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ENDOCRINE AND METABOLIC ASPECTS OF ADULT PRADER WILLI SYNDROME WITH SPECIAL EMPHASIS ON THE EFFECT OF GROWTH HORMONE TREATMENT

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Stockholm 2003

Soleno Therapeutics, Inc.
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Apotex v. Soleno
PGR2026-00034
Ex. 2057-001

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ISBN 91-7349-645-6

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Sokrates

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Abstract

Prader Willi Syndrome (PWS) is a complex genetic disorder characterized by muscular hypotonia, hyperphagia, obesity and behavioural problems. Partial growth hormone (GH) deficiency and hypogonadism are common. Results of several GH treatment studies in children with PWS have shown improvements not only in growth, but also in body composition, physical strength and agility. The partial GH deficiency seen in PWS might render these patients at risk of metabolic diseases in adult life and of reduced life span. The non-growth effects of GH treatment in PWS children have directed the interest towards the PWS adults in preventing the long-term consequences of GH deficiency. Until recently, neither the endocrine and metabolic consequences of the syndrome in adult patients, nor the potential effects of GH treatment have been known in detail.

Aims: To study endocrine, metabolic and psycho-social functions, in adult PWS patients and the impact of GH treatment on these parameters.

Patients and methods: We examined a cohort of 19 adult patients with clinical PWS (13 with PWS genotype) of which 17 (9 men and 8 women) with a mean age of 25 years and a mean BMI of 35 kg/m^2, subsequently completed a 12 months GH treatment trial.

Results and discussion: At baseline all but three patients were obese despite a strict diet. Waist/hip ratio was increased in all women, and the mean percentage body fat was high in both genders. The activity of the GH-insulin-like-growth-factor-I (IGF-I) system was impaired with low GH values, low total IGF-I and in relation to the obesity low levels of free IGF-I and non-suppressed IGF-binding-protein-1 (IGFBP-1). Approximately two thirds were biochemical hypogonadal. Bone mineral density (BMD) was low. Four patients had impaired glucose tolerance and 9 patients high homeostasis model assessment (HOMA) index, indicating insulin resistance. A moderate dyslipidemia was seen in seven patients. The 13 patients with genetically confirmed diagnosis were shorter and had significantly lower IGF-I. GH treatment showed beneficial effects on body composition with reduction in body fat and increase in lean body mass following 6 and 12 months of therapy with doses, that normalized total IGF-I levels. The effects were more pronounced in the patients with the PWS genotype. Carbohydrate and lipid metabolism was not significantly affected by GH treatment.

The aetiology of hyperphagia in PWS is not known. Examination of peptides involved in appetite regulation showed that leptin levels were high reflecting obesity, and as a consequence NPY levels were low. In view of the patients' adiposity circulating oxytocin levels were abnormally low and circulating ghrelin levels abnormally high. Therefore, oxytocin as well as ghrelin might be involved in the hyperphagia. The peptides involved in appetite regulation did not change during GH treatment. Psychological evaluation revealed positive effects on intellectual speed and flexibility, reaction time and motor speed. When GH was discontinued significant impairments in physical and social function as well as in the over-all functioning were seen, as judged from questionnaires to relatives and caretakers.

Conclusion: GH treatment might offer an opportunity to reduce some of the adverse consequences of the PWS syndrome. It should be remembered, however, that dysfunction of the GH-IGF-I axis and the potential effects hereof are only minor parts of the clinical syndrome. Larger and longer term studies on the effect of GH replacement in adult PWS patients should be carried out.

Key words: Prader Willi syndrome, adults, obesity, GH, IGF-I, psycho-social functioning, GH treatment

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Thesis

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I Höybye C, Hilding A, Jacobsson H, Thorén M. Metabolic profile and body composition in adults with Prader Willi syndrome and severe obesity. Journal of Clinical Endocrinology and Metabolism 2002; 87:3590-97.

II Höybye C, Hilding A, Jacobsson H, Thorén M. Growth hormone treatment improves body composition in adults with Prader Willi syndrome. Clinical Endocrinology (Oxf) 2003; 58:653-61).

III Höybye C, Frystyk J, Thorén M. The growth hormone-insulin-like growth factor axis in adult patients with Prader-Willi syndrome. Growth Hormone and IGF Research 2003; in press.

IV Höybye C, Barkeling B, Espelund U, Petersson M, Thorén M. Peptides associated with hyperphagia in adults with Prader Willi syndrome before and during growth hormone treatment. Growth Hormone and IGF Research 2003; in press.

V Höybye C, Thorén M, Böhm B. Cognitive, emotional, physical and social effects of growth hormone treatment in adults with Prader Willi syndrome. Journal of Intellectual Disability Research. Submitted.

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I Abbreviations

ACTH:	Adrenocorticotrophic hormone
BIA:	Bioelectric impedance analysis
BF%:	Body fat percent
BMC:	Bone mineral content
BMI:	Body mass index
DEXA:	Dual energy X-ray absorptiometry
DNA:	Desoxyribonucleic acid
FSH:	Follicle-stimulating hormone
GH:	Growth hormone
GHD:	Growth hormone deficiency
GHBP:	Growth hormone binding protein
HbA1c:	Glycosylated haemoglobin
HDL:	High density lipoprotein
HOMA:	Homeostasis model assessment
IGF-I:	Insulin-like growth factor I
IGF-II:	Insulin-like growth factor II
IGFBP-1:	IGF-binding protein-1
IGFBP-2:	IGF-binding protein-2
IGFBP-3:	IGF-binding protein-3
IQ:	Intelligence quotient
LDL:	Low density lipoprotein
LH:	Luteinizing hormone
Lp(a):	Lipoprotein(a)
OGTT:	Oral glucose tolerance test
PWS:	Prader Willi syndrome
RIA:	Radioimmunoassay
SD:	Standard deviation
SDS:	Standard deviation score
SNRPN:	Small nuclear ribonucleoprotein N
T3:	Triiodothyronine
T4:	Thyroxine
TSH:	Thyroid stimulating hormone
WISC-III:	Wechslers intelligence scale for children-third edition

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II Introduction

During the last decade our knowledge especially of the endocrine and metabolic aspects of Prader Willi syndrome (PWS) has increased and accordingly the treatment possibilities. This progress is the result of many years of meticulous clinical observations and research carried out primarily within paediatric endocrinology and clinical genetics. Recently the studies leading to the introduction of growth hormone (GH) treatment in PWS children have further extended our knowledge on the endocrine and metabolic consequences of the syndrome. Few studies have concentrated on PWS in adulthood and the potential impact of GH treatment in these patients.

III Prader Willi syndrome

III 1 Signs and symptoms

In 1956 three paediatricians in Switzerland, Prader, Labhart and Willi, made the first report of the disorder that was later to be called The Prader Willi syndrome (1).
PWS is a multisystemic disorder affecting approximately 1/15 000 newborns, characterized by short stature, muscular hypotonia, hypogonadism, mental retardation, behavioural problems and hyperphagia (2, 3). Dysmorphic features like narrow face, almond shaped eyes, smaller hands and feet than expected for height and age, are typical (1-4).
The signs and symptoms vary with age.
In the new-born the muscular hypotonia, the failure to thrive, feeding difficulties and slow weight gain as well as impaired growth are most striking. From the age of 2-4 years appetite becomes insatiable leading to extreme obesity, if not adequately treated. The muscular strength increases, but muscles are still weak and this affects development with delay in both psychological and motor milestones. Body composition is abnormal with more fat than lean body mass.
In the adolescent phase appetite continues to be excessive. Stature is short, and complete puberty is rarely seen. The average final adult height in PWS is about 2 standard deviations less than in a relevant reference population (4-7). Mood becomes increasingly unstable, especially if routines are changed.
In the adults behavioural problems are of major concern (8, 9). Temper outbursts, aggressive and deviant behaviour affecting psychosocial wellbeing are common. Most adults manage to have some occupation (sheltered) but are often unable to manage the challenge of living alone. The excessive appetite with the constant obsessive interest in food together with a more independent lifestyle and a more uncontrolled access to food highly increases the risk of morbid obesity in adulthood (Fig 1). Increased frequency of cardiovascular disease, pulmonary dysfunction, type 2 diabetes and dyslipidemia often lead to complete disability in the third decade of life and to early death (10-12).
Hyperphagia, partial GH deficiency and hypogonadism in PWS are thought to arise from developmental abnormalities in the hypothalamus, although their nature is unclear (3, 4). It has been assumed that the cognitive impairments and the observed deficiencies in gonadotropins and the GH/IGF-I axis are all interrelated through a common unknown mechanism that impairs brain function. However, neuroanatomy is normal in PWS, except for a reduced oxytocin and neuropeptide Y (NPY) cell number in the paraventricular nucleus (13).
GH deficiency in adults with other diseases than PWS is associated with abnormal body composition with increased body fat and decreased lean body mass (14). The physical and

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psychological capacities are impaired and they are often overweight with central adiposity. Muscle strength and bone mineral density are reduced. IGF-I is subnormal. Hyperinsulinaemia indicating insulin resistance is present and serum lipids are increased. Epidemiological data suggest that adults with hypopituitarism have reduced life expectancy, compared to healthy controls, with a greater than 2-fold increase in mortality from cardiovascular diseases (15, 16). GH deficiency has been proposed as an accounting factor, although it is still too early to establish if GH replacement will reduce cardiovascular mortality. In addition hypogonadism is a well-known risk factor of osteoporosis. Thus, some of the symptoms seen in PWS adults might be secondary to partial GH deficiency and hypogonadism.



