Skeletal Muscle Characteristics and Motor Performance After 2-Year Growth Hormone Treatment in Adults With Prader-Willi Syndrome

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Context: In adults with Prader-Willi syndrome (PWS), abnormal body composition with decreased lean body mass and skeletal muscle (SM) volume has been related to altered GH secretion and may possibly contribute to greatly reduced motor capacity.

Objective: The scope of the study was to test the hypothesis that GH treatment has favorable effects on SM characteristics and motor performance in adults with PWS.

Design, Setting, and Participants: Fifteen obese PWS subjects (nine males and six females; age range, 19–35 y; body mass index, 37.7–59.9 kg/m^2) were investigated before and after 12 (GH12) and 24 (GH24) months of GH treatment.

Main Outcome Measures: SM cross-sectional area and SM attenuation were determined with computed tomography at the lumbar and midthigh levels. Maximal isometric handgrip strength and isokinetic knee extension peak torque were measured. Motor performance was evaluated with different indoor walking tests, whereas exercise endurance was assessed with a treadmill incremental test to exhaustion.

Results: A condition of severe GH deficiency was found in six patients (40%). GH treatment significantly increased lean body mass (GH12, $P < .05$; GH24, $P < .05$), reduced percentage of body fat (GH12, $P < .05$; GH24, $P < .05$), and augmented SM cross-sectional area and SM attenuation of both lumbar (GH12, $P < .01$; GH24, $P < .001$) and thigh muscles (GH24, $P < .05$). Handgrip strength increased by 7% at GH12 ($P < .05$) and by 13% at GH24 ($P < .001$). Peak torque of knee extension extrapolated at zero angular velocity was significantly higher at GH24 ($P < .01$), and exercise endurance rose by 13% ($P < .05$) and 17% ($P < .05$) before exhaustion at GH12 and GH24, respectively, whereas no change was detected with walking tests. No significant difference in the response to GH treatment was detected between patients with and without GH deficiency.

Conclusion: Long-term GH treatment in adult PWS patients improves body composition and muscle size and quality and increases muscle strength and exercise tolerance independently from the GH secretory status. **(*J Clin Endocrinol Metab* 99: 1816–1824, 2014)**

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Abbreviations: ATTSM, attenuation of skeletal muscle; BM, body mass; BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; CSASM, cross-sectional area of skeletal muscle; CT, computed tomography; ECG, electrocardiogram; FM, fat mass; GH12, 12-month GH treatment; GH24, 24-month GH treatment; GHD, GH deficiency; HbA1c, glycosylated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; HR, heart rate; LDL-C, low-density lipoprotein cholesterol; PTKE, peak torque of knee extension; PWS, Prader-Willi syndrome; RPP, rate pressure product; SHG, handgrip strength; v, angular velocity of knee extension; WT, walking test.

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Prader-Willi syndrome (PWS) is a rare chromosomal disorder characterized by muscular hypotonia, dysmorphogenetic abnormalities, behavioral disturbances with cognitive impairment, hyperphagia leading most subjects to develop morbid obesity from early childhood, hypogonadism, and growth failure (1). Reduced GH response to different stimulation tests and low IGF-1 levels have been documented in both children and adults with PWS (2, 3). Impaired GH secretion in PWS adults is revealed not only by deficits in secretion amplitude parameters, but also by abnormalities in the shape of the secretory response as demonstrated by deconvolution analysis (4). Besides short stature, additional features possibly related to a condition of GH deficiency (GHD) are detectable in PWS from infancy to adulthood, including higher fat mass (FM) with distribution abnormalities and reduced lean body mass (BM), involving skeletal muscle tissue (5, 6). Although systematic studies on skeletal muscle size and quality are not available in PWS patients, reduced muscle mass with poor quality is presumed to be implicated in the considerable strength limitations observed in PWS (7) and may contribute to the overall poor motor function (8).

