-three children were evaluated at baseline. Twenty-nine of them were randomised into the GH group (n=15) or to a non-GH-treated control group (n=14). At baseline and after 1 year of follow-up, we used Bayley Scales of Infant Development (BSID II) for assessment of psychomotor development. Data were converted to percentage of expected development for age (%ed), and changes during follow-up were calculated. Median age was 2.3 years (interquartile range (iqr) 1.7 to 3.0) for the GH group and 1.5 years (iqr 1.2 to 2.7) for the control group (p=0.17). Compared with randomised controls, both mental and motor development improved significantly during the first year of study in the GH group. Median (iqr) change in mental development was +9.3% (-5.3 to 13.3) vs. -2.9% (-8.1 to 4.9) ($p<0.05$), and change in motor development was +11.2% (-4.9 to 22.5) vs. -18.5% (-27.9 to 1.8) ($p<0.05$), for the GH group and the control group, respectively. Children with a lower motor developmental age at start of study, improved more during GH treatment, compared with randomised controls, than children with a higher motor developmental age at start. In conclusion, 1 year of GH treatment resulted in significant improvement of both mental and motor development in PWS infants, compared with randomised controls.

Chapter 4

Several cases of unexpected death have recently been reported in both GH-treated and non-GH-treated young children with PWS. These unexpected deaths were often related to a complicated course of upper respiratory tract infections (URTIs). Some clinicians suggested a causal relationship between GH treatment and unexpected death. Data concerning effects of GH on respiratory parameters are, however, limited. We therefore evaluated respiratory parameters in 53 pre-pubertal PWS children (30 boys) before and during GH treatment. We performed complete polysomnography (PSG) before start with GH treatment and repeated PSG 6 months after start of GH treatment in 35 of them. Median (iqr) age at baseline was 5.4 (2.1 to 7.2) years and BMI was +1.0 standard deviation score (SDS) (-0.1 to 1.7). Apnea Hypopnea Index (AHI) was 5.1/h (2.8 to 8.7) (normal range 0 -1/h). Of these, 2.8/h (1.5 to 5.4) were central apneas and the rest mainly hypopneas. Median duration of longest apneas was 15.0 sec (13.0 to 28.0). AHI did not correlate with age and BMI, but central apneas decreased

with age (r=-0.34, p=0.01). During 6 months of GH treatment, AHI did not significantly change, from 4.8 (2.6 to 7.9) at baseline to 4.0 (2.7 to 6.2) (p=0.36). One patient died unexpectedly during a relatively mild URTI although he had a nearly normal PSG both prior to and during GH treatment. In conclusion, PWS children have a high AHI, mainly due to central apneas. Six months of GH does not aggravate the SRBD in young PWS children, but a (nearly) normal PSG, does not exclude a complicated course of a relatively mild URTI. Based on our data, monitoring of sleep-related breathing during URTI in PWS children should be considered.

Chapter 5

Recently, it has been reported that snoring related arousal indices are inversely associated with mental development in healthy infants. We therefore evaluated the association between psychomotor development and SRBD in PWS infants. We performed BSID II in 22 PWS infants. They had a median (iqr) age of 1.8 (1.1 to 3.4) years, and a BMI SDS of -0.5 (-1.3 to 1.6). We evaluated psychomotor development in relation to the results of PSG. Median (iqr) mental and motor development in this group of infants was 73.1% (64.3 to 79.6) and 55.2% (46.5 to 63.1) of normal children, respectively. All infants had SRBD, mostly of central origin. Only 4 infants had obstructive sleep apnea syndrome (OSAS). We did not find any association between the AHI and psychomotor development for the total group. However, the children with OSAS had a significantly delayed mental development of 65.5% (60.0 to 70.3) of normal, compared to those without OSAS. Children with OSAS had a median BMI SDS of 1.4 (0.1 to 1.6), which tended to be higher than in those without OSAS. Our data indicate that psychomotor development in PWS infants is not related to central SRBD. However, infants with OSAS have more severely delayed mental development, suggesting that PWS infants should be screened for OSAS.

