Long-term echocardiographic and cardioscintigraphic effects of growth hormone treatment in adults with Prader-Willi syndrome

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Context: In Prader-Willi Syndrome (PWS), an altered GH secretion has been related to reduced cardiac mass and systolic function compared to controls.

Objectives: To evaluate the cardiovascular response to a 4 yr GH therapy in adult PWS patients.

Study participants: Nine severely obese PWS adults (3 females, 6 males) and 13 age-, gender- and BMI-matched obese controls.

Methods: In an **open-label** prospective study, assessment of endocrine parameters and metabolic outcome, whole body and abdominal fat scans, echocardiography, radionuclide angiography in unstimulated and dobutamine-stimulated conditions were conducted at baseline and after 1 and 4 yr of GH treatment.

Results: GH treatment increased IGF-I ($P<0.0001$), decreased C-reactive protein levels ($P<0.05$), improved visceral fat mass ($P<0.05$), and achieved near-significant changes of fat and fat-free body mass in PWS patients. Left ventricle mass indexed by fat mass increased significantly after 1 yr and 4 yr of GH therapy ($P<0.05$) without evident abnormalities of diastolic function, while a trend toward a reduction of the ejection fraction was documented by echocardiography ($P=0.054$). Radionuclide angiography revealed stable values throughout the study both of the left and right ventricle ejection fraction, though being accompanied by a statistically not significant reduction of the left ventricle filling rate. A positive association between lean body mass and LVEF was evident during the study ($P<0.05$).

Conclusions: GH therapy increased cardiac mass of PWS adults without causing overt abnormalities of systolic and diastolic function. While the association between lean mass and left ventricle ejection fraction during GH therapy corroborates a favorable systemic outcome of long-term GH treatment in adults with Prader-Willi syndrome, subtle longitudinal modifications of functional parameters advocate appropriate cardiac monitoring in the long-term therapeutic strategy for Prader-Willi Syndrome.

Prader-Willi Syndrome (PWS) is a rare monogenic disorder associated with neuro-cognitive, musculoskeletal and obesity-related disorders, which contribute to enhanced propensity to cardio-respiratory and metabolic complications (1–3). Patients with PWS are considered to be growth hormone (GH)-deficient, although study find-

Abbreviations:

ings differ largely on the prevalence of this clinical feature in the adult population (4, 5). Overall, GH deficiency (GHD) is believed to influence the adult PWS phenotype, being associated with short stature, increased adiposity and decreased lean mass as opposed to essential obesity, where both lean body mass and fat mass are increased (6). GHD is a defined clinical syndrome associated with an unfavourable cardiovascular profile, which improves with GH therapy (7). The therapeutic rationale for using GH to treat patients with PWS stems both from the demonstration of quantitatively and qualitatively abnormal dynamics of GH secretion (8), and from observed clinical similarities between individuals with GHD without PWS and PWS patients, who are commonly found with reduced muscle strength, altered body composition, low energy expenditure, and reduced statural growth, even in the presence of obesity (9). Within the endocrine care community, increasing experience has accumulated over the past few years on the ability of GH treatment to alleviate some dysfunctions of PWS patients. In children with PWS, GH treatment normalizes stature and ameliorates body composition, fat utilization, and muscle strength (10). These effects are maintained during a 8 years GH treatment (11), and tend to perdure upon treatment discontinuation (12). To date, there are no controlled studies of uninterrupted GH treatment through childhood, adolescence, and the transitional period into adulthood in PWS. Despite a shorter clinical experience, there is a strong likelihood of a sustained benefit exerted by GH treatment also in PWS adults (9), where GH treatment can favorably decrease adiposity (13, 14) and improve muscle bulk, muscle power, and exercise tolerance (15). These changes are expected to favor the control of body mass in PWS. Oppositely, there is evidence that cessation of GH therapy can worsen BMI, thus that long-term use of GH may be advisable to maintain good body composition and prevent complications of obesity in adult PWS patients (16).

To date, only a limited number of studies investigated the cardiovascular features of PWS patients in detail. Physical examination, ECG recordings and transthoracic echocardiograms including two-dimensional speckle-tracking echocardiography have been used to assess cardiac anatomy and myocardial systolic function in children with PWS, and found frequent alterations in myocardial systolic function, while conventional echocardiographic findings failed to uncover ventricular systolic dysfunction (17). In a group of adult PWS patients, key evidence of significantly raised levels of the inflammatory marker hs-CRP, microcirculatory dysfunction and occasional sinus node dysfunction, all of which are associated with early sudden death, were documented (18). In addition, obese PWS adults have been found to harbor hypokinetic cardiac features resembling those of GHD, ie, lower systolic function, smaller left ventricle (LV) size and impaired chronotropic response to an adrenergic stimulus (19). Some of these features can improve with GH therapy (20), yet the chronic effects of GH administration on the cardiovascular system in the setting of PWS are completely unknown.