Several studies have documented the benefits of GH therapy in infants and children with PWS, including normalization of height and improved metabolic status in adulthood, increased lean mass, and decreased body fat (9–11). Recent reports have also documented in PWS adults these favorable findings observed in children. Increase in lean BM and reduction of body fat, without significant impairment of glucose homeostasis, are commonly reported in PWS adults after interventions ranging from 1- to 5-year treatment with GH (12–14), as confirmed by a recent meta-analysis (15). Moreover, Sode-Carlsen et al (16) also found that thigh muscle size increased in adult PWS patients after a 24-month GH treatment, but muscle functionality was only indirectly assessed. Indeed, studies testing the effects of GH treatment on motor performance in adult PWS patients are exceedingly scanty (17). To our knowledge, exercise capacity during a treadmill test after 1-year GH treatment in such patients was previously measured only in a study from our group, which reported a significant improvement (18). However, in that study, we did not analyze changes in muscle size and the strength production capability. Recently, Butler et al (19) did not detect significant changes in muscle strength during isotonic maximal tests in response to 12-month GH administration, despite significant increases of lean mass and spontaneous physical activity at moderate-vigorous intensity.

Taken together, these disparate findings indicate that GH therapy in PWS adults safely improves body composition and metabolic functions. However, a characterization of changes in muscle structure and functional features for effect of GH treatment is scarcely available, especially in relation to the improvements of motor function, for which reports are conflicting.

We therefore assessed in a group of PWS adults the functional impact of changes in skeletal muscle characteristics induced by 2-year treatment with GH, with the hypothesis that motor functionality is improved by GH supplementation.

Subjects and Methods

Subjects

Fifteen obese adult PWS subjects (nine males and six females; age range, 19–35 y; mean age ± SD, 26.1 ± 5.4 y) were consecutively enrolled into the study. Twelve patients had an interstitial deletion of the proximal long arm of chromosome 15 (del15q11-q13), whereas maternal uniparental disomy for chromosome 15 was found in the remaining three individuals. Eight males had previously undergone GH treatment but withdrew in all cases 2–4 years before starting the study. One patient had type 2 diabetes and was treated with insulin. All subjects showed normal findings in the main laboratory test, including adrenal and thyroid functions. All patients achieved a score > 24 in the Mini Mental State Examination (20), which warranted an intellective level allowing appropriate compliance. None of the subjects presented orthopedic conditions possibly limiting their capacity of movement.

The study was approved by the Ethical Committee of Istituto Auxologico Italiano. Written informed consent was obtained from the parents, and from the patients when applicable.

Anthropometric measurements

Weight and height were measured by standard techniques, and the body mass index (BMI) cutoff point of 30 kg/m^2 was used to define obesity. At baseline, all PWS patients were obese (BMI, 37.7–55.5 kg/m^2). Waist and hip circumferences and waist-to-hip ratio were also determined (21). BM, FM, and lean BM were measured by dual energy x-ray absorptiometry (GE Lunar).

Endocrine characterization and study design

At baseline, a standard GHRH + arginine test indicated that 40% of the participants presented a severe degree of GHD, GH levels being below the BMI-related cutoff point in six of them (22). In addition, basal IGF-1 serum levels were determined. Methodological details are given as Supplemental Data [published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org].

After baseline evaluation, patients received GH therapy (Genotropin; Pfizer) for 24 months at a mean starting dose of 0.019 ± 0.005 mg/kg/wk for the first month. The starting GH dose varied from 0.012 to 0.036 mg/kg/wk on the basis of the presence of edema, age, use of oral estrogen, and previous GH therapy and sensitivity. Subsequently, the dose was adjusted to maintain serum total IGF-1 within ±2 SD from an age-matched reference value to avoid overdosing. The mean GH dose was 0.034 ± 0.019 mg/kg/wk after the first 12-month GH treatment

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(GH12) period and 0.027 ± 0.012 mg/kg/wk after 24 months (GH24).

All outcome variables were determined before starting GH treatment (baseline) and at GH12 and GH24, with patients being admitted to the hospital for clinical measurements and functional testing.

Metabolic evaluation

At baseline and at the end of each study period, fasting blood samples were collected for determination of glucose, glycosylated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides, and C-reactive protein (CRP). Insulin levels were measured in 14 subjects (excluding the patient with diabetes).