Chapter 6

Neurobehavioural abnormalities, cognitive impairment and SRBD are all common features of PWS. In the general population consistent relations have been found between neurobehavioural abnormalities and cognitive deficits, and SRBD. We therefore evaluated cognition, behaviour and their associations with sleep-disordered breathing in pre-pubertal PWS children. We evaluated 31 pre-pubertal PWS children, aged 4.0 to 13.2 years. We assessed cognition by age appropriate Wechsler Intelligence Scale subtests, and neurobehavioural abnormalities with parent-questionnaires. We evaluated the results of cognition, behaviour and their associations with SRBD, as

measured with PSG. All cognitive subtests were significantly below 0 SDS, with the lowest median (iqr) scores for one of the performance subtests (Block design) of -2.7 SDS (-3.0 to -0.3). Notably, we did not find a consistent impairment of all cognitive subtests. In 60% of children, verbal subtests were less affected than performance subtests. Problem behaviour was scored mainly on subscales related to "emotions/ behaviour not adapted to the social situation" and "insensitivity to social information". These findings might be explained by the relatively high prevalence of uniparental maternal disomy in our patient group. All children had SRBD, with a median (iqr) AHI of 4.1/hour (2.6 to 7.9). One of the performance subtest-scores was significantly higher in children with better sleep efficiency, as measured with PSG. Autistic-like social impairment was associated with daytime sleepiness, as reported by parents. In contrast to our expectations, children with better sleep-related breathing had more behavioural abnormalities (particularly social relating behaviour). In conclusion, cognition is impaired in pre-pubertal PWS children. Neurobehavioural abnormalities are common, particularly autistic-like social impairment. Sleep efficiency index was associated with better performance on the Picture Arrangement/Completion subtests, and daytime sleepiness was associated with autistic like social impairment. In contrast, we could not confirm a positive association of neurobehavioural abnormalities with SRBD in PWS.

Chapter 7

Several endocrine abnormalities have been described in PWS, such as GH deficiency, and hypogonadotropic hypogonadism. However, data on thyroid hormone levels in PWS children are very limited. GH treatment has been suggested to unmask an underlying TSH deficiency and to increase peripheral conversion from T4 to T3. We therefore evaluated thyroid function in children with PWS, before and during GH treatment, compared with randomised controls. Serum levels of T4, free T4 (fT4), T3, reverse T3 (rT3) and TSH were measured in 75 PWS children. After one year, assessments were repeated in 57 of them. Thirty-four of them were treated with GH and 23 were followed as randomised controls. Median (iqr) age of the total group at baseline was 4.7 years (2.7 to 7.6). Median (iqr) TSH level was -0.1 SDS (-0.5 to 0.5), T4 level, -0.6 SDS (-1.7 to 0.0), and fT4 level, -0.8 SDS (-1.3 to -0.3), the latter two being significantly lower than 0 SDS. T3 level, 0.3 SDS (-0.3 to 0.9), was significantly higher than 0 SDS. After one year of GH treatment, fT4 decreased significantly from -0.8 SDS (-1.5 to -0.2) to -1.4 SDS (-1.6 to -0.7), compared with no change in untreated PWS children. T3 did, however, not change. In conclusion, we found fT4 levels within the normal range in

most PWS children at start of study. During GH treatment, fT4 decreased significantly to low-normal levels, but TSH levels remained completely normal. T3 levels were relatively high or normal, both before and during GH treatment, indicating that PWS children have increased T4 to T3 conversion. We recommend monitoring of thyroid hormone levels in PWS children, particularly during GH treatment.