The current study aimed to explore if long-term GH therapy modifies the clinical outcome in PWS adults by evaluating its efficacy on metabolic homeostasis, adiposity indices, and structural and functional cardiovascular features in obese PWS adults.

Patients and Methods

Study design. Data presented in the current study are based on a 4-year prospective study of 9 PWS adult subjects who took originally part to a 12-month trial performed on 13 patients and previously published (20). Of the initial group of 13 patients, 4 patients withdrew GH treatment within two years after starting therapy and refused to undergo the final cardiovascular testing after 4 years. Reasons for withdrawing GH were excessive gain of body mass due to insufficient familiar control (n = 2), lack of motivation (n = 1) and inadequate compliance to GH administration (n = 1). As such, current analysis refers exclusively to the 9 patients (3 females, 6 males; age 26.4 ± 3.7 years) who completed the whole study and includes results obtained at study entry, after 1 year and 4 years GH treatment. Of these, 2 patients underwent the radionuclide study only in unstimulated conditions, due to potential pharmacological interference with the dobutamine stimulus. As controls, 13 age-, sex-, and BMI-matched otherwise healthy individuals (6 females, 7 males; age 26.2 ± 3.1 year) were selected from the hospital patients and accepted to partake into the study. All patients and their obese control subjects were studied as in-patients, after two resting days and overnight fasting. The experimental procedure was approved by the ad hoc Ethical Research Committee of the Istituto Auxologico Italiano, Verbania, Italy. Written informed consent was obtained from the PWS patients and their parents or guardians, and from the obese participants. The study protocol conformed to the guidelines of the Additional Protocol to the European Convention on Human Rights and Biomedicine, concerning Biomedical Research.

Subjects. All subjects included in the study were Caucasian and aged over 18 years. All PWS patients exhibited the typical phenotype (2), suffered from childhood obesity and were undergoing strict parental guidance to achieve control of their feeding obsession. Cytogenetic analysis was performed in all subjects, and 7 carried the deletion of the paternal chromosome 15 (15q11-q13), while maternal uniparental disomy was diagnosed in the remaining 2. No study participant or their families reported on any previous heart problems. At baseline, all participants reported a stable body mass for the previous 3 months, none suffered from impaired thyroid, adrenal, renal or hepatic function nor had been previously subjected to any pharmacological or surgical treatment for loss of body mass. Four PWS patients had previously undergone GH treatment, withdrawn in

all cases 1–4 years before enrolment in the current study. Pituitary GH secretion was evaluated in PWS by dynamic testing with GHRH + arginine. The threshold of peak GH levels used for diagnosis of GHD was 4.1 μg/liter (21). Four out of 9 PWS patients satisfied the criterion of GHD. Since the original visit, all PWS patients had been regularly attending the hospital and subjected to EKG and cardiology consultation yearly to assess any variation that would advocate for therapy withdrawal while on GH treatment. At baseline, 2 patients suffering from arterial hypertension were receiving therapy with loop diuretics (n = 1) and angiotensin-converting enzyme (ACE) inhibitor plus calcium antagonist (n = 1). One hypertensive patient also suffered from type 2 diabetes mellitus and was insulin treated. All PWS were reported to have behavioral problems, and 4 subjects were treated with neuroleptics. All therapies did not vary both at 1 year and 4 years time-points, with the exception of 1 male who stopped neuroleptic therapy after 1 year of GH administration. One of two PWS women with primary amenorrhea was undergoing sex steroid substitutive therapy, and the remaining suffered from oligomenorrhea. There had been no changes in the replacement dose of hormones other than GH since the original trial. GH treatment was administered at bedtime, with a mean GH dose of 0.97 ± 17 mg/d after the first 12-month period. Subsequently, the dose was adjusted to maintain serum total IGF-1 levels within 2 SD from an age-matched reference value, so as to avoid overdosing. The final dose attained after 48 months in the PWS patients group was 0.40 ± 0.11 mg/d. Arthralgia and fluid retention were the only documented side-effects and were limited to the first month of treatment in 1 female patient. GH therapy was self-injected by one patient and administered by patient's parents or their caregiver in the remainders.

Anthropometric measures. Determination of stature and body mass were obtained in fasting conditions, after voiding. BMI was defined as body mass in kilograms divided by the square of stature in centimeters. Waist was measured as halfway between the costal edge and the crista. Hip was measured as the greatest circumference around the nates. Dual-energy x-ray absorptiometry (DXA) was used for assessment of body mass (GE-Lunar, Madison, WI), expressed as lean body fat in kilograms and fat body mass as percentage of total body mass. Abdominal visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) were measured by 6-mm single-slice L_4-level computed tomography (CT) using GE Hi-Speed DX/I with 6.4 CT scanner software (General Electric Medical Systems, Milwaukee, WI). Intra-abdominal fat was separated from retroperitoneal fat by drawing a line to the pericolic gutters. Sagittal diameter was measured as the anterior-to-posterior distance in millimeters at the disk level. A single image was obtained during suspended respiration. The within-subject variation for repeated analyses of fat measurement in our laboratory is < 1%. Arterial blood pressure (BP) was recorded in the sitting position after at least 30 minutes of rest. At least two measurements were taken, and the mean value was recorded.