Fig 1 Woman with Prader Willi syndrome.

III 2 Diagnosis

III 2.1 Holm's diagnostic criteria

For more than two decades the diagnosis of PWS was made based on clinical findings only. Because of the age dependent variability in symptoms and phenotype the clinical diagnosis is difficult. Consequently the diagnosis might easily been missed or given to patients without PWS. This led to the formulation of diagnostic criteria by Holm et al 1993 (2). These include both major and minor criteria, which are converted into a scoring system. Major criteria each weighted as one point comprise 8 items: Neonatal and infantile hypotonia, feeding problems in infancy with poor weight gain, excessive or rapid weight gain between one and 6 years of age in the absence of intervention, characteristic facial features, hypogonadism, mild to modest mental retardation and learning problems, hyperphagia and food obsession and cytogenetic findings with paternal deletion or other abnormalities of the Prader Willi gene. The minor criteria each weighted as 0.5 point comprise: Decreased fetal movements or weak cry in infancy, behavioural problems, sleep disturbances and apnea, short stature for genetic background, small and narrow hands with straight ulnar borders, eye abnormalities (esotropi, myopathi), viscous saliva, speech articulation defects and skin picking.
In childhood a total score of five is necessary for the diagnosis, whereas in adulthood a total score of eight is needed. In order to make the diagnosis in adulthood major criteria must comprise at least 5 points of the total score.

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III 2.2 Genetics

The genetic basis of PWS was unknown until the early 1980s (17). Since then the understanding has evolved, and it is now known that PWS results from a paternally derived deletion in chromosome 15 in the region q11-13 (15q11-q13), a maternal uniparental disomy of chromosome 15 (UPD 15) or an imprinting defect (ID) (17-20). UPD is present when both chromosomes in one pair are inherited from a single parent. The effect of UPD that has received particular attention is genomic imprinting, which modulates gene function as a result of the sex of the transmitting parent. In PWS paternal deletions occur in 70-75% of patients, UPD 15 in about 20-25% of patients, while PWS due to (ID) is rare (2-5%).
Methylation analysis (see assay section below) is a common standard genetic test. Methylation analysis detects all 3 groups of molecular defects mentioned above, but will not distinguish between them; to do this further analysis might be required. Fluorescence in situ hybridization (FISH) detects deletions, and analysis of polymorphic DNA markers reveals maternal UPD. If both these tests are negative an imprinting defect is likely.
The region 15q11-13 contains a cluster of genes which are expressed from the paternal or maternal chromosome only, but the clinical implications are incompletely known (19, 20). The area is believed to contain a specific gene or genes, which encode(s) one or more proteins important for brain development (20). Loss of this genetic information primarily affects the brain, with a special preference for the hypothalamus. Mouse models for each of the three main molecular classes (deletion, UPD and ID) of PWS now exist (20).

III 3 Treatment

PWS is a multisymptomatic condition and treatment therefore involves a multidiciplinary team with psychosocial advice, dietary treatment and regular exercise as cornerstone efforts. Also suitable occupation and appropriate accommodation are important. Cardiovascular disease, diabetes, hypertension, dyslipidemia and pulmonary dysfunction are treated according to conventional therapeutic modalities.
It has been assumed that the reason for the increased morbidity and mortality in PWS is the abnormal body composition, and attention has been directed towards the importance of a long lasting improvement of this. Since a few years childhood PWS has been a registered indication for GH treatment in USA and some countries in Europe. The GH replacement therapy in PWS children accelerates linear height velocity with optimization of final height, and has beneficial effects on body composition with increased lean body mass and decreased body fat as well as increased bone mineral content (4, 21). Hence, the anabolic non growth effects of GH seen in PWS children rise important questions about the potential beneficial effects of GH therapy in adult PWS.
Replacement therapy of the hypogonadism is not commonly used in PWS (4). No systematic studies exist. In PWS females it has been suggested that the amount of estradiol converted from testosterone in adipose tissue is enough for basic needs, but the potential risk of thromboembolic events has also been a concern. In PWS males reports of aggravation of temper outbursts and aggressiveness have diminished the use of testosterone replacement. However, due to the potential benefits, replacement therapy of the hypogonadism may still be given (if no contraindications) to avoid secondary diseases and to improve general wellbeing.

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IV Aims of the study

The aims of my study were, to characterize the patients at baseline and evaluate the impact of GH treatment to adult PWS patients on

- The GH-IGF-I axis
- Body composition
- Carbohydrate and lipid metabolism
- Hyperphagia and appetite regulating peptides
- Cognitive, emotional, physical and social function

V Patients and methods

V 1 Patients

Twenty consecutive adults with PWS were referred to us from other clinics or via the Swedish Prader Willi patient organisation (Paper I). The patients were diagnosed during childhood, except one woman, who was diagnosed at the age of 30 years. One patient suffered from chronic lymphatic leukaemia and was excluded from further investigations. Thus, 19 patients, 10 men and 9 women, 17-37 (mean 25) years of age were studied (Table 1).

TABLE 1 Characteristics of 19 adults with clinical Prader Willi syndrome

Gender Females/ Males	Age (years)	BMI (kg/m^2)	Holm's criteria (points)*	Methylation test ** positive/ negative	IGF-I SDS	Hypogonadism	OGTT****
F	19	38.1	12	positive	-1.8	Yes	Normal
F	19	34.4	11.5	positive	-1.7	Yes	Normal
F	22	33.2	11	positive	-0.3	Yes	Normal
F	23	36.5	10.5	negative	-1.7		Normal
F	25	31.3	8	negative	0.8		Normal
F	29	36.5	11	negative	-1.9	Yes	Impaired
F	31	31.4	11	positive	-1.9	Yes	Normal
F	32	50.4	11	positive	-3.5	Yes	Normal
F	37	49.1	11.5	positive	-4.4		Diabetes
M	17	36.9	10	positive	-3.8	Yes	Impaired
M	19	20.4	8	negative	0.0		Normal
M	20	21.2	12.5	positive	-2.0	Yes	Normal
M	22	44.4	12	positive	-3.2	Yes	Impaired
M	25	24.2	11	positive	-3.7	Yes***	Normal
M	25	39.3	8	negative	0.3		Normal
M	27	27.5	12.5	positive	-2.5		Normal
M	30	57.8	10	negative	-1.7	Yes	Normal
M	32	31.9	11.5	positive	-2.3	Yes	Normal
M	36	32.7	11.5	positive	-3.2	Yes	Impaired

*8 points are necessary for the diagnosis from 3 years of age to adulthood.
** Standard cytogenetic analysis.
*** Replacement therapy with oral testosteronundekanoat
**** OGTT (Oral Glucose Tolerance Test)

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The patients were clinically re-evaluated, and all fulfilled Holm's criteria for the clinical diagnosis. All patients underwent standard genetic testing with methylation analysis and 13 were methylation positive. Informed consent was obtained from the patients and their caretakers. The study was approved by the committee for medical ethics at the Karolinska Institute.
Four patients received medication for hypertension and one patient with insulin and metformin treated diabetes type II also suffered from cardiac insufficiency. Hypogonadism was diagnosed in 13 patients by signs and low serum concentrations of gonadal steroids and low to normal LH and FSH concentrations. One man received oral androgen substitution. Three men had normal testosterone values, and three women had irregular menstrual periods. The pubic hair development corresponded to Tanner stage 5, i.e. adult stage, in all patients. Serum T3, T4, TSH, prolactin and cortisol were within the respective normal range in all patients (Paper I).
The cohort was for practical and ethical reasons established according to clinical criteria only and 6 patients were found to be methylation negative. For all parameters evaluated in this study, comparison between the methylation positive and methylation negative patients have been performed. In general we did not find any difference between the groups concerning parameters tested for. Thus, in the following - unless otherwise specified - results are given from the entire cohort.
There may of cause be differences between the two subgroups unrevealed by our investigations and some patients in the methylation negative group may have unidentified chromosomal abnormalities. It should also be remembered that the sample size and heterogeneity within the two groups limit generalisations.

V_2 Study design

After baseline examinations the patients were randomized to treatment with either placebo or GH (Genotropin®, Pharmacia Corporation) as described in Paper II. Two of the initial 19 patients could not be evaluated. One patient developed psychosis during the placebo period, while another went into a period of excessive weight loss deemed unrelated to the intervention. Of the 17 patients who completed the study, 9 were assigned to GH treatment and 8 to placebo. The groups were similar regarding gender, age, scores according to Holm's criteria, genotype, BMI, IGF-I SDS, and frequency of hypogonadism. During the placebo controlled period patients were treated with 0.8 IU (0.26 mg) daily for one month, and then 1.6 IU (0.53 mg) daily for 5 months. Subsequently, all received active open label treatment for 12 months. GH treatment was then discontinued. Injections were administered subcutaneously in the evening, and during the open label period GH doses were individually titrated to keep serum IGF-I concentrations within the normal age related range. The GH dose at 12 months of active treatment was similar in males and females, the mean being 1.8 ±0.1 IU (range 1.6-2.0) and 1.2± 0.1 IU (range 0.8-1.6) respectively. Evaluation of the patients was performed at baseline and every 6 months during the study (Paper II), and questionnaires were delivered to relatives/caretakers 3 and 6 months after GH treatment was discontinued. Results are presented both in relation to placebo and for the combined group with 6 and 12 months of active treatment. In the placebo group the visit at six months was considered comparable to the visit at start in the GH group.