Chapter 8

Obesity and an abnormal body composition with relatively low LBM and high body fat percentage, all common factors in PWS, may predispose to the development of insulin resistance and of cardiovascular diseases. Adiponectin is an adipocytokine, which is considered protective with regard to diabetes mellitus and cardiovascular disease. We evaluated in pre-pubertal PWS children (1) adiponectin levels, body composition, carbohydrate metabolism, and triglyceride levels, (2) associations between adiponectin and body composition, carbohydrate metabolism and triglycerides, and (3) effects of GH treatment on these outcome measures. Twenty PWS children were randomised into a GH treatment group (n=10) and a non-GH treated control-group (n=10). We measured fasting levels of adiponectin, glucose, insulin, and triglycerides at start, after 1 and after 2 years. We measured body composition and fat distribution by DXA. At baseline, median (iqr) adiponectin levels, 17.1 mg/l (13.9 to 23.2) were significantly higher in PWS children than in healthy sex- and age-matched controls, 11.8 mg/l (9.7 to 12.5) ($p<0.005$). Body fat percentage was significantly higher than 0 SDS, 1.8 SDS (1.5 to 2.1) ($p<0.001$). Adiponectin levels were inversely associated with triglyceride levels ($r=-0.52$, $p=0.03$), and tended to be inversely related with body fat percentage and BMI. We did not find any correlation with fasting insulin or glucose levels, the insulin/glucose ratio or HOMA-index. After 1, and 2 years, adiponectin levels increased significantly in the GH group and did not change in the control group. In conclusion, adiponectin levels were increased, and inversely associated with triglyceride levels, in pre-pubertal, not overweight PWS children, although they had a relatively high body fat percentage. GH resulted in a significant increase of adiponectin levels, whereas no change was found in the control group. This is reassuring with respect to the development of insulin resistance during GH treatment in PWS children.

Chapter 9

In the General Discussion, we discuss our findings in relation to current literature. This chapter ends with clinical implications and considerations for future research.

Chapter 11

Samenvatting

Dit proefschrift beschrijft de resultaten van de Nederlandse groeihormoon (GH) studie bij kinderen met Prader-Willi syndroom (PWS). In deze studie werden verschillende aspecten van het PWS onderzocht, met name anthropometrie, psychomotorische ontwikkeling, cognitieve ontwikkeling, gedrag, slaap-gerelateerde ademhalingsstoornissen, schildklierfuncties en adiponectine spiegels, alsmede de efffecten van GH behandeling hierop.

Hoofdstuk 1

In dit hoofdstuk wordt een overzicht gegeven van de prevalentie, de oorzaak en het klinisch beeld van het PWS. Tevens worden de verschillende endocrinologische aspecten van het PWS beschreven. Er wordt een overzicht gegeven van de eerdere GH studies. Tenslotte worden de doelstellingen, de in- en exclusiecriteria en de opzet van de studie uiteengezet.

Hoofdstuk 2

Kinderen met PWS hebben een verminderde lengtegroei en een afwijkende lichaamssamenstelling. Zij hebben een relatief hoog vetpercentage en een lage vetvrije massa. Uit eerdere studies is gebleken dat tijdens GH behandeling de lengtegroei en de lichaamssamenstelling verbeterde. Echter, deze studies waren gedurende maximaal één jaar gecontroleerd en er werden relatief kleine patiëntengroepen beschreven. Bovendien hadden de meeste patiënten in deze studies ernstig overgewicht. Wij hebben daarom een gecontroleerde GH studie uitgevoerd bij 91 pre-pubertaire kinderen met het PWS (42 zeer jonge kinderen <3 jaar, 49 kinderen tussen 3-14 jaar). Wij onderzochten de effecten van GH op lengtegroei, lichaamssamenstelling en lichaamsproporties. Na onafhankelijke loting werden alle kinderen verdeeld in 4 groepen. Twintig zeer jonge kinderen en 27 kinderen tussen 3-14 jaar werden behandeld met GH (1 mg/m^2/day). Hun resultaten werden vergeleken met die van de 22 zeer jonge kinderen en 22 kinderen van 3-14 jaar die respectievelijk gedurende 1 en 2 jaar niet met GH behandeld werden (de controlegroep). We hebben jaarlijks anthropometrie en lichaamssamenstelling (met behulp van een DXA scan) bepaald. Gedurende de 1 (zeer jonge kinderen) en 2 jaar (kinderen 3-14) gecontroleerde studie vonden we een volledige normalisatie van lengte, body mass index (BMI) en hoofdomtrek. Lichaamssamenstelling (uitgedrukt in standard deviatie score, SDS, voor leeftijd en geslacht) verbeterde, maar werd niet volledig normaal tijdens GH behandeling. De vetvrije massa, nadat gecorrigeerd was voor de toegenomen lengte tijdens GH behandeling, was niet toegenomen bij de GH-behandelde kinderen, maar was afgenomen bij de kinderen in de controlegroep. GH

