

Visceral adipose tissue resides within the reference range in children with Prader-Willi syndrome receiving nutritional intervention on a regular basis

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Abstract. Nutritional intervention for maintaining an appropriate body composition is central to the management of Prader-Willi syndrome (PWS). Despite evidence that visceral adipose tissue (VAT) is associated with increased metabolic risks, the effects of nutritional intervention on fat distribution have not been evaluated for PWS children. We herein investigated fat distribution in 20 genetically diagnosed PWS children (9 males and 11 females); 17 of which received nutritional intervention with or without growth hormone (GH) treatment [GH-treated group ($n = 8$), GH-untreated group ($n = 9$)]. GH treatment continued for median of 4.9 years. GH treatment significantly increased height standard deviation score (SDS) whereas body weight SDS and body mass index SDS were not affected in GH-treated group. In GH-untreated group, height SDS significantly decreased during approximately 5 years of follow-up. Fat distribution was evaluated at the median age of 6.93 years in GH-treated group and 7.01 years in GH-untreated group. VAT was maintained within the reference range in both groups. Subcutaneous adipose tissue (SAT) was elevated in GH-untreated groups compared to reference values whereas it was not in GH-treated group. The remaining three subjects, who had never received nutritional intervention or GH treatment, showed increased VAT and SAT. In conclusion, nutritional intervention is beneficial in maintaining VAT within the reference range during childhood, although excessive nutritional intervention may cause unfavorable effect on linear growth.

Key words: Prader-Willi syndrome, Nutritional intervention, Growth hormone, Adipose tissue

PRADER-WILLI SYNDROME (PWS) is a disorder with multifaceted clinical characteristics, such as infantile hypotonia, intellectual and behavioral problems, and endocrine abnormalities [1]. The pathogenesis of PWS is based on the absence of the paternally inherited region of the 15q11–13 chromosome due to deletion, maternal disomy, imprinting defect, or chromosomal translocation. Most PWS patients are born with lower birth weight than their siblings [2], and later have infantile hypotonia and poor appetite, usually requiring tube feeding. By the end of the infantile period, feeding difficulties resolve and patients start gaining weight without significant changes

in appetite or caloric intake. Thereafter, increased interest for food accelerates weight gain. Reduced energy expenditure, disruption in hypothalamic pathways of satiety control, and aberrations in hormones regulating appetite have been implicated to cause obesity; however, the exact mechanism for the development of obesity is still largely unknown [3].

Metabolic complications are more frequently observed in obese PWS patients than non-obese PWS patients [4]. Since obesity-associated complications such as obstructive sleep apnea, hypertension, and cardiovascular disease are the main cause of death in adult PWS patients [5, 6], weight control is central to the management of PWS. There is evidence for the beneficial effect of growth hormone (GH) therapy on weight control in both pediatric and adult PWS patients [7, 8]. Several randomized clinical trials (RCTs) have shown that GH therapy not only improved height velocity but also reduced fat

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mass and increased lean body mass (LBM) in pediatric PWS patients [9–17]. Similar benefits were also observed in adult PWS patients [7, 18, 19]. In addition to GH therapy, nutritional intervention is essential to protect PWS patients from developing obesity and caloric restriction has been a successful nutritional strategy to maintain normal body mass index (BMI) in PWS children and adolescents [20, 21].

Accumulating evidence indicates that metabolic risk factors are more strongly associated with visceral adipose tissue (VAT) than subcutaneous adipose tissue (SAT) [22]. This is also true in PWS patients [23, 24], although PWS adult women have lower VAT than age, sex, and BMI-matched obesity controls [25]. These findings indicate the importance of analyzing fat distribution in PWS; however, the effectiveness of nutritional intervention on fat distribution has never been evaluated so far. Based on this, we herein investigated fat distribution by computed tomography (CT) for quantification of VAT and SAT to determine the effects of nutritional intervention on fat distribution in PWS children, and found that VAT was maintained within the reference range in those who regularly received nutritional intervention.

Materials and Methods

Editorial policies and ethical considerations

This study was approved by the Ethical Committee of Osaka Women's and Children's Hospital (approval No. 1070).

Subjects and study design

This retrospective study analyzed the medical records of genetically diagnosed PWS patients between November 1981 and March 2018 at Osaka Women's and Children's Hospital. Genetic diagnosis of PWS is based on the methylation analysis; however, due to the lack of insurance coverage of this test during the study period in Japan, the diagnosis was performed by a fluorescence *in situ* hybridization (FISH) analysis using small nuclear ribonucleoprotein-associated polypeptide N (SNRPN) probe and clinical evaluations. When deletion was not observed by a FISH analysis, methylation test was performed. Thereafter, uniparental disomy (UPD) was investigated by the DNA polymorphism analysis. A total of 20 subjects were included (9 males and 11 females); 17 of which received nutritional intervention regularly. The remaining three subjects had never received nutritional management or GH treatment. The former group was subdivided into two subgroups based on the presence or absence of GH treatment. GH treatment in PWS children is only approved in Japan for children with a short stature defined as height standard deviation score