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V 3 Methods

V 3.1 Anthropometric methods

Physical examination included measurements of height, weight, waist and hip circumferences and blood pressure. BMI and waist-hip ratio were calculated. Waist and hip circumferences were measured in the standing position. Waist was measured as halfway between the costal edge and iliac crest. Hip was measured as the greatest circumference around the buttocks. The cut-off level for waist/hip ratio was defined as >1.0 in men and >0.8 in women, because higher ratios are associated with an increased risk of cardiovascular disease (22, 23).

V 3.2 Body composition studies

Body fat was determined by Dual Energy X-ray Absorptiometry, DXA (Hologic QDR 4500, Hologic, inc., Waltham, MA, USA) according to a standard procedure described earlier (24). This method estimates total body fat, but also regional fat distribution in trunc and extremities. Lean body mass and bone mineral density (BMD) of total body, lumbar spine (L1-L4), femoral neck and trochanter region were assessed by the same instrument. The BMD values in the patients were compared with data from reference material provided by the manufacturer. The BMD values were expressed as standard deviations scores (SDS) from the mean of age and gender matched reference material (Z-scores) and SDS from the mean of young adults (T-scores). The WHO definitions of osteoporosis and osteopenia were applied; i.e. BMD between –1.0 and –2.5 SDS from the mean of young adults at any measured site was defined as osteopenia. Values below –2.5 SDS were defined as osteoporosis (25).

V 3.3 Growth hormone stimulation tests

Spontaneous GH secretion was measured by continuous venous blood sampling with 20 min intervals using a withdrawal pump (Swemed lab, Model 3003) from 8 pm to 8 am (n=12). Maximal GH level was measured after stimulation tests with either insulin induced hypoglycaemia (n=9) and/or arginine infusion (n=18). The stimulation tests were performed according to standardized protocols (Paper I), and severe GHD was defined as <3µg/L (14). The insulin tolerance test required presence of hypoglycaemic symptoms and blood glucose values lower than 2.2 mmol/liters following insulin administration.

V3.4 Oral glucose tolerance test (OGTT)

Oral glucose tolerance test (OGTT) was carried out at baseline and thereafter every 6 months during the study. The patients ingested 75 g glucose dissolved in water, and blood glucose was measured before ingestion and 120 min after (WHO). Impaired glucose tolerance was defined as 120 min glucose level between 7.8 and 11.0 mmol/L and diabetes as glucose level >11.1 mmol/L. The Homeostasis model assessment (HOMA) index=insulin/ ($22.5e^{-\ln glu}$) using single fasting sample was calculated as an estimation of insulin resistance (26, 27). We used 2.77 as threshold for insulin resistance, as suggested in the Bruneck study (28).

V 3.5 Appetite regulating peptides

To further evaluate the aetiology of the hyperphagia peripheral levels of peptides with known association to eating behaviour were measured. Oxytocin levels were monitored prior to GH treatment only, while ghrelin, NPY and leptin levels were monitored both prior to and after 6 and 12 months of GH treatment.

V 3.6 Cognitive, emotional, physical and social evaluation

The patients' cognitive, emotional, physical and social status was described using neuropsychological tests and questionnaires (Paper V).

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A battery of eleven neuropsychological tests was selected and employed at baseline and 18 months. The time limit for each assessment needed to be considered in these particular patients and did not allow the use of a full cognitive scale test. After 6 months tests with parallel versions and those measuring reaction time and tapping speed were repeated without risking bias due to remembrance.
The test battery included the SPIQ (Speedy performance-test of IQ) (29) word comprehension test, which measures the ability to combine words with pictures by pointing to one of four alternatives. The test has two parallel versions.
The Block Design test (30) is a test of perceptual organisation. The patient builds a copy of an abstract design out of a given amount of blocks.
The Coding test (30) evaluates working memory, flexibility and fine motor performance. Digits presented in random order are each given an unique sign. The patient is asked to fill in an empty space with the correct sign for each digit.
The Bender Gestalt Test (31) is a copying test of nine abstract designs demanding visual perception, fine motor function and a capacity to organise the material and close the parts of each item to a "gestalt".
In the Draw a Man test, the patient draws a person of optional sex and character (32).
Tests of executive functions included the verbal fluency tests of semantic categories and phonological issues (31). In the first test the patient names as many animals and then things to eat and drink as possible. The second task is to name as many words as possible beginning with "f", "a" and "s". The other executive test used was the Trail Making Test (TMT-A for numbers and TMT-B for numbers and letters) (31). The patient combines, consecutively, randomly scattered digits as fast as possible. Then the task is made more difficult as the patient has to switch between digits and letters, combining them consecutively. It is necessary to know the alphabet and to have an active working memory to perform the task. These tests have parallel versions.
To measure mental and motor speed we used two computerized tests (33). In the Simple Reaction-Time Measurements, auditory stimuli are presented at random intervals by the computer and the patient press a button as fast as possible with the right and left index finger, respectively, when hearing a sound. The Finger Tapping Task measures motor speed and motor fluency in five consecutive trials for each index finger separately.
We included a learning and memory test, the Luria Word learning and retention test. The patient listens to ten read words (all nouns) and then says all he/she can remember. This procedure is repeated until all words are remembered or maximum ten times. After about 30 minutes the patient is asked to say all the words again, now without hearing them first. Three parallel versions exist for these tests.
Standardised questionnaires reflecting mental health and psychosocial adjustment do not exist for this group of patients. Therefore, three specific questionnaires were composed especially for our study (Paper V).
The first questionnaire was a self-evaluation questionnaire answered by the patients at the end of each test situation at baseline, 6 and 18 months. This questionnaire included domains of mental flexibility, emotional status, physical performance and social behaviour. The responses were converted to scores from one to four, with four being the most positive. The ratio of the individually obtained scores divided with the total possible scores was then calculated.
The second questionnaire evaluated the same domains as the first questionnaire, but was answered by the relatives/caretakers. The responses were converted to scores and ratios in the same way.
The third questionnaire dealt with possible changes in cognitive, emotional, physical and social functions evaluated by relatives/caretakers during the 6 months period, when GH

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treatment was discontinued. After three and six months the relatives/caretakers evaluated whether the patient's behaviour was unchanged or more or less aggravated (Paper V).

V 3.7 Assays

Standard genetic testing was performed using southern blot analysis after cleavage with methylation sensitive restriction enzymes (CfoI and BglII) (34). The absence of unmethylated PW71 DNA fragment was defined as methylation positive, and diagnostic of PWS.
In paper I and II total IGF-I was determined by radioimmunoassay (RIA) (35). Normal range of IGF-I in adults was established in healthy subjects (36) and were expressed as concentrations and standard deviation scores (SDS) from the age related mean. IGFBP-1 was analysed by the method described by Povoa and co-workers (37).
In paper III total IGF-I and total IGF–II, free IGF-I, GHBP, IGFBP-1 and 2 were analysed with assays as previously described (38-41). The reference levels (mean ± SD) of these assays in young adults were: total IGF-II: 825±107 µg/L, free IGF-I: 0.95±0.44 µg/L, GHBP: 1.71±0.47 nmol/L, IGFBP-1: 44± 24 µg/L and IGFBP-2: 764±316 µg/L. IGFBP-3 was determined by a commercial assay (DSL Inc., Webster, TX, USA). According to the supplier the concentration of IGFBP-3 in healthy controls was: 3500±900 µg/L.
Correlation between the two methods measuring total IGF-I was good ($r=0.99$, $P<0.0001$) as well as for the methods measuring IGFBP-1 ($r=0.89$, $p<0.0001$). The relations to the reference populations were similar for the different methods.
GH and insulin were measured by fluoroimmunoassays (Delfia hGH and autodelfia insulin respectively, Wallac Oy, Turku, Finland). Reference value for fasting serum insulin was <144 pmol/L.
Serum concentrations of T4, T3, TSH, cortisol, FSH, LH, testosterone, estradiol as well as prolactin were measured using standard laboratory methods.
Oxytocin was determined in EDTA plasma by radioimmunoassay (RIA) after SEP-PAK_{18}-extraction (Waters Corporation, Mass., USA) as described previously (42). The detection limit was 3.2 pmol/L. In a previous study the normal mean ± SE was 15 ± 5 pmol/L (42).
Serum ghrelin was determined by a novel RIA, using recombinant human octanoylated ghrelin and ^{125}I-labelled ghrelin. The assay was calibrated against a commercially available RIA kit from Phoenix Pharmaceuticals (Belmont, CA, USA), and compatibility was complete. The lower detection limit was estimated to ~0.125 µg/L, and the within assay coefficient of variation averaged less than 5%. All samples were measured in one assay in duplicate. Normal values according to the Phoenix kit were 0.20-0.80 µg/L.
Serum leptin was measured with Human Leptin RIA kit (LINCO research, Inc. St. Charles, Missouri, USA). Mean reference leptin values ±SD in individuals with BMI 18-25 kg/m^2 were for men: 3.8 ± 1.8 µg/ L and for women: 7.4 ± 3.7 µg/L.
NPY was determined in plasma by RIA after SEP-PAK_{18}-extraction. The detection limit was 1.90 pmol/L and normal plasma levels were below 50 pmol/L.
Evaluation of serum lipids included analysis of triglycerides, total cholesterol, high density lipoproteins (HDL) and low density lipoprotein (LDL) cholesterol and lipoprotein (a), (Lp (a)) (43). Serum cholesterol and triglycerides were measured with colorimetric methods (Vitos 900, Johnson and Johnson, Ortoclinical Diagnostics, Rochester, NY) and HDL with direct colorimetry (Hitachi 911, Hialeah, FL). LDL concentration was calculated according to the formula suggested by Friedewald et al (44). Lp (a) was analyzed with nephelometry (NIH Image, Immunochemistry System, Beckman Instrument INC., Fullerton, CA).