behandeling heeft dus een gunstig effect op de vetvrije massa. Insulin like growth factor I (IGF-I) steeg bij de meeste kinderen tot suprafysiologische waarden tijdens de behandeling. Uit onze resultaten blijkt dat 2 jaar GH behandeling bij kinderen met PWS lengte, alsook BMI en hoofdomtrek normaliseert en hun lichaamssamenstelling en lichaamsproporties verbetert. Omdat de IGF-I spiegels fors stijgen bij een aantal PWS kinderen, lijkt monitoring van IGF-I spiegels tijdens GH behandeling aangewezen.

Hoofdstuk 3

Eerdere GH-studies bij PWS hebben voornamelijk de effecten van GH op antropometrische parameters en lichaamssamenstelling beschreven. Gegevens over het effect van GH op de psychomotorische ontwikkeling van zeer jonge kinderen met PWS zijn zeer beperkt. Wij onderzochten de psychomotorische ontwikkeling van 43 zeer jonge kinderen met PWS bij het begin van de studie, 29 van hen werden opnieuw geëvalueerd na 1 jaar. De GH groep bestond uit 15 kinderen en de onbehandelde controlegroep bestond uit 14 kinderen. We hebben de psychomotorische ontwikkeling geëvalueerd met behulp van de Bayley Scales of Infant development (BSID II), bij het begin van de studie en na 1 jaar. De gegevens werden omgerekend naar het percentage van de verwachte ontwikkeling op basis van leeftijd. De verandering (ten opzichte van de normale progressie) in ontwikkeling gedurende het jaar follow-up werd berekend. De leeftijd (mediaan, interkwartielbereik, ikb) van de GH groep was 2.3 jaar (1.7 tot 3.0) en van de controlegroep 1.5 jaar (1.2 tot 2.7) ($p=0.17$). Tijdens het jaar follow-up was zowel voor de motorische ontwikkeling als voor de mentale ontwikkeling een significant betere progressie in de behandelgroep dan in de controlegroep. De verandering (mediaan, ikb) in de mentale ontwikkeling was +9.3% (-5.3 tot 13.3) in de GH-groep en -2.9% (-8.1 tot 4.9) in de controlegroep, ($p<0.05$). De verandering in motorische ontwikkeling was +11.2% (-4.9 tot 22.5) vs. -18.5% (-27.9 tot 1.8) ($p<0.05$) voor respectievelijk de GH-groep en de controlegroep. Kinderen met een lagere motorische ontwikkelingsleeftijd bij de start van de studie hadden tijdens GH een sterkere progressie van hun motoriek dan kinderen met een hogere motorische ontwikkelingsleeftijd bij start. Uit onze studie blijkt dat zowel de motorische als de mentale ontwikkeling van kinderen met PWS gunstig beïnvloed wordt door GH behandeling.

Hoofdstuk 4

Recent zijn een aantal beschrijvingen van onverwacht overlijden van zowel GH-behandelde als niet-GH-behandelde jonge kinderen met PWS gepubliceerd. Deze