Cardiac analyses. Assessment procedures have been previously described (19) and will be briefly summarized. Echocardiography was performed by M-mode, two-dimensional, and pulsed Doppler studies (Hewlett-Packard Sonos 2500, Andover, MA) by one echocardiographer throughout the course of GH treatment. The following measurements were recorded on M-mode

tracing: interventricular septum thickness (IVST; millimeters), left ventricular (LV) posterior wall thickness (LVPWT, millimeters), LV end-diastole diameter (LVEDD, millimeters), LV end-diastole volume (LVEDV, millilitres); calculation of the LV mass (LVM, grams) was made using the Devereux's formula according to the Penn convention with the following regression-corrected cube formula: LVM = 1.04 [(IVST + LVEDD + LVPWT)^3) - (LVEDD)^3] - 14 g, and calculated after correction for body surface area (BSA) (LVM$_{BSA}$, grams per square meter), height$^{2.7}$ (LVM$_{h2.7}$), or percent fat mass (LVM$_{FM}$, grams, percent). Doppler studies provided indices of ventricular filling that were derived from the mitral flow velocities curves, ie, maximal early diastolic flow velocity (E, centimetres per second), maximal late diastolic flow velocity (A, centimetres per second), peak E/A wave velocity ratio (normal value 1 or more), and the deceleration time of early filling (DT, milliseconds). Equilibrium radionuclide ventriculography was performed at rest and during dobutamine infusion. Acquisitions were obtained with patients in supine position in the left anterior oblique best septal view with a large field-of-view camera (Apex 409, Elcsint, Haifa, Israel) equipped with a parallel-hole high-sensitivity collimator. Data were collected in minilist mode to compensate for heart variability during acquisition. Dobutamine infusion was performed in 5-minute steps; image acquisition was obtained during the last 3 minutes of each step. LV ejection fraction (LVEF, percent) was computed on the basis of relative end-diastolic and end-systolic counts. In all participants, framing rate was sufficiently high to allow calculation of diastolic parameters. Accordingly, peak filling rate (PFR) was computed from the first derivative of a third-order polynomial function fitted to the first two thirds of the diastolic portion of the LV time-activity curve by a least squares technique, normalized for end-diastolic volume (EDV) and expressed as EDV per second. As normal, the following values were taken in consideration: LVEF 50% or more in basal conditions with 5% increments or more during dobutamine, and PFR 2.5 EDV/sec or more.

Hormone assays. Measurements were performed using commercially available kits. All samples were analyzed separately at baseline and at the 1 year and 4 years points. GH levels were measured by chemiluminescence (Immulite 2000 analyzer, Diagnostic Products Corp., Los Angeles, CA) having a sensitivity of 0.01 μg/liter and intra- and interassay coefficients of variation (CVs) of 2.9–4.2 and 4.2–6.5%, respectively. Total IGF-I levels were assayed by chemiluminescence IGF-I immunoassay by Liaison (Nichols Advantage, San Juan Capistrano, CA), having a sensitivity of 6 μg/liter, intraassay and interassay CVs of 4.8 and 6.7%, respectively. Serum insulin levels were measured by chemiluminescence (Immulite 2000). Enzymatic methods (Roche Molecular Biochemicals, Mannheim, Germany) were used for determination of blood glucose, total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides. Insulin resistance was measured by homeostatic model approach [HOMA, insulin (μU/mL) * blood glucose (mmol/L)/22.5]. Ultrasensitive C-reactive protein (CRP) was measured by HS Roche kit.

Data analysis Results are presented as mean±SEM. Two-tailed Mann-Whitney and paired Student's *t* test were used for comparison between PWS and control group, and in PWS during GH therapy, respectively. Analysis of variations was used to calculate

differences between baseline, 1 year and 4 years time-points. Relationships between variables were analyzed using Pearson's correlation analysis after merging datasets from the three time-points, to make the analysis numerically more consistent. Analyses were performed using SPSS 18.0 (SPSS, Inc., Chicago, IL). Significance was accepted for $P < .05$.

Results

Hormone and body composition investigations. No PWS patient who completed the study complained of significant side effects nor developed adverse events during the period of GH treatment, and none withdrew therapy. Compared to obese controls, PWS patients showed significantly lower IGF-I levels which steadily increased at the 1- and 4-year follow-up during GH treatment ($P = .0001$ vs. baseline for both) and proportionately to the GH treatment dose (Table 1). Indirect adiposity measures, ie, waist and waist-to-hip ratio, were similar between PWS and controls, and were unchanged by the end of the study. Inversely, DXA measured a markedly different accumulation in fat and fat-free body mass between PWS and controls. At the end of the treatment period, a near-significant increase of lean body mass occurred in the PWS group ($+3$ kg; $P = .053$ vs. baseline), which was associated to a slight reduction of the percent body fat at this time-point ($P = .08$). By abdominal CT scan, we found no

difference in SAT or VAT accumulation between groups, while a marked reduction of VAT was seen at the 1 year and 4 years follow-up in all but one PWS male patient (cumulative variation vs. baseline: −30.1% and −30.3%; $P = .007$ and $P = .01$, respectively). GH therapy elicited variable reductions of SAT compartment (-8.9% vs. baseline), albeit unexpectedly increasing in one male and one female patient (4% and 28% by the end of the study).