Skeletal muscle characterization

In all participants, computed tomography (CT) images were acquired by a GE tomograph (GE Healthcare) and consisted of a series of three contiguous transaxial slices, imaged as 512 × 512 voxel matrices with 5-mm slice thickness and 0.89-mm pixel size, centered on the fourth lumbar vertebra, identified with preliminary scout imaging. The skeletal muscle cross-sectional area (CSASM) of paraspinalis muscles, quadratus lumborum, and psoas muscle were calculated after interactive identification and segmentation of muscle tissue on axial slices. In seven subjects (four males and three females), CSASM was also assessed at the midthigh level of the dominant leg. Attenuation of skeletal muscle (ATTSM) tissue was determined in the same slices used for area measurement. The Hounsfield values obtained in five different quadrangular regions of interest (20–30 mm^2), randomly positioned excluding intermuscular and visible im adipose tissue, were averaged for each muscle. All image analyses were performed using the public domain software package Medical Image Processing, Analysis and Visualization-MIPAV (Center for Information Technology, National Institutes of Health; http://mipav.cit.nih.gov, last accessed September 27, 2013).

Strength measurement

Maximal individual voluntary isometric strength of the hand (handgrip strength [SHG]) and forearm muscles was determined with a handgrip dynamometer (Baseline Hydraulic Hand Dynamometer; Fabrication Enterprises) by averaging the best result of three consecutive trials obtained with both hands. Maximal voluntary isokinetic peak torque of knee extension (PTKE) muscles of the dominant limb was assessed with an isokinetic dynamometer (Cybex Norm; CSMi) at 60, 180, and 240°/s of angular velocity (v) during knee extension. The best of three consecutive measurements was considered in every condition.

Motor performance evaluation

Walking velocity determined by photocells was assessed during 10- and 20-m walking tests (WTs), accomplished indoors at the maximal attainable velocity along a flat corridor after the operator's starting signal. The 20-m WT was performed with an inversion of direction after the first 10 m. Agility was assessed with a modification of the 10-m WT, entailing three consecutive approximately 90° changes of direction in correspondence to three marking cones at regular intervals on the floor (slalom WT). The tests were performed on different days and in random order.

Exercise endurance and cardiocirculatory testing

Cardiodynamic responses to exercise endurance were estimated with a modification of the Bruce treadmill stress test to exhaustion, suitable for the limited capabilities of patients with PWS. A Marquette motorized treadmill (series 2000) was used together with a Marquette Max Personal electrocardiogram (ECG) instrumentation (Marquette General Health System). Patients started the test at least 2 hours after a light breakfast at a velocity of 1.5 km/h and 0° incline; settings were increased by 0.3 km/h and 1° per minute, respectively, and the test continued until exhaustion, as indicated by limiting symptoms such as fatigue, dyspnea, or muscular pain. ECG, heart rate (HR), and oxygen saturation (pulse oxymeter NPB 295; Nellcor Puritan Bennett Inc) were continuously monitored throughout the entire test. Arterial blood pressure (BP) was measured with a standard electronic clinical device at baseline in a standing position on the treadmill and every 2-minute interval during the test. The product of HR × peak systolic BP (rate pressure product [RPP]) was used as an index of myocardial oxygen requirements during exercise. In no instance did tests have to be discontinued due to abnormalities in ECG or BP or a lack of patient compliance.

Statistical methods

All values are given as means ± SD. The significance of differences between the average values obtained for the whole group at baseline and at the end of each study period (GH12 and GH24) was assessed with a Wilcoxon signed rank test. Regression line equations were calculated with the least-square method, and the differences between regression lines were tested using conventional regression equation comparison procedures (23). *P* values less than .05 were considered statistically significant.

Results

A preliminary analysis comparing individuals with and without GHD did not reveal any significant difference for biochemical parameters, body composition, muscle characteristics, strength development, and exercise tolerance at baseline or after GH treatment. Similarly, we have detected no difference between GH-naive patients and subjects previously treated with GH. The patients were therefore analyzed as a whole group.

BM and body composition

Table 1 shows the average values of BM, body composition, and indices of adiposity distribution observed at baseline and after GH. Although BM and adiposity distribution were unaltered, lean BM increased and FM percentage decreased at GH12 and GH24.