onverwachte overlijdens werden dikwijls in verband gebracht met complicaties van bovenste luchtweginfecties. Sommige clinici suggereerden een causaal verband tussen GH behandeling en onverwacht overlijden. Er zijn echter thans weinig gegevens beschikbaar over de effecten van GH behandeling op de ademhaling bij PWS. Wij hebben daarom slaap-gerelateerde ademhaling gemeten bij 53 kinderen met PWS (30 jongens) voor en 6 maanden na start met GH behandeling, door middel van volledige polysomnografie (PSG). Na 6 maanden GH behandeling werd PSG herhaald bij 35 kinderen. De leeftijd (mediaan, ikb) was 5.4 (2.1 tot 7.2) jaar en BMI was +1.0 SDS (-0.1 tot 1.7). Apnea Hypopneu Index (AHI) was 5.1/u (2.8 tot 8.7) (normaalwaarden: AHI <1/u). Centrale apneus kwamen het meest voor, 2.8/h (1.5 tot 5.4) en obstructieve apneus kwamen minder voor. De duur (mediaan, ikb) van de langst gemeten apneu was 15.0 sec (13.0 tot 28.0). Er was geen verband tussen de totale AHI en leeftijd of BMI, maar met toenemende leeftijd daalde het aantal centrale apneus (r=-0.34, p=0.01). Na 6 maanden GH behandeling was er geen significante verandering in AHI, van 4.8 (2.6 tot 7.9) tot 4.0 (2.7 tot 6.2) (p=0.36). Eén patiënt in onze studie is onverwacht overleden tijdens een relatief milde bovenste luchtweginfectie. Hij had een bijna-normale PSG, zowel voor als tijdens GH behandeling. Concluderend hebben kinderen met PWS slaap-gerelateerde ademhalingsstoornissen, met een hoge AHI, voornamelijk ten gevolge van centrale apneus. Tijdens GH behandeling treedt er geen verergering op van slaap-gerelateerde ademhalingsproblemen, maar een (bijna) normale PSG sluit een gecompliceerd verloop van een relatief milde bovenste luchtweginfectie niet uit. Onze onderzoeksresultaten suggereren dat monitoring tijdens luchtweginfecties bij kinderen met PWS overwogen zou moeten worden.

Hoofdstuk 5

Er zijn aanwijzingen dat bij gezonde zeer jonge kinderen de mentale ontwikkeling een omgekeerd verband vertoont met de arousal index, gerelateerd aan snurken. Wij onderzochten bij kinderen met PWS de relatie tussen psychomotorische ontwikkeling, gemeten met BSID II, en slaap-gerelateerde ademhalingsstoornissen, gemeten met PSG. Wij onderzochten een groep van 22 kinderen met PWS. Deze groep had een leeftijd (mediaan, ikb) van 1.8 (1.1 tot 3.4) jaar en een BMI SDS van -0.5 (-1.3 tot 1.6). De mediaan (ikb) voor de mentale en motorische ontwikkeling in deze groep kinderen was respectievelijk 73.1% (64.3 tot 79.6) en 55.2% (46.5 tot 63.1), van de verwachte ontwikkeling voor de leeftijd. Alle kinderen hadden slaap-gerelateerde ademhalingsstoornissen, meestal van centrale oorsprong. Vier kinderen hadden obstructief slaapapneu syndroom (OSAS). We vonden geen verband tussen de AHI

en de psychomotorische ontwikkeling in de totale groep. De kinderen met OSAS hadden echter een significant lagere mentale ontwikkeling dan kinderen zonder OSAS, namelijk 65.5% (60.0 tot 70.3) van het normaal te verwachten ontwikkelingsniveau. De kinderen met OSAS hadden een BMI SDS van 1.4 (0.1 tot 1.6). Dit is hoger (hoewel niet statistisch significant) dan bij kinderen zonder OSAS. Onze resultaten tonen geen verband tussen psychomotorische ontwikkeling en centrale slaap-gerelateerde ademhalingsstoornissen. Echter, kinderen met OSAS hebben een ernstigere vertraging van de mentale ontwikkeling. Op basis van deze resultaten zou screening voor OSAS bij jonge kinderen met PWS zinvol zijn.