(SDS) being equal to or lower than -2.0 . The SDS for the application of GH treatment was determined based on the normal growth standards for Japanese children that were available at that time [26, 27]. No patients have received GH treatment based on the diagnosis of GH deficiency. Baseline data for anthropometric measurement was obtained before initiation of GH treatment in the GH-treated group [$n = 8$, 2.16 (1.46–2.82) years old], and at the corresponding age in the GH-untreated group [$n = 9$, 2.01 (1.85–3.42) years old]. In order to evaluate the effects of nutritional intervention and GH treatment on body composition, we retrospectively evaluated anthropometric measurements, fat distribution by abdominal CT, and body composition by dual-energy X-ray absorptiometry (DXA) after GH treatment in the GH-treated group [6.93 (6.00–7.81) years old] and at the corresponding age in the GH-untreated group [7.01 (6.25–10.47) years old]. Anthropometric measurements and fat distribution by abdominal CT were also evaluated in patients who had never received nutritional intervention ($n = 3$).

Data collection

Standing height and weight wearing lightweight clothes were measured using a digital scale (TANITA DC-250). BMI was calculated by dividing body weight (BW) (kg) by height (HT) squared (m^2). For research purpose, HT-SDS, BW-SDS, and BMI-SDS was calculated based on normal growth standards for Japanese children, taken from year 2000 national survey data [26, 28]. SAT (cm^2) and VAT (cm^2) at the level of the umbilicus were determined by abdominal CT (Toshiba Medical System TCT-900S before February 2004, Toshiba Medical System Aquilion 16 from February 2004 to March 2012, Toshiba Medical System Aquilion ONE from March 2012). The method to evaluate VAT and SAT were not changed during study period. DXA was utilized to evaluate fat mass (FM), %FM, LBM, %LBM, muscle mass (MM), %MM, and bone mineral parameters including bone mineral content (BMC) and bone mineral density (BMD) of the lumbar spine (L2–4) (Holgic QDR-4500A before February 2012, Holgic Horizon A after March 2012). Cross-calibration was performed to maintain consistency between devices. Quality control was performed using Step Phantom scanning. Bone mineral apparent density (BMAD), defined as $BMC/bone\ area^{3/2}$, that is used to measure volumetric bone density were also evaluated [29]. IGF-1 levels were determined by an immunoradiometric assay using somatomedin CII SIEMENS before March 2012, and IGF-1 IRMA DAIICHI after April 2012. The values of IGF-1 measured with the former kit was converted using the following equation to maintain consistency between two kits: $y =$

$0.924x + 11.871$ (x : the value by former kit, y : converted value). This equation was calculated by comparing the IGF-1 levels measured by these two kits from 57 samples. IGF-1 SDS was determined according to standardized centile curves for Japanese, taken from national survey data [30]. Circulating levels of AST, ALT, total cholesterol (TC), and HbA1c were also evaluated. AST and ALT levels were determined using Shikarikiddo AST and ALT before July 2016, and quick auto neo AST JS and ALT JS after July 2016, respectively. TC levels were determined using T-CHO(S) A-N/B-N before March 2007, and determiner L TC II after April 2007. HbA1c levels were determined using latex agglutination before March 2013, and TOHSO after April 2013. The value of HbA1c (JDS) was converted to that of HbA1c (NGSP) by the following equation to maintain consistency: $NGSP(\%) = 1.02 \times JDS(\%) + 0.25\%$ [31].

Nutritional intervention

Nutritional intervention was performed by managerial dieticians every few months throughout the study period after the patient was diagnosed with PWS or following their first visit to our hospital. During infancy, nutritional management was based on the support to give adequate nutrition to maintain the growth and the total intake of calories was gradually increased to meet the requirement for appropriate growth and development. When the phase of hypotonia with feeding difficulty was over [2], calorie restriction was initiated as a nutritional intervention. Nutritional intervention consisted of a daily calorie intake of less than 10 kcal per cm of height with age-appropriate protein (13–20%), carbohydrate (50–60%), and fat (20–30%) proportions as well as substantial vitamins and minerals. In addition, an educational program was concurrently initiated to help the patients' parents understand age-dependent typical behavior and eating habits of PWS children. The importance of performing regular physical activity was also educated. All subjects were not physically disabled and regularly joined the physical education class at schools.

Statistical analysis

Variables were expressed as the median (interquartile range) except for VAT and SAT and statistical analysis to compare the variables between prior to and after GH treatment in GH-treated group and between corresponding ages in the GH-untreated group was performed using Wilcoxon signed-rank test. VAT and SAT were presented as the mean \pm standard deviation (SD) to maintain consistency with a previous report that showed age- and sex-dependent reference values for VAT and SAT [32], and the statistical analysis was performed using 2-tailed Student's *t*-test. These analyses were performed using JMP software version 14 (SAS Institute, Cary, NC). *P*-values less than 0.05 were considered significant.