Clinical chemistry

The values of biochemical parameters measured at the different periods of the study are also presented in Table 1. Average blood glucose levels were within the normal limits at baseline and remained as such throughout the study. Although no significant change was observed in fasting insulin

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Table 1. Average Values ± SD of Parameters Related to BM, Adiposity, and Body Composition and Biochemical Parameters Determined in 15 Patients With PWS Before (Baseline) and at Different Times After the Onset of GH Treatment

	Baseline	GH12	GH24
BM and body composition			
BM, kg	108.4 ± 15.6	107.3 ± 18.9	109.6 ± 20.9
BMI, kg/m^2	45.0 ± 4.7	44.6 ± 6.6	45.1 ± 7.3
Lean BM, kg	45.6 ± 8.5	48.0 ± 7.6[a]	48.4 ± 9.0[a]
FM, %	55.8 ± 4.9	52.8 ± 4.9[a]	53.9 ± 5.0[a]
WC, cm	126.7 ± 10.7	122.7 ± 13.6[a]	124.9 ± 15.1
WHR	0.94 ± 0.08	0.93 ± 0.07	0.95 ± 0.07
Clinical chemistry			
Glucose, mmol/L	4.78 ± 1.11	5.07 ± 1.08	4.80 ± 1.15
Insulin, μU/mL	12.2 ± 6.1	16.0 ± 10.5	14.1 ± 7.8
HOMA-IR	2.46 ± 1.18	3.53 ± 2.61	2.93 ± 1.90
HbA1c, %	5.8 ± 0.7	5.5 ± 0.4[a]	5.6 ± 0.7
TC, mmol/L	4.67 ± 0.99	4.80 ± 0.86	4.76 ± 0.94
LDL-C, mmol/L	3.08 ± 0.81	3.18 ± 0.79	3.19 ± 0.83
HDL-C, mmol/L	1.29 ± 0.35	1.39 ± 0.33[a]	1.28 ± 0.36[d]
Triglycerides, mg/dL	107.5 ± 26.0	108.3 ± 36.4	109.1 ± 40.7
CRP, mg/L	1.8 ± 1.4	0.9 ± 1.1[c]	0.8 ± 1.0[b]
IGF-1, μg/L	97.1 ± 50.9	312.5 ± 132.7[c]	241.1 ± 99.8[c,d]

Abbreviations: WC, waist circumference; WHR, waist-to-hip ratio; TC, total cholesterol.

Significance of differences is assessed with Wilcoxon signed rank test (significantly different from baseline: [a] $P < .05$; [b] $P < .01$; [c] $P < .001$; significantly different from GH12: [d] $P < .05$).

and homeostatic model assessment of insulin resistance (HOMA-IR), in five patients at baseline and in six at GH12 and GH24, HOMA-IR was above 2.77 (24). However, a transient significant decrease in average HbA1c was observed at GH12. All lipid biomarkers were within the normal range throughout the study, although HDL-C transiently increased at GH12. The lack of effect of GH therapy on total or LDL-C seems to be explained by the normal values of these lipid biomarkers at baseline. Starting from an abnormally high value, CRP decreased significantly at both GH12 and GH24, whereas IGF-1 values increased throughout the whole period of treatment.

Skeletal muscle characteristics

Supplemental Figure 1 shows the result of lumbar and thigh muscle segmentation before and after GH treatment in a single representative subject, whereas the average values of CSASM and ATTSM determined at lumbar and midthigh levels in the investigated PWS patients are presented in Table 2. Overall, the treatment with GH induced significant increases in muscle size and attenuation at GH12 and GH24.

Strength measurement

Average values of maximal SHG observed before and at different times after GH treatment are presented in Figure

Table 2. Average Values ± SD of CSASM and ATTSM in the Lumbar Region Determined in 15 Patients and at Midthigh in Seven Patients With PWS Before (Baseline) and at Different Times After the Onset of GH Treatment