V 3.8 Statistics

Results are presented as mean ± SEM unless otherwise stated. When values were normally distributed comparisons within and between groups were carried out with paired and

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unpaired Student's t-test. Otherwise the Mann-Whitney Rank Sum test was used. Linear regression analysis was performed by Pearson's Correlation when values were normally distributed; otherwise with Spearman's Correlation. Changes over time were analysed by one-way repeated measures analysis of variance (ANOVA) or Wilcoxon's matched pair test. χ^2-test was used for comparison between ratios.

VI Results and discussion

VI 1 GH-IGF-I axis

VI1.1 Baseline data

The majority of our patients had low spontaneous and low stimulated GH secretion (Paper I). GH responses to provocative stimuli were heterogeneous. The peak GH response was below the accepted cut-off of 3 µg/L defining severe GHD (14) in 9 out of 18 patients. In the other 9 patients, values ranged between 3.7 and 16.0 µg/L (mean 7.7 µg/L). The peak spontaneous GH concentration between 8 pm to 8 am was <3µg/L in 10 of the 12 patients, where these measurements were available. The remaining 2 patients had 7.4 and 7.7 µg/L respectively. In accordance with the low GH levels, average total IGF-I was subnormal. The values were below –2SDS of the mean of healthy age-matched subjects in 8 patients, between mean and –2SDS in 9 patients, while two had higher values (Table 1).

To further characterize the IGF system activity we measured free IGF-I, total IGF-II, GHBP and IGFBP-1, -2 and -3 (Paper III). At baseline serum free IGF-I did not differ from controls. Mean ± SEM was 1.02 ± 0.12 µg/ L versus 0.95 ± 0.15 µg/ L. Total IGF-II was within the reference range in 9 patients and subnormal in 8 (mean 704 ± 45 µg/L). IGFBP-1 showed a wide variation with the range 9-64 µg/ L. IGFBP-2 was normal in 5 patients and subnormal in 12 (mean 158 ± 24 µg/L). IGFBP-3 was normal in all patients (mean 3887 ± 243 µg/L).

Free IGF-I was positively correlated to total IGF-I ($r=0.78$, $p<0.05$), IGFBP-2 ($r=0.57$, $p<0.05$) and IGF-II ($r=0.69$, $p<0.03$), while no relationship to IGFBP-3 was found. Furthermore negative correlations were found between free IGF-I and IGFBP-1 ($r=-0.70$, $p<0.03$) and between IGFBP-1 and insulin ($r=-0.49$, $p<0.05$). No other correlations were found between insulin and the IGF-system components measured. There were no correlations between free IGF-I and BMI, % body fat or lean body mass. GHBP values were elevated with values above the reference range in 6 patients. We found positive correlations between GHBP and BMI ($r=0.49$, $p<0.05$), % body fat ($r=0.62$, $p<0.01$) and insulin ($r=0.59$, $p<0.05$).

There were no significant differences in total or free IGF-I between methylation positive and negative patients (Paper III). Nor did we see any significant difference in total IGF-II, IGFBP-1, -2 or -3 or GHBP. There was a trend that the methylation positive group had lower total IGF-I levels as well as total IGF-II and IGFBP-3, while GHBP was higher, all indicating a more severe GH deficiency in the methylation positive group.

The interpretation of variables related to the activity of the GH-IGF-I axis in PWS is complicated by the frequent presence of obesity. In obese, but otherwise healthy subjects, the circulating GH-IGF-system is characterised by low levels of GH, normal levels of total IGF-I, high levels of free IGF-I, total IGF-II, IGFBP-3 and GHBP, whereas IGFBP-1 and -2 are suppressed (45). In GH deficiency GH levels are low, free and total IGF-I, IGF-II and IGFBP-3 are low, while IGFBP-1 and -2 are elevated. In childhood PWS GH, total IGF-I and total IGF-II have been reported to be low, while IGFBP-3 has been reported to be normal (4, 46-54). The relative changes in some of the key parameters of the GH-IGF-I axis in simple obesity, GH insufficiency and PWS are summarised in Table 2.

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TABLE 2 Abnormalities in growth hormone (GH) secretion, total and free insulin-like growth factor (IGF) I as well as binding protein (BP) 1 in simple obesity, GH deficiency (GHD) and Prader Willi syndrome (PWS).

	GH secretion	Total IGF-I	Free IGF-I	Insulin	IGFBP-1
Obesity	↓	→	↑	↑	↓
GHD	↓	↓	↓	↓	↑
PWS	↓	↓	→	→↓	→

IGF-I circulates bound to a number of different binding proteins of which 6 high affinity proteins (IGFBP-1-6) have been identified and fully characterized. The IGF binding proteins 1-6 modify the actions of IGFs. IGFBP-3 is the quantitatively most important binding protein carrying around ¾ of the total IGF-I and its production is thought to be primarily under the influence of GH. By contrast insulin is regarded the principal regulator of hepatic production of IGFBP-1 (55). Subjects with simple obesity usually have normal levels of serum total IGF-I, despite low GH levels (56). This may be caused by the hyperinsulinaemia, suppressing circulating levels of IGFBP-1, and hereby increasing free (i.e. bioavailable) IGF-I (Fig 2) and hence the negative feedback on the hypothalamic-pituitary level (45, 56, 57). It could be argued, that the low spontaneous and stimulated GH secretion found in PWS patients are the result of co-existing obesity. However, in our obese PWS adults we found low GH levels, low total IGF-I levels and in relation to the obesity, low free IGF-I, low total IGF-II and non-suppressed IGFBP-1. These results are consistent with partial GH deficiency in adult PWS. Levels of IGFBP-3 were normal in our patients. The levels of IGFBP-3 in partial GHD is not known, but the reason for the normal levels we found, could be that other factors than GH are involved in the regulation of IGFBP-3 levels.

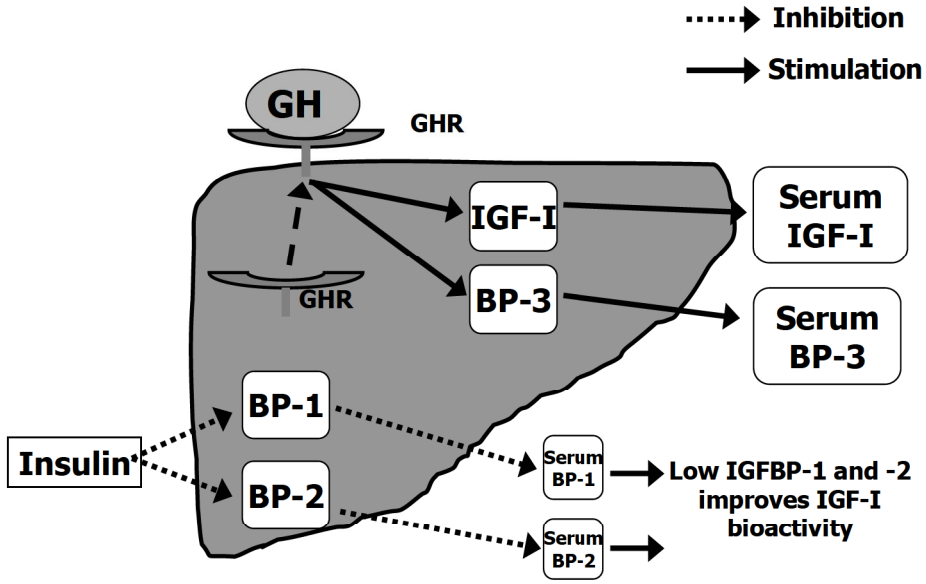


Fig 2 The regulation of the hepatic IGF-I synthesis.
Growth hormone (GH), GH receptor (GHR), GH binding protein 1, 2 and 3 (BP-1, BP-2, BP-3), insulin-like growth factor-I (IGF-I).