Hoofdstuk 6

Gedragsproblemen, cognitieve beperkingen en slaap-gerelateerde ademhalings-stoornissen zijn veelvoorkomende kenmerken van het PWS. In de algemene populatie bestaat er een sterk verband tussen gedragsproblemen, cognitieve beperkingen en slaap-gerelateerde ademhalingsproblemen. Wij onderzochten bij de pre-pubertaire kinderen met PWS het gedrag, de cognitieve ontwikkeling en het verband met de slaap-gerelateerde ademhalingsproblemen. Wij hebben bij 31 pre-pubertaire kinderen met PWS in de leeftijd van 4.0-13.2 jaar de cognitie bepaald met behulp van subtesten van, voor de leeftijd geschikte, Wechsler intelligentietesten. Het gedrag hebben we geëvalueerd met behulp van vragenlijsten voor ouders. Vervolgens hebben we gekeken of er een verband bestond tussen de resultaten van deze testen en die van de PSG. Alle cognitieve subtesten waren significant lager dan 0 SDS, met de laagste score (mediaan, ikb) voor één van de performale subtesten (Blokpatronen), -2.7 SDS (-3.0 tot -0.3). Opvallend was dat de cognitieve beperkingen niet harmonisch verdeeld waren. Zestig procent van de kinderen scoorden beter op de verbale dan op de performale subtesten. Gedragsproblemen werden gerapporteerd voornamelijk op de subschalen “emoties/gedrag niet aangepast aan de sociale situatie” en “ongevoeligheid voor sociale informatie”. Deze bevindingen zouden veroorzaakt kunnen zijn doordat er zich in deze groep een relatief hoog percentage kinderen bevindt met uniparentele maternale disomie. Alle kinderen hadden een slaap-gerelateerde ademhalingsstoornis, met een (mediaan, ikb) AHI van 4.1/u (2.6 tot 7.9). Op een van de performale subtesten werd significant beter gescoord door kinderen met een hogere slaap efficiëntie index, gemeten door middel van PSG. Autistisch aandoend sociaal gedrag was gerelateerd aan slaperigheid overdag, zoals gerapporteerd door de ouders. In tegenstelling tot onze verwachtingen, hadden kinderen met betere slaap-gerelateerde ademhaling meer gedragsproblemen (in het bijzonder problemen met sociaal-gerelateerd

gedrag). Concluderend vonden wij bij kinderen met PWS dat gedragsproblemen regelmatig voorkwamen, met name autistisch aandoend sociaal gedrag en dat er een disharmonische beperking van de cognitie was. Een hogere slaap efficiëntie index was geassocieerd met een betere score op de "Plaatjes ordenen/Onvolledige tekeningen" subtest, en slaperigheid overdag was geassocieerd met autistisch aandoend sociaal gedrag. Daarentegen konden we het positieve verband tussen slaap-gerelateerde ademhalingsstoornissen en gedragsstoornissen, bij PWS niet bevestigen.

Hoofdstuk 7

Bij PWS zijn er een aantal endocrinologische afwijkingen beschreven, onder welke GH deficiëntie en hypogonadotroop hypogonadisme. Echter, er zijn thans heel weinig gegevens beschikbaar over de schildklierfuncties bij PWS. Er wordt verondersteld dat er een verband is tussen GH behandeling en de schildklierfuncties. GH behandeling zou een latente TSH deficiëntie kunnen openbaren. Daarnaast zou GH behandeling de perifere omzetting van T4 naar T3 bevorderen. Wij onderzochten de schildklierfuncties bij kinderen met PWS voor en tijdens GH behandeling, in vergelijking met onbehandelde patiënten. T4, vrij T4 (vT4), T3, reverse T3 (rT3) en TSH spiegels werden gemeten bij 75 kinderen met PWS (mediaan (ikb) leeftijd 4.7 (2.7 tot 7.6) jaar). Na een jaar werden de spiegels opnieuw bepaald bij 57 kinderen. Vierendertig kinderen werden met GH behandeld, en 23 kinderen werden gedurende dit jaar gevolgd als controlegroep. Mediaan (ikb) TSH spiegel was -0.1 SDS (-0.5 tot 0.5), T4 spiegel, -0.6 SDS (-1.7 tot 0.0), en vT4 spiegel, -0.8 SDS (-1.3 tot -0.3). T4 en vT4 spiegels waren beiden significant lager dan 0 SDS en de T3 spiegel was significant hoger dan 0 SDS. Na 1 jaar GH behandeling daalde de vT4 spiegel significant, van -0.8 SDS (-1.5 tot -0.2) tot -1.4 SDS (-1.6 tot -0.7), terwijl er geen verandering optrad bij de controlegroep. De T3 spiegels veranderden niet. Onze resultaten laten zien dat T4 spiegels binnen normale grenzen waren bij de meeste kinderen met PWS. Tijdens GH behandeling trad er een daling op van de vT4 spiegel, maar TSH bleef volledig normaal. T3 spiegels waren juist relatief hoog of normaal. Dit wijst erop dat kinderen met PWS een verhoogde omzetting van T4 naar T3 hebben. In het bijzonder tijdens GH behandeling, lijkt het aangewezen om schildklierfuncties bij kinderen met PWS te bepalen.