At baseline, we found no difference between PWS and controls in glucose levels, while insulin concentrations were significantly lower in the former group (Table 1). GH therapy was associated with a transient increase in glucose levels at the 1 year visit ($P = .006$), then returning to values not dissimilar from baseline concentrations at the 4 years visit. Insulin levels increased in three PWS patients during therapy and remained stable in the remaining ones by the study end. HOMA-IR values showed a trend toward an increase at the 1 year and 4 years follow-up and were, cumulatively, subordinated to measures of adiposity (BMI: r = 0.70, $P < .0001$; percent fat body mass: r = 0.41, $P < .05$). However, final indices of glucose homeostasis were not different from those measured in controls (Table 1). CRP levels decreased significantly in PWS treated with GH after 1 ($P = .001$) and 4 years ($P = .03$). All parameters of lipid homeostasis except for HDL-cholesterol were (nonsignificantly) healthier in PWS subjects

Table 1. anthropometric and biochemical results (**mean±sem**) obtained in 9 PWS patients at baseline and after 1 and 4 yr of GH treatment. Comparative data from 13 obese control subjects are included.

Parameters	Control subjects (n = 13)	PWS patients (n = 9) baseline	GH treatment 1 yr	GH treatment 4 yr	P value (ANOVA)
Body mass (kg)	125.4 ± 3.6	108.7 ± 3.6[b]	110.4 ± 5.7[a]	110.4 ± 6.4[a]	0.9
Body mass index (kg/m^2)	43.0 ± 1.4	44.9 ± 1.6	45.5 ± 2.4	45.5 ± 2.5	0.9
Waist/hip	0.91 ± 0.02	0.94 ± 0.02	0.93 ± 0.02	0.94 ± 0.01	0.9
IGF-I (μg/liter)	155.3 ± 13.8	86.0 ± 9.6[b]	347.7 ± 36.2[bd]	197.9 ± 18.9[bd]	0.0001
Plasma glucose (mg/dl)	85.7 ± 3.1	85.1 ± 8.5	95.2 ± 7.9[d]	89.4 ± 10.1	0.7
Insulin (mUI/ml)	15.6 ± 2.0	9.5 ± 1.6[a]	15.8 ± 4.4	14.4 ± 3.5	0.4
HOMA-IR	3.3 ± 0.5	2.0 ± 0.4	3.8 ± 1.1	3.0 ± 0.7	0.3
C-reactive protein (mg/dl)	0.97 ± 0.22	0.98 ± 0.16	0.43 ± 0.13[c]	0.57 ± 0.16[d]	0.04
Triglycerides (mg/dl)	172.4 ± 27.8	115.4 ± 15.4	116.1 ± 13.9	98.6 ± 8.9	0.6
Total-cholesterol (mg/dl)	215.5 ± 13.3	177.1 ± 15.0	184.7 ± 14.0	178.0 ± 12.7	0.9
LDL-cholesterol (mg/dl)	148.2 ± 10.7	118.8 ± 12.3	124.9 ± 13.0	114.3 ± 14.0	0.8
HDL-cholesterol (mg/dl)	45.6 ± 2.6	45.3 ± 4.1	69.7 ± 3.0	55.2 ± 7.9	0.4
Fat mass (%)†	46.3 ± 1.8	55.5 ± 1.8[b]	53.1 ± 1.9[a]	52.9 ± 1.4[a]	0.4
Fat-free mass (kg)†	60.5 ± 3.7	47.7 ± 2.4[a]	49.8 ± 2.3[a]	50.7 ± 2.5[a]	0.6
SAT (cm^2)‡	675.5 ± 33.6	693.8 ± 36.2	635.1 ± 37.1	622.6 ± 42.7	0.4
VAT (cm^2)‡	150.1 ± 24.8	158.1 ± 23.9	110.6 ± 19.5[d]	111.5 ± 18.3[c]	0.2
APD (cm)‡	30.1 ± 6	29.9 ± 1.0	29.6 ± 1.42	30.3 ± 1.2	0.9

For significance: differences between controls and PWS are indicated as a ($P < 0.05$), b ($P < 0.01$); differences within the PWS group between 1 yr and 4 yr GH treatment and *vs.* baseline are indicated by ANOVA and as d ($P < 0.05$), e ($P < 0.01$).

Abbreviations: HOMA-IR and -IS, homeostatic model approach insulin resistance and sensitivity; SAT and VAT, abdominal subcutaneous and visceral adipose tissue; APD, abdominal antero-posterior diameter.