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VI $_{1.2}$ Effects of GH treatment

As in childhood PWS, GH treatment in our patients disclosed the expected rise in IGF-I (Paper II, III) (Fig 3).

During the placebo controlled period in our study mean total and free IGF-I increased in the GH treated group while there was no change in the placebo group. The changes between the two groups were significantly different (Paper II, III). In the entire group, free and total IGF-I increased significantly ($p<0.01$) during the following GH treatment period and were within the age-related reference range at 12 months in 16 patients (Fig 3).

No significant changes in either total IGF-II or GH and IGF binding proteins were seen during GH treatment (Paper III).

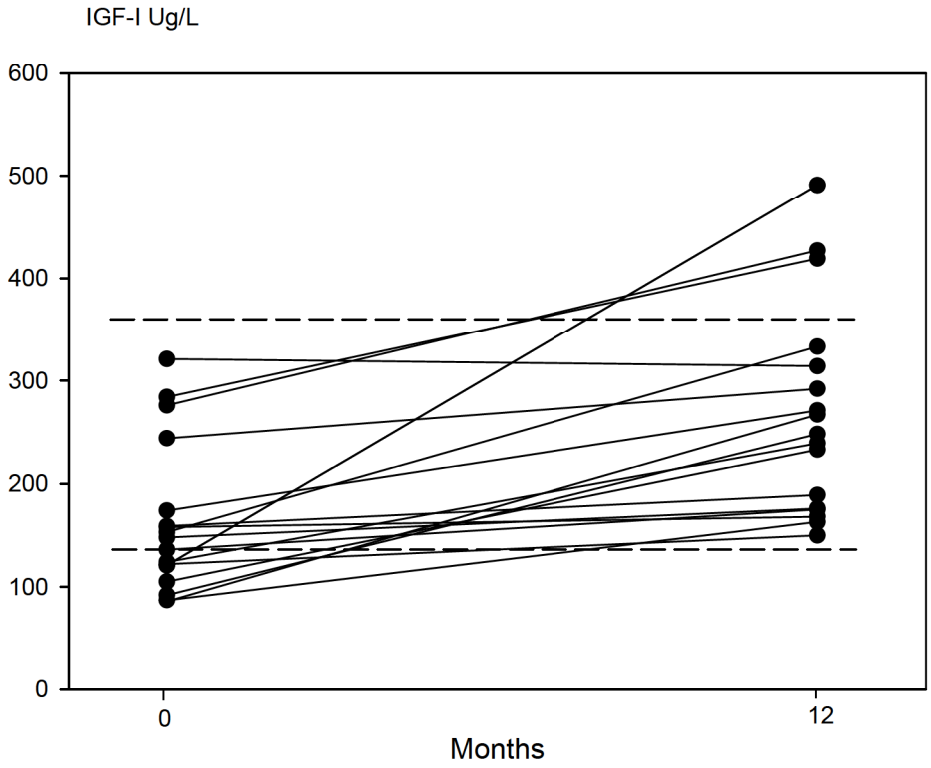


Fig 3 Serum IGF-I in 17 patients with Prader Willi syndrome at baseline and after 12 months GH therapy. Normal range is indicated with dotted lines.

VI$_{1.3}$ Conclusion

There is an impairment of the GH/IGF-I axis in both children and adults with PWS as judged from reduced spontaneous as well as stimulated GH-secretion. These findings may be seen in obesity but low circulating total IGF-I, normal free IGF-I, low total IGF-II and non-suppressed IGFBP-1 are consistent with the concept that PWS patients have a partial GH deficiency due to a hypothalamic pituitary insufficiency. GH replacement normalizes IGF-I levels.

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VI 2 Body composition: - body fat, lean body mass, BMD

VI 2.1 Baseline data

VI 2.1.1 Anthropometry

PWS has since long been known to be associated with short stature (1). In our cohort height ranged between 148 cm and 165 cm (mean 155 cm) in the females, and between 151 cm and 170 cm (mean 161 cm) in the males (Paper I). The corresponding height SDS were –3 to –0.5, mean –1.5, and –4 to –1, mean –2.5. Mean BMI was 35.6 kg/m^2 (n=19) (Table 1). Only three males had normal BMI, all the other patients had elevated BMI and the highest value found was 57.8 kg/m^2. Waist/hip ratio varied between 0.82 and 0.96, (mean 0.89) in the females and 0.81 to 1.01, (mean 0.90) in the males, i.e. all women had a ratio >0.8, while all but one man had a ratio <1.0. This indicates a similar fat distribution in the two sexes. Thus, the normal distinction in fat distribution between genders is less pronounced in PWS - most likely due to hypogonadism.

VI 2.1.2 Body composition and bone mineral density

The original description of the Prader Willi syndrome included obesity as one of the major clinical features (1). Total body fat has consistently been reported to be increased in children with PWS in conjunction with reduced lean body mass (4, 58). In accordance with this our patients showed a high percentage body fat, mean 49.4±2.5% (Paper I). Mean lean body mass in our patients was 38.4±2.1 kg. Goldstone and co-workers found that visceral fat estimated with MRI in adult PWS females was significantly reduced compared to other obese females, which were not reflected by a difference in waist/hip ratio (59). They also found that the proportion of visceral fat in PWS females was similar to non-obese controls. If this is true for the PWS males as well, the metabolic consequences of the increased fat mass might be less hazardous than previously thought. In fact a recent study in obese postmenopausal women showed that despite high levels of body fat women with lower amounts of visceral adipose tissue had a favourable metabolic profile, as compared to women with higher amounts of visceral adipose tissue (60, 61).

In our patients BMD was lower than in sex and age matched normal subjects (Paper I). Mean levels expressed as SDS of age matched reference material (Z-score) were –0.65±0.18 ($p=0.01$) in total body, -1.2±0.25 ($p<0.001$) in lumbar spine, -1.5±0.21 ($p<0.001$) in femoral neck and –1.06±0.28 ($p<0.001$) in trochanter region. According to the WHO criteria (25), four patients had osteoporosis (T-score below –2.5 SDS) and another 11 had osteopenia (T-score between –1 and –2.5 SDS). These results are compatible with our knowledge from studies in PWS children (4).

BMD has been shown to be reduced in adults with GHD (62), and our findings might be the result of a relative GHD as well as of hypogonadism representing a potential risk for fractures. It should be remembered however, that when assessing BMD using DXA technique short stature may lead to underestimation of BMD. On the other hand obesity tends to overestimate BMD.

VI 2.2 Effects of GH treatment

VI 2.2.1 Anthropometry

The mean BMI was unchanged both during the placebo-controlled part of the study and in the combined group (Paper II). Also mean waist-hip ratio remained constant. This is in contrast to the marked reduction in BMI reported for GH trials in children with PWS (4, 11). It is likely, however, that BMI is of limited value in assessing body composition in patients with PWS, because of the extreme relationship between body fat and lean body mass.

VI 2.2.2 Body composition and bone mineral density

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In the paediatric studies highly significant improvements of body composition towards normalisation have been reported (4). In the 6 months placebo controlled part of our trial in adult PWS patients we found a significant reduction in percent body fat in the GH treated group ($p<0.05$), while lean body mass increased albeit not reaching statistical significance (Paper II). No changes were seen in the placebo treated group.
The effect of GH was then evaluated in the combined group (n=17). Total body fat was significantly reduced after 6 ($p<0.05$) and 12 months ($p<0.01$). The mean reduction of total body fat was 2.5%, ranging from –12.8% to 5.6% (Paper II), (Fig 4). Regional analyses of the results obtained from DXA scanning showed that there was a significant reduction in percentage truncal fat after 6 and 12 months treatment ($p<0.01$) while the reduction in the right arm did not reach significance ($p=0.082$). Mean fat reduction at 12 months was 3.7 % in the trunk and 3.4% in the right arm.
Lean body mass increased significantly at 6 and 12 months compared to baseline ($p<0.05$), (Paper II), (Fig 5). The change in lean body mass between start of treatment and 12 months varied from –10.7 kg to 10.6 kg, mean 2.2 kg. The patient who decreased 10.7 kg in lean body mass had chronic oedema, which worsened shortly before the control at 12 months. His diuretic treatment was intensified, and during this period his weight loss was 13 kg.

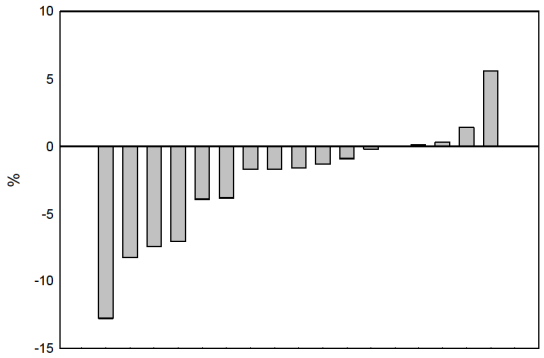


Fig 4 Changes in body fat (%) between baseline and 12 months growth hormone (GH) treatment in 17 adults with Prader Willi syndrome.