	Baseline	GH12	GH24
CSASM, cm^2			
Paraspinalis muscles	41.05 ± 10.16	42.83 ± 10.78[b]	43.97 ± 11.49[c,d]
Quadratus lumborum muscle	11.19 ± 3.05	11.62 ± 2.54	11.94 ± 2.84[b]
Psoas muscle	25.75 ± 4.69	26.16 ± 4.96	25.93 ± 4.62
All lumbar muscles	76.50 ± 14.15	79.06 ± 14.04[c]	80.25 ± 15.00[c]
Quadriceps muscle	61.82 ± 12.32	63.93 ± 11.46[a]	64.19 ± 11.55[b]
All thigh muscles	123.6 ± 19.8	126.7 ± 19.3	126.9 ± 19.2[a]
ATTSM, HU			
Paraspinalis muscles	38.61 ± 9.11	44.17 ± 8.25[b]	45.57 ± 7.68[c]
Quadratus lumborum muscle	37.09 ± 6.96	41.51 ± 5.68	42.49 ± 5.71[a]
Psoas muscle	48.44 ± 3.32	50.05 ± 3.09	50.04 ± 2.86
All lumbar muscles	41.83 ± 5.68	45.64 ± 5.88[b]	46.49 ± 5.49[c,d]
Quadriceps muscle	41.06 ± 6.99	41.20 ± 5.92	45.54 ± 3.77[a,d]
All thigh muscles	38.86 ± 5.01	37.99 ± 4.76	40.53 ± 3.87[a,d]

Significance of differences is assessed with Wilcoxon signed rank test (significantly different from baseline: [a] $P < .05$; [b] $P < .01$; [c] $P < .001$; significantly different from GH12: [d] $P < .05$).

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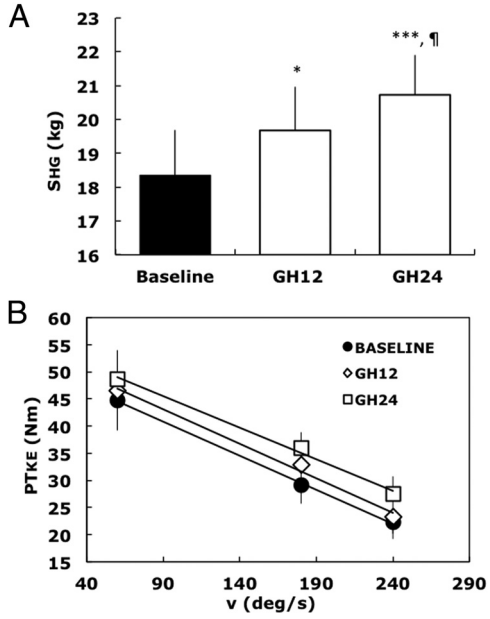

Figure 1. Average values of maximal SHG (A) and maximal PTKE (B) in 15 patients with PWS before (baseline) and after GH12 and GH24. Vertical bars represent 1 SD. Significance of differences from pretreatment values of SHG: *, $P < .05$; ***, $P < .001$; from GH12: ¶, $P < .05$ (Wilcoxon signed rank test). PTKE is plotted against angular velocity (v), and multiple regression comparison analysis indicated a significant increase in regression line elevation at GH24.

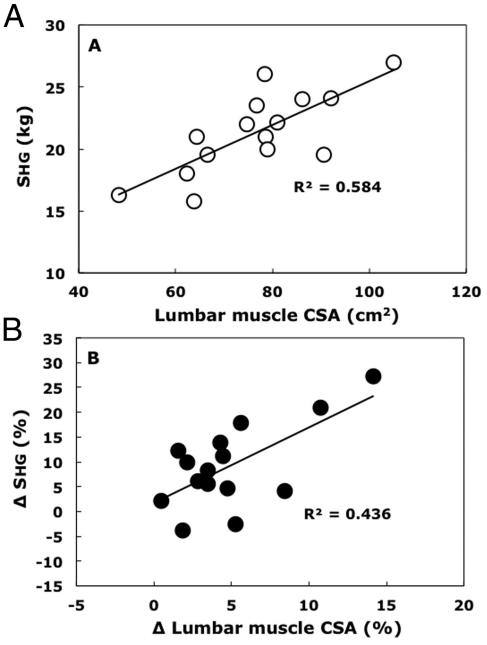

Figure 2. A, The relationship between single subject maximal SHG and lumbar muscle cross-sectional area (CSA) in baseline conditions before the start of GH treatment. B, The individual changes in SHG observed between baseline conditions and 24 months of GH treatment (ΔSHG) are plotted against the changes in lumbar muscle CSA (ΔCSA) in the same interval. Regression line through the points and R^2 values are also shown.