Hoofdstuk 8

Obesitas en een afwijkende lichaamssamenstelling, met een relatief lage vetvrije massa en een relatief hoog lichaamsvetpercentage, zijn veelvoorkomende kenmerken van het PWS. Deze factoren vormen een verhoogd risico op de ontwikkeling van

insulineresistentie en van cardiovasculaire ziekten. Adiponectine is een hormoon dat afgescheiden wordt door de vetcellen en een beschermende werking zou hebben tegen het ontstaan van diabetes mellitus en cardiovasculaire ziekten. Wij onderzochten bij pre-pubertaire kinderen met PWS (1) adiponectine spiegels, lichaamssamenstelling, koolhydraatmetabolisme, en triglyceride spiegels, (2) het verband tussen deze parameters en (3) effecten van GH behandeling op deze parameters. Twintig kinderen met PWS werden gevolgd in de GH behandelgroep (n=10), of in de controlegroep (n=10) gedurende 2 jaar. Wij hebben de adiponectine, glucose, insuline, and triglyceride spiegels gemeten bij start van de studie, na 1 jaar en na 2 jaar. Lichaamssamenstelling werd bepaald met behulp van een DXA scan. Bij start waren adiponectine spiegels (mediaan, ikb) 17.1 mg/l (13.9 tot 23.2) voor de totale groep, wat significant hoger is dan bij gezonde kinderen met hetzelfde geslacht en leeftijd, 11.8 mg/l (9.7 tot 12.5) ($p<0.005$). Het lichaamsvetpercentage was significant hoger dan 0 SDS bij de kinderen met PWS, 1.8 SDS (1.5 tot 2.1) ($p<0.001$). Adiponectine spiegels waren omgekeerd gerelateerd aan triglyceride spiegels, ($r=-0.52$, $p=0.03$), en er was een tendens tot een omgekeerde relatie met lichaamsvetpercentage en BMI. We vonden geen verband met nuchtere inuline of glucose spiegels, de insuline/glucose ratio of de HOMA-index. Na 1 en 2 jaar, stegen adiponectine spiegels significant in de GH group, terwijl er geen verandering optrad bij de controlegroep. Onze resultaten toonden dat jonge, pre-pubertaire kinderen met PWS hoge adiponectine spiegels hadden en dat deze omgekeerd gerelateerd waren aan de triglyceride spiegels. Deze kinderen hadden geen ernstig overgewicht, maar wel een verhoogd lichaamsvetpercentage. Tijdens GH behandeling trad er een verdere stijging op van de adiponectine spiegels, terwijl er geen sprake was van verandering in de controlegroep. Deze bevindingen zijn geruststellend voor wat betreft de ontwikkeling van insulineresistentie tijdens GH behandeling bij kinderen met PWS.

Hoofdstuk 9

In de algemene discussie worden de resultaten van de verschillende studies besproken en vergeleken met de huidige literatuur. Aan het eind van dit hoofdstuk wordt een overzicht gegeven van de klinische implicaties en worden er suggesties gedaan voor toekomstig onderzoek.