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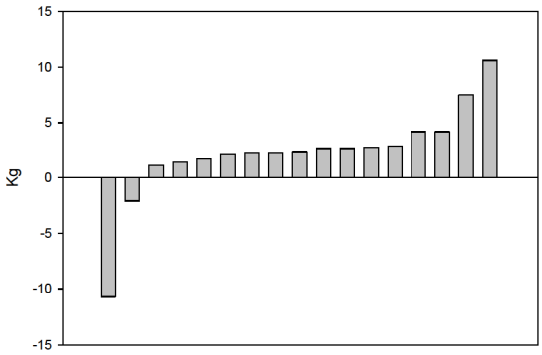


Fig 5 Changes in lean body mass (kg) between baseline and 12 months growth hormone (GH) treatment in 17 adults with Prader Willi syndrome

The reduction in percentage body fat and the increase in lean body mass in females and males between baseline and 12 months were not significantly different (p=0.15 and p=0.08, respectively). At 12 months the mean reduction in fat was –1.0 % in the females and –4.0 % in the males, while the mean increase in lean body mass was 2.5 kg in the females and 2.0 kg in the males. There was no correlation between IGF-I at baseline and increase in lean body mass during GH treatment.

The mean decrease in percentage body fat of 2.5 % and mean increase in lean body mass of 2.2 kg in our study, are similar to the findings following GH treatment in adults with severe GHD (63-65). The effect on body fat was, however, smaller than that described in similar trials in children with PWS (4, 21, 63). In these studies, the GH doses used were higher when adjusted for body weight, leading to higher levels of IGF-I.

Abdominal obesity often predominates in GHD but in PWS there is a significant fat accumulation in arms and legs, which was also shown in our study. As previously mentioned a recent study showed that abdominal fat in adult PWS women is mostly localized subcutaneously (59). If this can be generalised to adult PWS men, the slower or reduced mobilisation of subcutaneous fat could be an explanation why the changes in anthropometry in our patients were relatively modest.

BMD did not change significantly during 12 months of GH treatment, neither in the femoral neck nor in the lumbar spine. However, 12 months GH treatment is a short time for evaluation of GH's effects on BMD. Similar results were found in a study of adults with GHD (62).

Physical performance was not examined systematically, but anecdotally both the patients, their relatives and caretakers frequently reported improved physical capabilities which might reflect the improvement in lean body mass.

VI $_{2.3}$ Conclusion

BMI is highly increased and body composition abnormal in children and adults with PWS showing accumulation of fat, in particular in the periphery and reduced lean body mass. GH

Ex. 2057-022

treatment improves body composition in children as well as adult PWS patients with effects being more pronounced in the paediatric group. Bone mineral density is reduced in PWS and is not influenced by 12 months GH treatment.

VI $_3$ Carbohydrate and lipid metabolism

VI $_{3.1}$ Baseline data

Adults with GHD are characterized by insulin resistance (67, 68). The incidence of glucose intolerance and type 2 diabetes is considered to be high in PWS (10, 66). In both conditions it has been assumed that the reason is obesity causing insulin resistance. Studies in patients with PWS have, however, demonstrated normal or increased insulin sensitivity (10, 69) and low insulin levels compared to other obese subjects (45). The risk of developing diabetes in PWS might, therefore, be lower than previously suspected.

In our study insulin levels were normal, varying between 29 and 151 pmol/L with a mean of 80±10 pmol/L (Paper I). OGTT confirmed diabetes in one patient, showed impaired glucose tolerance in 4 patients, while it was normal in the remaining 14 patients. Insulin concentrations were similar in patients with normal and impaired OGTT. Using the HOMA index (26, 27) and the value of 2.77 as threshold for insulin resistance (28) 5 women and 4 men had values indicating increased insulin resistance. The patient with the highest index also had impaired OGTT. The other 3 patients with impaired OGTT had values below 2.77.

Dyslipidemia is frequently found in GHD adults (70-72), and GHD is associated with increased plasma cholesterol levels due to an elevation of LDL-cholesterol (70, 71). In the majority of our patients lipid profiles were within recommended limits not associated with increased cardiovascular risk (Paper I). This may be due to the very strict diet prescribed and (successfully) implemented in our patients. Lp(a), is an independent risk factor for cardiovascular disease not affected by diet or statins (73, 74), but mainly influenced by hereditary factors (71). Estrogens can lower Lp(a) concentrations (73). Five of our PWS patients had increased Lp(a) levels (>0.3 mmol/L), and three of them were women. They did not differ in oestrogen levels from the rest of the women.

In a study by Bonora et al (28) it was found that the prevalence of insulin resistance in persons with BMI>25 kg/m^2 was 43% in the absence of other metabolic disorders, increasing to 100% in the presence of 4 metabolic disorders. In our cohort one patient with low HDL (0.9 mmol/L) and normal LDL (2.1 mmol/L), another patient with low HDL (0.9 mmol/L) and increased LDL (3.4 mmol/L) and a third patient with increased total cholesterol (5.7 mmol/L) as well as LDL (3.9 mmol/L) had impaired glucose tolerance. Seven patients with HOMA index >2.77 had modest dyslipidemia in one or more of the measured lipids. These patients might, therefore, be at risk of developing cardiovascular disease. It can be argued, that in the patients, where we do not find any overt metabolic disorder, the intense efforts to reduce weight might be unnecessary at least with respect of preventing cardiovascular disease. However, the situation is complex in PWS and because of the hyperphagia and the reduced metabolic rate (75, 76) already established lifestyle intervention must be continued awaiting studies in larger cohorts.

VI $_{3.2}$ Effects of GH treatment

GH is known to have diabetogenic effects (77). Hence, GH treatment in itself might imply a further risk of diabetes. During the twelve months of GH intervention we saw no differences in mean glucose values. One patient however, had fasting blood glucose of 7.8 mmol/L and HbA_{1c} of 7.1% after 6 months of placebo treatment. Both values reverted to normal levels after 6 months on GH treatment, and remained normal for the rest of the study period. Insulin levels were 29-244 pmol/L (mean 76 ±9.2), and did not change during the treatment period, consistent with the previously mentioned unchanged IGFBP-1 levels (Paper II, III).

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In the 17 patients evaluated in the treatment trial OGTT was impaired in one, three and five patients at baseline and after 6 and 12 months treatment, respectively. However, two of the patients who became glucose intolerant showed an increase in body fat. It is therefore difficult to evaluate if the glucose intolerance in these cases was caused by the increased body fat, the GH treatment or a combination of the two. Individual insulin levels during the OGTT varied, but the insulin value calculated as area under curve (AUC) during 120 min did not change significantly during the GH treatment. Using the HOMA model (26, 27), no significant change in beta cell function or insulin resistance could be detected (p=0.771 and p=0.608, respectively). Mean beta-cell function was far above the assumed normal 100%, and mean HOMA index below threshold for insulin sensitivity. At baseline 7 patients and after 6 and 12 months of GH treatment 5 patients had a HOMA index above 2.77. Two patients displayed reduced HOMA indices during treatment. None of the patients developed overt diabetes during the treatment period. Neither baseline insulin levels nor 120 minutes AUC during OGTT correlated with BMI. Thus, there was no pronounced adverse effect of GH treatment on glucose and insulin homeostasis (Paper II).
In general, serum triglycerides, total cholesterol, HDL and LDL cholesterol showed insignificant changes within the normal range during the study period. Lp(a) levels, which were above 0.30 g/L in 5 patients, varied between 0.02-1.2 g/L (mean 0.35 ±0.16), and the individual values showed very small variations during the treatment period (Paper II).

VI$_{3.3}$ Conclusion
Due to the excessive obesity associated with PWS, the risk of diabetes is a priori high. IIowever, normal- or even relatively low levels- of circulating insulin are found in children as well as adults with well managed PWS. Furthermore, we found circulating lipids in adult PWS to be essentially normal. Thus, since these patients are in general normotensive (see below), the metabolic syndrome X seems not to be a common feature in this particular type of obesity. The carbohydrate and lipid metabolism were not adversely affected by GH treatment to a significant degree.

VI $_4$ Hyperphagia and appetite regulating peptides
VI $_{4.1}$ Baseline data
VI $_{4.1.1}$ Considerations on the interactions between appetite regulating peptides and hyperphagia
Knowledge of the hormones, neurotransmitters and neural pathways regulating energy homeostasis is rapidly increasing. However, at present it is not known whether the hyperphagia in PWS is caused by increased hunger or decreased satiation (78).
NPY and oxytocin are important hormones regulating hunger and satiation (79, 80), NPY being orexigenic and oxytocin anorexigenic. Other peptides involved in appetite regulation are leptin and ghrelin. Leptin is synthesised and secreted exclusively by adipose tissue and is associated with anorexigenic abilities (81). Ghrelin is mainly synthesised and secreted from the upper gastrointestinal tract (82), and is a strong orexigen (83-85). Ghrelin has recently been found to circulate in abnormally high concentrations in PWS patients (86, 87).
Animal experiments have shown, that lesions in the parvocellular oxytocin neurons in the paraventricular nuclei (PVN) in rat cause overeating and obesity (88) and that central administration of oxytocin to rats has anorectic effects (89). A previous study has shown, that the oxytocin producing neurons in the PVN are reduced in PWS (13), thus, potentially linking reduced oxytocin to hyperphagia. On the other hand, plasma oxytocin levels have been reported to be higher in simple obesity than in normal weighted controls (53±6 pmol/L versus 15±5 pmol/L) (42) - and these results have recently been confirmed (K Uvnäs-Moberg et al, personal communication).