1A. Significant increases of SHG are detectable at GH12 and continue up to the end of the treatment period (GH24). The average values of PTKE obtained before treatment and at GH12 and GH24 are plotted as a function of v during knee extension in Figure 1B. According to the muscle force-velocity relationship, PTKE decreased with v in all conditions according to the linear regression equation: $\text{PTKE} = -a\,\text{v} + b$. Knee angle at which PTKE was attained was independent from v and was unaffected by GH treatment, being in the mean 127.3 ± 8.2° (90° = leg perpendicular to ground; 180° = fully extended knee). Although after the onset of GH treatment average PTKE increased at all v by an amount not statistically significant, a multiple comparison of the regression equations describing the relation between PTKE and v at different times of treatment revealed no significant difference between the slope coefficients ($-a$) but a significant increase in elevations (coefficients b), which represent the value of PTKE at $\text{v} = 0$ ($P < .01$). Compared to the baseline reference, a Tukey test for multiple contrast of elevations indicated a significant increase of this coefficient at GH24 ($P < .01$).

Taking lumbar muscle size as a general indicator of the degree of muscle development, Figure 2A shows the functional relationship between a single subject's SHG and CSASM at baseline. In Figure 1B, the strength improvements at GH24 are plotted against the corresponding changes in CSASM, indicating the relationship between structural and functional improvements.

Walking and treadmill stress tests

Average velocities before the beginning of GH treatment were 2.66 ± 0.41, 2.22 ± 0.36, and 1.11 ± 0.14 m/s for the 10-m WT, 20-m WT, and slalom WT, respectively. No significant velocity change was detected in the execution of any test at any time after GH treatment onset.

On the contrary, a significant increase in time leading subjects to exhaustion during the incremental treadmill stress test was evidenced both at GH12 and GH24 with respect to pretreatment values. None of the other considered cardiocirculatory parameters, including RPP, whose value indicated a moderate to high level of hemodynamic activation, were changed significantly at exhaustion for effect of treatment, as reported in Table 3.

Because the same maximal HR is attained independent from changes induced on test duration before exhaustion by effect of the intervention, a simple model can be devised. Because, during an incremental test, exercise intensity is proportional to the ongoing time of the test itself, a diagram representing the trend of HR as a function of exercise intensity can be depicted (Figure 3). The diagram shows that after GH treatment, the same exercise intensity is accomplished at a lower HR and that a higher exercise intensity can be sustained before subjects are led to exhaustion, indicating a net increase in exercise capacity.

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Table 3. Average Values ± SD of Time of Exhaustion and Different Cardiocirculatory Parameters Observed After a Treadmill Stress Test Performed by 15 Patients With PWS Before and at Different Times After the Onset of GH Treatment

	Baseline	GH12	GH24
Time of exhaustion, min	9.42 ± 2.54	10.62 ± 3.05[a]	10.97 ± 3.36[a]
HR, beats/min	151.8 ± 12.5	152.9 ± 9.1	157.1 ± 11.2
BP, mm Hg	147.5 ± 13.6	151.0 ± 20.2	145.0 ± 10.8
O_2 SAT, %	91.1 ± 4.8	92.2 ± 5.0	92.6 ± 3.9
RPP, beats/min ∗ mm Hg	22 344 ± 2290	23 165 ± 3894	22 796 ± 2091

Abbreviations: BP, systolic blood pressure; O_2 SAT, arterial blood oxygen saturation.

Significance of differences is assessed with Wilcoxon signed rank test (significantly different from baseline: [a] $P < .05$).

Discussion

Several studies provided evidence that GHD may be present in a remarkable number of PWS adults (3, 14, 25, 26), with consistent abnormalities in both amplitude and kinetics of stimulated GH secretion (4). According to appropriate BMI-related diagnostic cutoff limits of GH peak response during dynamic test (22), a condition of severe GHD was evidenced also in 40% of our subjects, the remaining part presenting GH values in the low-normal range. In addition, below-normal IGF-1 values were present before therapy in all but one patient. Therefore, the exploration of the effects of treatment with GH in this kind of patient appears well grounded, although the lack of cutoff points for BMI much higher than 30 kg/m^2 suggests caution in interpreting these results. It is, however, noteworthy that the improvements observed in the different outcomes presently investigated were independent from the GH secretory status assessed before supplementation, with important implications for the treatment of PWS with GH. In contrast to Coupaye et al (11), who reported an improved body composition and metabolic status several years after discontinuing GH therapy, the endpoints analyzed at the baseline and the results obtained in our study were not influenced by previous GH treatment.