†, as determined by total-body DXA

‡, as determined by abdominal CT scans

than obese controls. Despite the lack of significant modifications of lipid levels during the course of GH therapy, HDL-cholesterol levels increased by 22% by the study end in PWS. Neither the GH dose at each time point nor the attained IGF-I levels were associated to any metabolic variables obtained during the study period.

Cardiovascular exams. Diastolic and systolic BP were stable during the course of GH treatment (Table 2). Echocardiography showed no hypertrophic effect of GH on the interventricular septum and posterior wall of the LV. However, an increase in LV end-diastole diameter occurred after 1 year and 4 years, which was reflected in the increase of LV mass$_{FM}$ after 1 year (delta, 11.9%; P = .02 vs. baseline) and 4 years of GH therapy (delta, 11.6%; P = .07 vs. baseline). Correlation analysis on cumulative longitudinal data displayed a direct association between the LV mass$_{FM}$ and lean body mass (r = 0.62, P = .001) achieved during the treatment period (Figure 1). Doppler

analysis revealed a normal systolic function (eg, LV EF $\geq$ 55%) in all PWS subjects throughout the study, and final values did not differ from those of controls, despite a reduction of LV EF at 1 year and 4 years visit (P = .03 and P = .02, respectively). Diastolic function was preserved (eg, E/A ratio $\geq$ 1) in all PWS patients during GH therapy, and individual values of the E/A ratio were inversely related to BMI (r=-0.64, P < .0001; Figure 2) and HOMA-IR (r=-0.55, P = .003). A decline of the deceleration time was seen after 1 year and 4 years of therapy (P = .02 and P = .04, respectively).

Radionuclide angiography analysis was obtained throughout the study in unstimulated conditions (ie, baseline) and after β-adrenergic stimulus, and documented an overall broad stability of LV systolic and diastolic activities during the GH treatment course in PWS. Left ventricle EF did not change significantly during treatment (Table 2), and correlated positively to the lean body mass (Figure 3)

Table 2. echocardiography and radionuclide angiography results (**mean**[plusmn]**SEM**) obtained in PWS patients at baseline and after 1 and 4 yr of GH treatment. Comparative data from obese control subjects are included.

| Parameters	Control subjects (n = 13)	PWS patients (n = 9)			
			GH treatment		P value
		baseline	1 yr	4 yr	(ANOVA)
Echocardiography					
LVPWT (mm)	10.1 ± 0.3	9.2 ± 0.4	8.9 ± 0.2	9.0 ± 0.4	0.8
IVST (mm)	9.5 ± 0.3	9.6 ± 0.3	9.1 ± 0.4	9.7 ± 0.4	0.7
LVM (g)	163.5 ± 7.6	133.7 ± 6.7[a]	146.6 ± 5.5	150.1 ± 9.0	0.3
LVM$_{BSA}$ (g/m^2)	70.6 ± 2.9	64.6 ± 3.0	71.2 ± 1.7	72.8 ± 3.9	0.2
LVM$_h$ (g/h$^{2.7}$)	39.5 ± 1.9	40.3 ± 1.6	44.5 ± 1.8	45.5 ± 2.8	0.3
LVM$_{FM}$ (g%)	3.7 ± 0.3	2.4 ± 0.1[b]	2.8 ± 0.1[ac]	2.8 ± 0.1[a]	0.2
LVEDD (mm)	47.0 ± 1.1	43.0 ± 1.4	47.1 ± 1.2[c]	46.2 ± 1.2	0.1
LVEDV (mL)	46.3 ± 1.6	47.1 ± 3.2	49.7 ± 2.8	50.6 ± 2.9	0.8
LVEF (%)	60.6 ± 1.5	64.0 ± 1.5	59.0 ± 1.7[c]	60.0 ± 1.5[c]	0.2
E/A	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	0.7
DT (msec)	175.4 ± 4.1	192.1 ± 7.2	170.6 ± 4.2[c]	168.9 ± 2.8[c]	0.02
Radionuclide angiography					
LVEF$_{BAS}$ (%)	58.6 ± 1.5	55.7 ± 1.7	54.8 ± 2.0	54.9 ± 1.5	0.9
LVEF$_{DOB}$ (%)	76.4 ± 1.1	72.1 ± 2.2	69.3 ± 1.7[d]	67.9 ± 1.4[d]	0.4
δ LVEF (%)	31.3 ± 3.1	30.3 ± 2.7	30.9 ± 6.7	26.4 ± 1.4	0.8
RVEF$_{BAS}$ (%)	44.9 ± 1.7	42.5 ± 2.2	39.4 ± 1.2[c]	43.7 ± 2.0	0.4
RVEF$_{DOB}$ (%)	61.7 ± 1.4	55.7 ± 1.8	62.1 ± 1.8	57.0 ± 3.6	0.4
δ RVEF (%)	42.6 ± 3.5	35.3 ± 8.1	58.0 ± 4.2[a]	30.1 ± 2.9[c]	0.04
PFR$_{BAS}$ (EDV/s)	2.9 ± 0.2	2.6 ± 0.2	2.6 ± 0.2	2.5 ± 0.1	0.8
PFR$_{DOB}$ (EDV/s)	4.5 ± 0.3	4.1 ± 0.3	3.4 ± 0.2[a]	3.6 ± 0.3[a]	0.4
δ PFR (%)	56.2 ± 7.2	67.4 ± 14.1	48.3 ± 20.6	57.5 ± 11.6	0.8
HR$_{BAS}$ (bpm)	68.3 ± 2.7	62.9 ± 3.8	63.9 ± 2.9	62.6 ± 3.6	0.9
HR$_{DOB}$ (bpm)	128.7 ± 2.5	107.4 ± 7.7[a]	108.6 ± 6.7[a]	116.3 ± 4.5[a]	0.7
δ HR (%)	92.9 ± 8.9	60.9 ± 10.4	67.9 ± 8.1	81.8 ± 6.5	0.4
DBP$_{BAS}$ (mmHg)	78.5 ± 1.8	77.5 ± 1.2	76.3 ± 1.4	77.9 ± 1.9	0.8
DBP$_{DOB}$ (mmHg)	83.1 ± 2.8	78.6 ± 1.9	78.6 ± 1.9	78.8 ± 4.1	0.9
SBP$_{BAS}$ (mmHg)	125.8 ± 3.6	120.0 ± 2.3	122.5 ± 3.0	121.4 ± 6.5	0.9
SBP$_{DOB}$ (mmHg)	145.4 ± 8.2	141.4 ± 6.7	133.6 ± 4.1	132.9 ± 3.1	0.7