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Circulating ghrelin levels are low in patients with adiposity as compared to normal weight adults (90, 91). In normal subjects a tendency towards lower plasma ghrelin levels was found under conditions of positive energy balance, and a tendency towards higher levels under conditions of negative energy balance, suggesting a dynamic relationship between ghrelin and energy balance (92). Furthermore, it has been shown that feeding decreases plasma levels of ghrelin in humans (84).
Leptin inhibits the gene expression of NPY in the arcuate nucleus (80), and there is a strong positive correlation between leptin levels and % body fat (93). The hyperphagia in PWS could theoretically be due to increased secretion of NPY at the CNS level due to defect leptin signalling. However, Goldstone et al. (94) have recently in a post-mortem material shown, that NPY immunocytochemistry staining and NPY mRNA expression, were reduced in obese PWS adults. This suggests normal response in PWS to peripheral signals, such as elevated leptin secondary to obesity.

VI 4.1.2 Circulating levels of oxytocin, ghrelin, leptin and NPY
In our group of adult obese PWS patients we found levels of oxytocin within and levels of ghrelin just above the normal range, while levels of leptin were high and NPY within the lower normal range at baseline (Paper IV). There were no significant correlations between oxytocin and body weight, BMI, body fat, insulin and IGF-I. Furthermore, there were no significant correlations between oxytocin and ghrelin, leptin or NPY. Mean serum ghrelin was 0.87 ± 0.12 µg/ L (normal range 0.20-0.80 µg/ L) and did not significantly correlate with either body weight, BMI, body fat, insulin and IGF-I, or with oxytocin, leptin or NPY. Mean serum leptin was 47.8±29.1µg/L and showed significant positive correlations with body weight, BMI and body fat. Leptin did not significantly correlate with insulin, IGF-I, oxytocin, ghrelin or NPY. Mean plasma NPY was 13±1pmol/L and did not correlate with body weight, BMI, body fat, insulin, IGF-I, oxytocin, ghrelin or NPY (Paper IV).
The physiological significance of normal oxytocin levels in PWS patients remains to be clarified, but normal oxytocin values in PWS instead of the expected obesity related high values are in concordance with oxytocin being an anorexigenic peptide.
The ghrelin values reported in our patients are not as high as the values reported by Cummings et al and DelParigi et al (86, 87), but similar to the levels reported by Haqq et al in PWS children (95). The reason for these discordant results is not clear at present. Variations in results comparing values from different assays of this relatively new hormone have to be considered. Our samples were obtained during standardized conditions at fixed hours in order to reduce variability. However, a number of known and unknown factors contribute to the short-term oscillations of ghrelin- including caloric restrictions and interaction with other peptides such as leptin. Since ghrelin was not completely suppressed,- which theoretically might be expected in these grossly obese individuals, -our results are not inconsistent with the suggestion that ghrelin is one of the significant contributors to hyperphagia in PWS. The negative correlation between ghrelin and insulin, BMI, fat mass and leptin shown in other studies (87, 96) did not reach statistical significance in our patients.
Our patients had high leptin values that were significantly related to percentage body fat suggesting the existence of a central leptin resistance. The same results have been found in children with PWS (97). Impaired leptin secretion is therefore not the explanation of hyperphagia in our cohort. Peripheral NPY levels were not elevated in our patients and although we do not have any measurements of NPY at the hypothalamic level, we suggest that also at the CNS level NPY concentrations are normal or low. Accordingly NPY is not a likely candidate of being among the orexigenic peptides leading to hyperphagia in PWS.

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VI 4.2 Effects of GH treatment

VI 4.2.1 Circulating levels of ghrelin, leptin and NPY

GH is an important regulator of glucose, lipid and amino acid metabolism (77), and could theoretically increase the metabolic rate through an increase in lean body mass. It is not known in detail if GH treatment changes the levels of ghrelin, leptin or NPY in PWS. A previous study has shown that GH treatment of other GH deficient patients does not change ghrelin levels (96). We were unable to find significant changes in ghrelin, leptin or NPY level in our patients during GH treatment. Oxytocin was only measured at baseline due to insufficient sample material.

VI 4.3 Conclusion

This group of obese adult PWS patients had low plasma oxytocin and elevated serum ghrelin relative to their obesity. Changes in the concentrations of these peptides may therefore be involved in promoting hyperphagia. Leptin levels were in general high reflecting obesity and as a consequence NPY levels were low. Levels of leptin, NPY or ghrelin were not changed by GH treatment in adult PWS patients.

VI 5 Cognitive, emotional, physical and social function

VI 5.1 Baseline data

Maladaptive behaviour, including temper outbursts, social unawareness, mood instability and stubbornness are frequent in PWS (2, 9, 12, 98-100). Many individuals need psychiatric support during several periods of their lives due to symptoms such as compulsive behaviour, depression or psychotic periods. Some degree of mental retardation is described in almost all PWS patients (12, 101). Mean intelligence quotient (IQ) is reported to be around 65. Speech development is commonly delayed with difficulties in articulation, phonation and fluency. Paper V demonstrated no major differences in baseline data between the patients assigned to GH or placebo treatment in medical or demographic characteristics. The patients showed mildly to moderately decreased results in all tests as compared to normal individuals. Cognitive level at baseline was estimated by one test of verbal comprehension (the SPIQ test) and two sub-tests of perceptual organisation (the Block Design and Coding tests), and IQ ranged between 40 and 90, median value 50-60. The patients on placebo scored a little better in two of the neuropsychological tests and had a better reaction time when using the right hand. The results in the females were almost always ahead of those of the males, and the females scored significantly better in two of the neuropsychological tests. There was no difference between patients with PWS genotype and those without.

The patients' responses at baseline to the first questionnaire revealed a very positive self-evaluation, although the majority admitted a desire for less weight. Twelve patients had a friend, and nine would like to live for themselves. We did neither find any differences in responses to the first questionnaire between the patients with or without PWS genotype nor between men and women at baseline (Paper V).

Eighteen responded the second questionnaire (Paper V). In 11 cases parents answered the questionnaire, and in one case an older brother. The remaining questionnaires were answered by caretakers. Among the four domains both the patients and the relatives/caretakers gave the somatic status the lowest scores, while the relatives/caretakers evaluated behaviour in mental, emotional and social domains poorer than the patients did. There were no differences in relation to gender or genotypes.

Ex. 2057-026

VI $_{5.2}$ Effects of GH treatment

After 6 months there were significant improvements in TMT B test and in reaction time for both right and left hand in the GH treated group, while the result of the tapping test was impaired for the left side in the placebo treated group (Paper V).

After 18 months, the group treated with GH for 12 months surpassed the group treated for 18 months in the Coding test, while both groups improved their results in the Block Design test and reaction time for the right hand. On the other hand significantly lower reaction time for the left hand and a better performance on the tapping test with the right hand were only seen in the group treated with GH for 18 months. The significant changes were seen predominantly in the males (Paper V).

The patients' responses to the self-report questionnaire during GH treatment did not change from the answers at baseline. Unfortunately, we did not receive sufficient responses from relatives/caretakers to the second questionnaire during this period to be able to make statistical calculations. Evaluation of the 6 months period, which followed immediately after cessation of GH treatment by means of the third questionnaire demonstrated that 1/3 of the patients, had impairment in mental and emotional domains both at 3 and 6 months. Half of the patient group showed impairment in physical status after 3 months and 2/3 after 6 months ($p<0.01$). Two thirds of the patients showed impairment in social behaviour both at 3 ($p=0.03$) and 6 months ($p=0.045$). The relatives/caretakers evaluated almost 2/3 of the patients to have a decreased over-all functioning after 3 months without GH treatment, augmenting to ¾ after 6 months ($p<0.01$), as compared to their capacity during the GH treatment period (Paper V).

Two men in our cohort became increasingly aggressive and had many more temper outbursts 1½ and 2 months, respectively, after discontinuation of GH treatment. One of them devastated his room and started beating his caretakers. In both cases the mood became more stable and less aggressive after reinstitution of GH therapy.

We did not expect that GH treatment would change the PWS stigmata or the complex cognitive processing. We were, however, able to demonstrate some increments in intellectual speed and flexibility as well as decreased reaction time, in accordance with the anecdotal improvements in quality of life reported about most of our patients. In most of the neuropsychological tests, results were better after 18 months than at baseline, but the number of patients may have been inadequate to show significant differences. On the other hand the impairment in physical, social and the over-all functioning, seen after GH treatment was discontinued, is indirectly indicating an effect of GH.

VI $_{5.3}$ Conclusions

This study showed that our patients had the expected mild to moderate mental retardation. The results of some of the cognitive tests improved during GH treatment implying a potential for further mental development. In addition improvements in reaction time and motor speed were seen throughout the treatment period. At baseline our questionnaires did not give the impression that these adult PWS patients had major cognitive, emotional, physical or social problems, neither according to themselves nor their relatives/caretakers. No changes were seen in the self reported questionnaires during GH treatment. However, the patients' physical and social status as well as their over-all capacity declined significantly, according to relatives/caretakers, when GH treatment was discontinued. The deterioration associated with cessation of GH therapy is in our opinion very suggestive of improvements taking place during GH treatment.