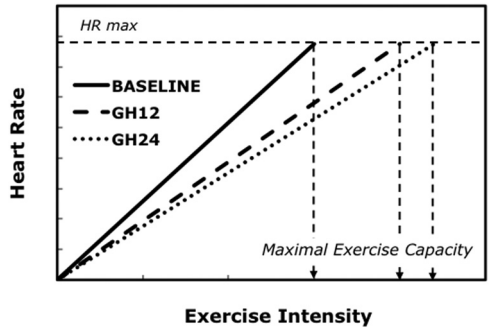

Figure 3. Diagrammatic representation of HR trends as a function of exercise intensity during treadmill test to exhaustion before starting treatment (continuous line) and after different times of treatment (dashed and dotted lines). The model assumes that, during an incremental test, exercise intensity is proportional to the time of the test.

These discrepancies could be related to both the small number and the different degree of obesity of our patients as well as to the different GH dosages.

From a functional standpoint, PWS individuals in the present study also had a seriously reduced capacity of static and dynamic strength production. Both maximal SHG and PTKE were very low in respect of normal or obese subjects (27–29), indicating that different muscle groups of upper and lower limbs are analogously implicated in the loss of strength associated with PWS.

Endocrine and metabolic responses to GH treatment

Our current results show that GH therapy promptly led to age-normalized IGF-1 levels in all cases. The incidence of altered glucose metabolism is considered to be high in PWS, particularly after the pubertal age (1). Therefore, the potentially diabetogenic effect of GH treatment in such patients should be carefully evaluated. In our investigation, GH therapy slightly impaired glucose homeostasis; glucose, insulin, and HbA1c levels remained within the normal limits during the whole period of therapy, despite a nonsignificant increase in fasting glucose, insulin, and HOMA-IR. Moreover, the only patient with type 2 diabetes at baseline did not require any modification of insulin therapy throughout the study period. Based on these findings, we therefore conclude that GH administration, which normalizes IGF-1 in obese PWS adults, does not seem to elicit pronounced adverse effects on glucose and insulin homeostasis.

Increased levels of CRP, a well-recognized marker of inflammation, have been significantly correlated with negative cardiovascular events, particularly in patients with weight excess (30). Raised levels of CRP have also been demonstrated in subjects with PWS (31). A significant reduction of CRP levels was observed at the different times of our study, as previously reported after 1 year of GH therapy (32). Thus, it is conceivable that the cardiovascular risk associated with the obesity-related inflammatory

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state may be partially contrasted by GH therapy in PWS patients.

Effect of GH treatment on skeletal muscle characteristics

Sound evidence indicates that CT imaging is a reliable method to estimate skeletal muscle size and composition (33, 34). Using this approach, we found evidence of a significant increase in CSASM of lumbar muscles (a muscle group involved in important postural actions during different locomotor activities and specific trunk movements) as well as of thigh muscles, after relatively prolonged GH treatment.

Such a size increase was also accompanied by an increase in ATTSM. Indeed, attenuation characteristics of tissues in vivo detected through the Hounsfield units scale of CT imaging provide the basis to assess the lipid content in muscle. In fact, due to the widely lower attenuation coefficient of fat, the interindividual variation of ATTSM reflects the degree of lipid infiltration (35), which increases with body adiposity and older age (36, 37).

Besides the changes in body composition consisting of a reduction of body fat percentage and increase of absolute lean BM (15), the present investigation also demonstrates a significant increase in skeletal muscle size at both the lumbar and thigh levels after GH treatment, which confirms the observations made by Sode-Carlsen et al (16) on thigh muscles and extends this finding also to lumbar muscles. Moreover, we also report a significant improvement in muscle composition with a substantial decrease of lipid infiltration in muscle tissue, which predicts improvements also in muscle function. Indeed, after accounting for the size of muscle mass, low values of ATTSM are associated with reduced motor performances (38, 39), besides an increased metabolic risk (37). Thus, qualitative attributes of muscle composition may contribute importantly to functional performance.

Effect of GH treatment on skeletal muscle strength and motor function

Compared with baseline values, we detected a significant increase in maximal SHG at GH12, which further increased at GH24, the changes in functional performance being correlated with increases in overall muscle size (Figure 2). Similarly, GH treatment positively affected isokinetic strength, which represents the capability of force production in dynamic conditions, as indicated by a significant shift to the right of the relationship between PTKE and v detected at GH24 in comparison with baseline conditions.