For significance: a (P < 0.05) and b (P < 0.01), obese controls *vs.* PWS patients; c (P < 0.05) and d (P < 0.01), 1-yr and 4-yr GH treatment *vs.* baseline in PWS patients.

Abbreviations: LV and RV, left and right ventricles; PWT, posterior wall thickness; IVT, interventricular septum thickness; LVM, left ventricle mass; LVMi, LVM indexed by body surface area; LVM$_h$, LVM indexed by height$^{2.7}$; LVM$_{FM}$, LVM indexed by percent fat mass; LVEDD, LV end-diastole diameter; LVEF, LV ejection fraction; E/A, early-to-late mitral peak flow velocity; DT, deceleration time; RVEF, right ventricle ejection fraction; PFR, peak filling rate; HR, heart rate; DBP and SBP, diastolic and systolic blood pressures; BAS, unstimulated conditions; DOB, after dobutamine stimulation.

both when assessed in unstimulated and dobutamine-stimulated conditions (r = 0.45 and r = 0.47, respectively; $P < .05$ for both). Echocardiography- and angiography-derived measures of LV EF obtained throughout the treatment period were positively associated (r = 0.55, $P = .004$). Peak EF of the LV showed a trend toward a decrease in PWS subjects and was, by the study end, lower than in controls. Diastolic function, as assessed by the LV peak

filling rate, near-significantly decreased during the study period ($P = .067$).