Ex. 2057-027

VII Comparison between PWS-patients with and without chromosomal abnormalities

All our patients fulfilled Holm's criteria for the Prader Willi diagnosis but only thirteen patients had a pathological (positive) methylation test while the remaining six patients were methylation negative (Paper I). The mean Holm score for the methylation positive Prader Willi patients was 11.5±0.19 points, and for the methylation negative patients 9.3±0.57 points. The scores were evenly distributed between major and minor points in both groups. We compared the patients with PWS genotype i.e. positive methylation test to those who had negative methylation test. The methylation positive group consisted of 13 patients, 7 men and 6 women, and the methylation negative group of 6 patients, 3 men and 3 women (Table 1). The groups were similar with respect to age, BMI, waist/hip ratio and percent body fat. Although the GH response to arginine was not significantly different between the groups, total IGF-I serum concentrations were significantly higher in the methylation negative patients, and they had also a tendency to a higher height SDS score (Paper I). The lumbar spine BMD Z-values were significantly lower (p=0.005) in the methylation positive patients, while the results obtained at the other measurement sites (femoral neck, trochanter region and total body) showed no significant differences between the two groups (Paper I). The groups had similar mean blood pressure, HbA1c, insulin, leptin and lipid levels, except Lp(a) which was significantly higher in the methylation negative group. The baseline HOMA index (26, 27) was not significantly different between the two groups. Also similar results from the psychological tests and appetite regulating peptides were seen.
During GH treatment the mean IGF-I increase at 12 months was 114 ±30 µg/L in the methylation positive group and 85 ±33 µg/L in the methylation negative group. The difference between the groups was not significant (p=0.543).
In the methylation positive patients the percentage body fat decreased significantly at 6 and 12 months (mean -3.8±1.3%) while the reduction in the methylation negative group was smaller and did not reach statistical significance. The concomitant change in lean body mass was 3.7 ±0.9 kg and -0.4 ±2.2 kg, respectively. Thus, despite the comparability with respect to BMI, body composition and metabolic parameters (except for IGF-I levels) and despite a similar increment in IGF-I, significant effects on body composition were seen only in the methylation positive group. The reason for this is not clear, but patients carrying the PWS genotype may have a higher GH sensitivity which is not necessarily displayed in the IGF-I concentrations.

VIII Safety

GH treatment was generally well tolerated. Side effects were mild and could be attributed to fluid retention. Two women developed oedema during the treatment period. The oedema reversed when the GH dose was reduced. One man with chronic peripheral oedema needed intensified diuretic treatment after 10 months of GH treatment. Except for impaired OGTT in 5 patients (one patient with existing impaired glucose tolerance at study entry normalised during GH treatment), no other adverse events were recorded. In particular, no patients developed diabetes. Blood pressure (125±10/75±5 mmHg at baseline) and routine chemistry (haemoglobin, electrolytes, creatinine and liver transaminases) were unchanged and normal during the treatment period, with the exception of two women who had slightly decreased haemoglobin concentrations at study start.

Ex. 2057-028

IX General Conclusions

Adult PWS patients suffer from partial GH deficiency, as evidenced by reduced spontaneous and stimulated GH secretion, low total IGF-I levels and normal concentrations of free IGF-I. The abnormal body composition seen in these patients can in part be explained by the reduced activity of the GH-IGF-I axis.
GH treatment to adults with PWS normalises the impairment of the GH-IGF-I axis and body composition improves.
The carbohydrate and lipid metabolism are not significantly altered by GH treatment.
Plasma oxytocin was abnormally low and serum ghrelin abnormally high in relation to obesity; therefore both peptides might be involved in promoting hyperphagia. GH treatment did not influence circulating levels of ghrelin, leptin and NPY.
Significant effects in intellectual speed and flexibility and improvements in reaction time and motor speed were seen during GH treatment. Impairment was demonstrated in physical, social and over-all functioning, when GH treatment was discontinued.
It is justified to carry out larger studies, to document the long-term effects of GH replacement, in order to further evaluate this potentially beneficial treatment in adults with PWS.

Ex. 2057-029

X Svensk sammanfattning

Prader Willi syndrom (PWS) är en genetisk sjukdom karakteriserad av muskulär svaghet, överätning (hyperfagi), fetma och beteenderubbningar. Partiell brist på tillväxthormon (GH) och nedsatt nivå av könshormoner är vanligt, vilket kan innebära en risk för sjukdom senare i livet. Flera studier av GH behandling till barn med PWS har visat förbättrad längdtillväxt, kroppssammansättning och kondition. Effekterna av GH behandling till PWS barn har väckt intresset för behandling av vuxna med PWS för om möjligt förhindra långsiktiga konsekvenser av GH brist. Fram tills nyligen har varken konsekvenserna av syndromet hos vuxna eller effekterna av GH behandling till dessa varit kända i detalj.

Syfte: Att undersöka hormoner, ämnesomsättning och psykosociala funktion hos en grupp vuxna med PWS före och under behandling med GH.

Patienter och metoder: Nitton vuxna med kliniskt diagnosticerad PWS undersöktes initialt. Tretton hade kromosomförändringar, som vid PWS. Sjutton (9 män, 8 kvinnor), medel ålder 25 år och medel BMI 35 kg/m^2, genomförde 12 månaders behandling med GH.

Resultat och diskussion: Alla, utom 3 män, var överviktiga trots strikt diet. Midja/höft kvoten var ökad hos alla kvinnor och procenten kroppsfett var hög hos båda könen. Aktiviteten av GH och GH-beroende tillväxtfaktorer, t.ex. insulinliknande tillväxtfaktor(IGFI)-I var låg. Cirka 2/3 hade låga koncentrationer av könshormon. Bentätheten var sänkt. Fyra patienter hade nedsatt glukostolerans, moderata förändringar i blodfetter fanns hos 7 patienter. Insulinnivåerna var låga med hänsyn till övervikten. Patienter med kromosomförändringar var kortare och hade signifikant lägre IGF-I.

Tolv månaders GH behandling med doser, som normaliserade IGF-I, reducerade kroppsfettet och ökade fettfria kroppsmassan signifikant utan förändring av totala kroppsvikten. Störst effekt sågs hos patienterna med kromosomförändringar. Kolhydrat- och fettomsättningen påverkades inte under behandlingen.

Orsaken till hyperfagi vid PWS är inte känd. Ett flertal peptid hormoner är normalt involverade i reglering av hunger och mättnad. Vi fann ingen störning i relationen mellan fettvävens hormon leptin och aptitökända hormonet NPY. Däremot var hormonet oxytocin, som är aptithämmande , lågt i relation till patienternas övervikt, och det aptitstimulerande hormonet ghrelin från magsäcken förhöjd. Oxytocin och ghrelin kan därför tänkas vare av betydelse för ätbeteendet vid PWS. Peptiderna förändrades ej under GH behandlingen.

Den intellektuella processens hastighet och flexibilitet ökade och reaktionstiden och motoriska funktioner förbättrades under GH behandlingen. Patienternas kapacitet försämrades, när de slutade med GH.

Slutsats: GH behandling kan förbättra flera av de kliniska symptomen och förändringar i hormoner och ämnesomsättning, som ses vid PWS. Det är dock viktigt att komma ihåg att GH-IGF-I systemet och effekterna av detta endast är en mindre del av problemen vid PWS. Det uppmanas till större och längre varande studier.

Ex. 2057-030

XI Acknowledgements

I am deeply indebted to many who has supported me, assisted me and in different ways made this work possible. My most sincere gratitude I wish to express to:

All Prader-Willi adults, their relatives and caretakers for their kindness and interest in participating in my studies.

Marja Thorén, my supervisor for encouraging me, for generous support, for constructive criticism and for always having the time to discuss.

Anna-Lena Hulting, my co-supervisor for friendship, support and for creating an inspiring atmosphere of daily work.

Kerstin Brismar and Suad Efendic for always being interested in my work and for providing a stimulatory research environment at the Department of Molecular medicine.

Agneta Hilding, Hans Jacobsson , Jan Frystyk, Ulrik Espelund, Maria Petersson and Birgitta Böhm my co-authors for invaluable comments and cooperation, and Agneta Hilding in addition for always having the time to give me advices on graphic illustrations and statistics.

Per Hellström, Claude Marcus and Jens Sandahl Christiansen for stimulating scientific discussions.

Kerstin Hall for interest and help with statistics.

Eva Grenbäck my friend and roommate for support, enthusiasm and our many talks about very important and less important things in life.

Anette Härström for taking excellent care of the patients.

Kerstin Florell for helping me creating figures and pictures.

Sibylla Philipsson for professional secretarial assistance.

Inga-Lena Wiwall and Elisabeth Noren-Krog for important work in the laboratory.

All staff and colleagues in the Department of Endocrinology and Diabetology for friendly and helpful assistance.

Victor and Hans for patience and love.

The studies were supported by grants from the Karolinska Institute, the Swedish Research Council (no 4224) and Pharmacia Sweden.

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