Although the effect of GH treatment in adult PWS patients on functional properties of skeletal muscle has been scarcely investigated, our findings concerning muscle size increase and strength improvement are in substantial agreement with observations deriving from the Scandinavian study (16). In this study, however, the increase in muscle strength was not directly measured, but indeed was inferred on the basis of the observed increase in peak expiratory flow. By contrast, Butler et al (19) failed to detect significant improvements of strength assessed with one-repetition maximum test at bench and leg press machines after GH12 in adult PWS cases, despite increases of spontaneous physical activity and quality of life. Possibly those PWS patients lacked a certain amount of skillfulness required for achieving a proper lifting technique and attaining reliably measurable strength changes.

In terms of motor capabilities, we did not detect changes in walking performance during different indoor tests. Due to the relevant components of coordination, agility, and attentive capacity required in the accomplishment of the WTs, especially the slalom WT, the patients, who were typically endowed with poor gross motor skills (40), may have been unable to improve their walking performance during these tasks involving rapid changes of direction and brisk accelerations and decelerations along predetermined tracks.

On the contrary, however, GH treatment induced a significant improvement in exercise tolerance during the aerobic stress test on the treadmill. Under these conditions, the duration of the test before exhaustion increased significantly after GH treatment, whereas cardiocirculatory parameters, including HR attained at the end of the test, remained unchanged. Indeed, a complete evaluation of cardiac hemodynamics during the treadmill stress test was investigated in the present study, and available information indicates a good preservation of cardiac functionality at exhaustion, especially in terms of myocardial oxygen requirements during exercise, as represented by RPP trends that were unaffected by GH treatment. These results are in substantial agreement with those obtained by Marzullo et al (32), who detected an increase of overall cardiac mass without negative effects on left ventricle diastolic and systolic function after GH12 in adult obese PWS patients. According to the model depicted in Figure 3, GH treatment results in a lower rate of heart frequency rise during the incremental test up to the maximal attained value, so that the same exercise intensity is accomplished at a lower HR and a higher exercise intensity is sustained before the subjects are led to exhaustion.

Such a result, indicating an increase in exercise capacity, is in agreement with our previous study (18) that tested the changes of exercise capacity after GH12 in adult PWS patients and may support the findings of the positive impact of GH supplementation on the levels of spontaneous

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daily physical activity (20) and physical functioning and well being (41) of adult PWS patients. However, because we did not explore the mechanisms ultimately leading to improved exercise capacity, we can only hypothesize the basis of this phenomenon. On one hand, the improved strength capacity may substantially contribute to a better exercise tolerance during a task with a considerable strength requirement like locomotion on incline performed during the stress test. Strength training has been observed to increase exercise capacity and improve vagal modulation of HR at submaximal exercise intensities in young and healthy but inactive men (42). Moreover, quadriceps strength is recognized to be correlated with exercise capacity levels in patients with poor performance (43). On the other hand, maximal aerobic capacity may have been increased due to peripheral factors such as the augmented volume of muscles and mitochondrial mass available for oxidative processes, seeing that no improvement seems to be attributable to cardiocirculatory factors that were substantially unchanged after GH treatment. In this respect, Esposito et al (44) showed that improvements in muscle structure have positive effects on peripheral convective and diffusive oxygen transport and utilization, so that the GH-related changes in the muscles of PWS patients presently investigated may also have induced an increase in maximal aerobic capacity of those muscles. However, because we did not determine the subjects' maximal oxygen uptake, such a metabolic conclusion should be made with some caution.

In conclusion, 24-month GH treatment of adult PWS patients at appropriate dosage tailored to individual response has positive effects on body composition and increases skeletal muscle size, reducing lipid content, with an increase of strength performance in different muscle groups of upper and lower limbs along with improved exercise tolerance. On the other hand, GH therapy does not appear to induce appreciable derangements of glucose homeostasis, and a positive effect on CRP levels is observed. These findings may be important in the strategic perspective for the use of GH supplementation in the treatment of PWS adults, especially in view of the present result indicating that improvements deriving from GH supplementation are not dependent on a concomitant condition of GHD.

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