There were no differences in GH therapy-associated metabolic and cardiovascular outcomes in PWS patients when stratified by GHD, genetic setting or gender (data not shown). No relationship between GH dose or attained IGF-I levels and any cardiac variables obtained during the study was also observed.
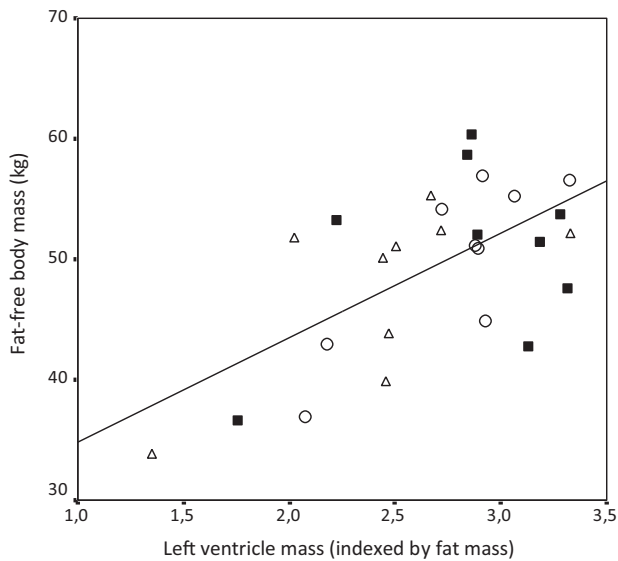
Figure 1. bivariate correlation analysis calculated on cumulative longitudinal data collected in 9 adult PWS patients, measured between free-fat body mass (kg, DXA) and LV mass indexed by fat mass (%, echocardiography) obtained at baseline (triangles), after 1 year (circles) and after 4 years GH treatment (squares). One fit line is expressed for whole data set.
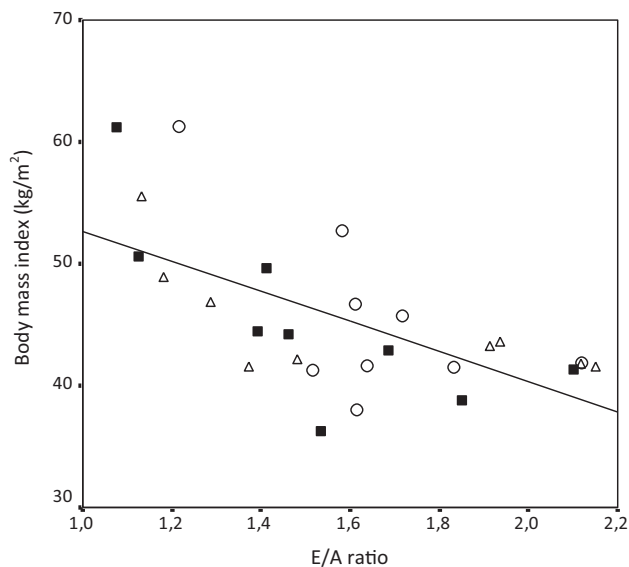
Figure 2. bivariate correlation analysis calculated on cumulative longitudinal data collected in 9 adult PWS patients, measured between BMI (kg/m^2) and early to late mitral flow velocities (E/A, echocardiography) obtained at baseline (triangles), after 1 year (circles) and after 4 years GH treatment (squares). One fit line is expressed for whole data set.
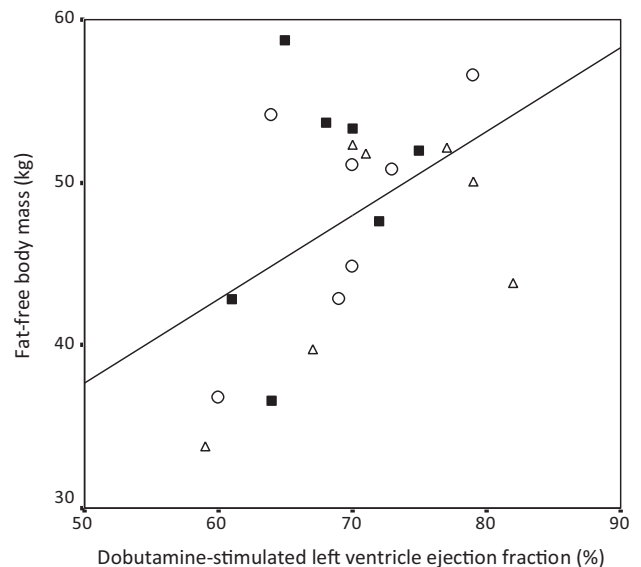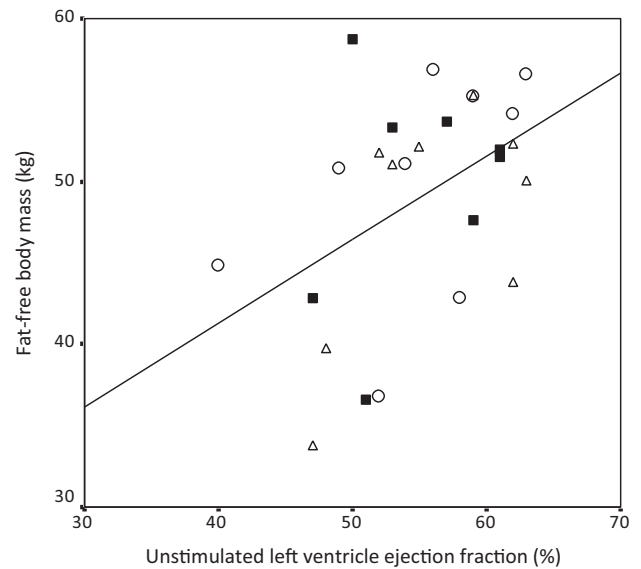
Figure 3. bivariate correlation analysis calculated on cumulative longitudinal data collected in 9 adult PWS patients, measured between free-fat body mass (kg, DXA) and unstimulated (top panel) or dobutamine-stimulated (lower panel) LV EF (%, radionuclide angiography) obtained at baseline (triangles), after 1 year (circles) and after 4 years GH treatment (squares). One fit line is expressed for whole data set.

Discussion

Our study in a selected sample of severely obese adults with PWS found that 4 years treatment with GH improved lean body mass, decreased visceral adiposity and reduced proinflammatory markers associated with obesity. During the course of GH treatment, an increase in LV mass was observed in PWS patients and all functional variables assessed by echocardiography and radionuclide angiography remained within the normal range, despite the evidence of a trend toward a decline of diastolic and systolic indices of the LV. These results suggest that long-term GH administration can contribute to stabilize cardiovascular structure of PWS adults and contrast the inherent negative outcome associated with the disorder.