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Curriculum vitae of Dederieke Festen

Dederieke Festen was born in Leidschendam on April 10th, 1974. In 1992, she graduated from high school (Christelijk Lyceum, Apeldoorn), and started her medical training at the Catholic University of Leuven (Belgium). After obtaining her medical degree in June 2000, Dederieke started to work as a resident in pediatric psychiatry at the RMPI (Rotterdams Medisch Pedagogisch Instituut) in Barendrecht. In April 2001, she started as a research fellow at the Dutch Growth Foundation in Rotterdam (supervisor Prof. dr. A.C.S. Hokken-Koelega), which has resulted in the present thesis. In February, 2008 she will start her clinical trainingship for physicians for persons with intellectual disability (supervisors Drs. F.V.P.M. Ewals) at the Erasmus Medical Center, Rotterdam and at Stichting IPSE, Nootdorp (supervisor Dr. M.A.M. Tonino). Dederieke lives in Rotterdam, together with her husband Jan Pieter Maes and their son, Otto.

List of publications

***D.A.M. Festen*, A.W. de Weerd, R.A.S. van den Bossche, K. Joosten, H. Hoeve, A.C.S. Hokken-Koelega** Sleep-related breathing disorders in pre-pubertal children with Prader-Willi Syndrome and effects of growth hormone treatment. *J Clin Endocrinol Metab (2006): 91 (12):4911-4915*

***D.A.M. Festen*, A.W. van Toorenenbergen, H.J. Duivenvoorden, A.C.S. Hokken-Koelega** Adiponectin levels in pre-pubertal children with Prader-Willi Syndrome before and during growth hormone therapy. *J Clin Endocrinol Metab (2007): 92 (4): 1549-1554*

***D.A.M. Festen*, M. Wevers, A.W. de Weerd, R.A.S. van den Bossche, H.J. Duivenvoorden, B.J. Otten, J.M. Wit, A.C.S. Hokken-Koelega** Psychomotor development in infants with Prader-Willi Syndrome and associations with sleep-related breathing disorders *Pediatr Res (2007): 62 (2): 221-224*

***D.A.M. Festen*, T.J. Visser, B.J. Otten, J.M. Wit, H.J. Duivenvoorden, A.C.S. Hokken-Koelega** Thyroid hormone levels in children with Prader-Willi Syndrome before and during growth hormone treatment. *Clin Endocrinol (2007): 67 (3): 449-456*

***D.A.M. Festen*, M. Wevers, A.W. de Weerd, R.A.S vanden Bossche, H.J. Duivenvoorden, A.C.S. Hokken-Koelega** Cognition and behavior in pre-pubertal children with Prader-Willi Syndrome and associations with sleep-related breathing disorders. S*ubmitted*

***D.A.M. Festen*, M. Wevers, A.C. Lindgren, B.Böhm, B.J. Otten, J.M. Wit, H.J. Duivenvoorden, A.C.S. Hokken-Koelega** Mental and motor development before and during growth hormone treatment in infants and toddlers with Prader-Willi syndrome. *Clin Endocrinol (2007), in press*

***D.A.M. Festen*, A.C.S. Hokken-Koelega** Breathing disorders in Prader-Willi Syndrome: The role of obesity, GH treatment and upper respiratory tract infections *Exp Rev Clin Endocrinol Metab (2007): 2 (4): 529-537*

***D.A.M. Festen*, M. van Eekelen, B.J. Otten, J.M. Wit, H.J. Duivenvoorden, A.C.S. Hokken-Koelega** Randomized controlled growth hormone trial: Effects on anthropometry, body composition, and body proportions in a large group of children with Prader-Willi Syndrome. *Submitted*

M. den Brinker, B. Dumas, T.J. Visser, W.C. Hop, J.A. Hazelzet, *D.A. Festen*, A.C. Hokken-Koelega, K.F. Joosten Thyroid function and outcome in children who survived meningococcal septic shock. *Intensive Care Med, Jul 2005, 31 (7): 970-976*