Obesity is a hallmark of PWS and a prominent cause of cardiovascular complications leading to premature mortality. Early dietary treatment is effective in avoiding excessive gain of body mass in patients with PWS, but results in shorter stature unless GH treatment is commenced (22). Although many studies published to date have reported an overall narrow prevalence of cardiovascular events in PWS (23–26), this inference is possibly flawed by the premature mortality affecting PWS, which could mask the development of cardiovascular accidents later in life. In such context, obesity-related cardiovascular and respiratory diseases are actually a major cause of death, both during child- and adulthood (27), and the reportedly increased prevalence of diabetes mellitus further enhances their unfavorable cardiovascular profile (26–30).

The current study used an echocardiographic and scintigraphic methodology to investigate the long-term cardiovascular outcome of GH treatment in adults with PWS. Favorable inotropic effects of GH therapy have been consistently described in hypopituitary populations with GHD, and interventional long-term studies uniformly stressed the importance of GH replacement to normalize LV wall thickness, end-diastole volume and stroke volume (31). In addition, GH treatment increases LV end-diastole diameter, and this effect possibly originates from an increased plasma volume (32). A previous retrospective analysis by Hauffa et al in PWS children documented a trend for a positive effect of GH therapy on LV end-systolic diameter, a contractility index, compared to GHD controls (33). Our analysis in PWS adults identified a peculiar profile of cardiac response during GH therapy, which consisted of an incremental variation of LV mass determined by the increase of end-diastole diameter, while no change in LV cardiac walls thickness could be documented. Although there were no systolic or diastolic abnormalities occurring with GH therapy, the LV EF appeared to decline by the study end. It is worth noting that

the analytical solution provided by echocardiography is faster and the examination is easily performed in the routine practice, but its accuracy is possibly affected by the abnormal body conformation and excessive adiposity of PWS patients. When a more sensitive functional analysis was used, eg, radionuclide angiography, again we did not document diastolic and systolic dysfunctions during the GH treatment period. However, values of peak systolic and diastolic function (ie, LV EF and filling rate measured during dobutamine) averagely decreased during the study, albeit remaining within the normal range for the whole study duration. While these contradictory data convey the impression of a peculiar pattern of cardiac response in PWS that may not entirely fulfill Laplace's law, relatively to the effects of GH (34), it should be borne in mind that our study included a limited sample and that dobutamine, herein used as a proxy of the stress test to compensate for motor disabilities of PWS patients, may not provide a sufficient stimulus in obese PWS subjects to illustrate GH effects on cardiac function adequately.

To further explain the atypical pattern of response of PWS adults, as compared to GH-treated hypopituitary GHD populations (31), a number of phenotypical and functional characteristics should be considered. First, there is postmortem evidence in a PWS toddler with sudden cardiac death of increased cardiac collagen deposition and band necrosis (35). In children with PWS, perturbations in the sympathetic nervous system, autonomic dysfunction, as well as inherent low cardiovascular fitness have been reported (36). Finally, studies in adults with PWS disclosed the existence of early vascular changes in the forearm microcirculation (18), decreased chronotropic and inotropic response to β-adrenergic stimulus (19), impaired autonomic regulation (37), increased risk of sleep apnea as well as abnormal arousal and impaired cardiorespiratory responses to hypoxia (38). In frail PWS individuals, these abnormalities can provoke cardiorespiratory failure necessitating treatment by oxygen therapy, adaptive servoventilation, medications, diet therapy and rehabilitation (39). Although current data necessarily warrant confirmation in larger prospective investigations, we are inclined to consider GH treatment as a valid supportive care for long-term cardiovascular stability in PWS, which is chronically linked to increased cardiovascular risk.

In addition to the previous, positive changes of other systemic correlates could develop with GH therapy and, hence, beneficially act on the cardiovascular outcome of PWS patients. Improvements of body composition have long been recorded in PWS children treated with GH treatment, which has been thus proposed to delay the onset of obesity into adolescence (40–43). In long-term investiga-

tions in PWS children, GH treatment for up to 6 years improved fat mass, motor development and muscle strength (10, 44). In adults with the disease, GH therapy for up to 2 years decreased abdominal fat content and increased muscle fat content, muscle mass and functions (14, 15, 43, 45, 46), and advantaged the sustainable physical workload (47). Our results confirm the benefits of GH therapy on body composition and visceral fat and, like other long-term studies (11), suggest that GH effects occurred as early as after 1 year of therapy, and remained stable thereafter. GH-dependent improvements in body composition could benefit exercise capacity and help to contrast obesity, as well as influence the function of cardiac parameters, as implied by the associations found in our analysis between LV parameters and fat free mass or BMI. Because insulin resistance increased during the study, there is still a need for serial evaluations of metabolic homeostasis during GH therapy. In addition, the potential effect of gonadal steroid replacement on our findings remains to be elucidated.

In conclusion, current data suggest that long-term GH administration can favor preservation of cardiac and metabolic parameters in adult PWS patients. In association with strategies for controlling body mass, GH treatment can be seen as a critical upholder of physiological homeostasis, and could create extended benefits for cardiovascular health in adults with the disease. Cautiously, nonsignificant modifications of functional parameters point out a need for appropriate cardiac monitoring during long-term management of Prader-Willi Syndrome.

Acknowledgments

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