Results

Characteristics of subjects

We retrospectively analyzed medical records and identified 17 PWS patients who received nutritional intervention from a managerial dietician every few months following their diagnosis of PWS or on their first visit to our hospital. In eight subjects, GH treatment was initiated by the age of 3 and continued for more than three years (GH-treated group). The remaining nine subjects had never received GH treatment (GH-untreated group). Characteristics of these subjects are shown in Table 1. All the patients received nutritional intervention regularly. The starting age of nutritional intervention was shown in Table 1. GH treatment was initiated at the age of 2.16 (1.46–2.82) years old and continued for 4.89 (4.06–5.42) years in the GH-treated group (Table 2), among which four had deletion type, two had uniparental disomy, and two had imprinting defects (Table 1). In the GH-untreated group, seven subjects had deletion type and two had imprinting defects (Table 1).

The alterations in anthropometric measurements during nutritional intervention

For evaluation of anthropometric measurements during nutritional intervention, the HT-SDS, BW-SDS for age

Table 1 Clinical characteristics of subjects

	GH-treated	GH-untreated
Number	8	9
Sex: male/female	3/5	4/5
Genotype		
deletion (%)	4 (50)	7 (78)
uniparental disomy (%)	2 (25)	0
imprinting defect (%)	2 (25)	2 (22)
Starting age of nutritional intervention (month)	22 (3.5–44)	13.5 (8–46)

Table 2 Anthropometric measurements of GH-treated and GH-untreated subjects

	GH-treated			GH-untreated		
	Pre (<i>n</i> = 8)	Post (<i>n</i> = 8)	<i>p</i> value	Pre (<i>n</i> = 9)	Post (<i>n</i> = 9)	<i>p</i> value
Age at evaluation (years)	2.16 (1.46–2.82)	6.93 (6.00–7.81)		2.01 (1.85–3.42)	7.01 (6.25–10.47)	
HT-SDS	–2.34 (–2.80––1.82)	–1.41 (–2.23––0.85)	0.036	–1.07 (–1.57––0.52)	–2.02 (–2.61––1.11)	0.0077
BW-SDS for age and sex	–2.47 (–3.11––1.22)	–1.61 (–2.87––0.22)	0.21	–1.64 (–2.48––0.73)	–1.19 (–2.10––0.56)	0.68
BW-SDS for height and sex	–0.43 (–0.96––0.59)	–0.49 (–0.71––0.24)	0.16	–0.56 (–1.21––0.31)	0.25 (–0.50––0.72)	0.14
BMI-SDS	–0.53 (–1.41––0.56)	–0.89 (–1.17––0.11)	0.16	–1.49 (–1.83––0.24)	–0.34 (–0.98––0.40)	0.21
IGF-1 SDS	–0.73 (–2.17––0.07) (<i>n</i> = 6)	2.50 (0.95–3.40) (<i>n</i> = 7)	0.028*	–0.47 (–1.09––0.22) (<i>n</i> = 6)	–2.30 (–2.95––1.65)	0.046*

Pre and Post in GH-treated group represent the age of GH commencement and after GH treatment, respectively.

Pre and Post in GH-untreated group represent the corresponding ages for the age at GH commencement and after GH treatment in GH-treated group, respectively.

HT, height; BW, body weight; BMI, body mass index; IGF-1, insulin-like growth factor 1

Significant values are in bold. *: statistical analysis was performed based on data from 6 subjects.

and sex, BW-SDS for height and sex, BMI-SDS, and IGF-1 SDS were investigated prior to and after the initiation of GH treatment in GH-treated group and at corresponding ages in the GH-untreated group (Table 2). All subjects were at the prepubertal stage. Median value of HT-SDS at the initiation of GH treatment in GH-treated group was smaller than that at a corresponding age in GH-untreated group. GH treatment significantly increased HT-SDS and this was associated with significant increase in IGF-1 SDS, whereas BW-SDS for height and age or BMI-SDS were not affected (Table 2). In GH-untreated group, HT-SDS significantly decreased during approximately 5 years of follow-up and this was associated with significant decline in IGF-1 SDS (Table 2). In the current study, we did not perform statistical analysis between GH-treated and GH-untreated groups because GH treatment is only approved in PWS children with short stature in Japan and this may create the selection bias, resulting in the misunderstanding of the data.

Amount of VAT was maintained within the reference range in subjects receiving nutritional intervention on a regular basis

We analyzed the body composition in PWS subjects regularly receiving nutritional intervention by DXA and CT analyses. As shown in Table 3, median value of %FM was smaller, whereas median values of the %LBM and %MM were higher in the GH-treated group. Median values of lumbar spine BMC and BMD were similar between two groups, but median value of BMAD of the lumbar vertebrae was higher in GH-untreated group.

Statistical analysis was not performed because GH treatment is only approved in PWS children with short stature in Japan and this may create the selection bias, resulting in the misunderstanding of the data. We performed CT analysis to evaluate fat distribution and found that VAT was not significantly different compared to an age- and sex-matched reference values both in GH-treated or GH-untreated groups (Table 4) [32]. SAT in GH-treated group was not different from reference value, whereas SAT in GH-untreated group was greater compared to age- and sex-matched Japanese reference values (Table 4). These findings indicate that VAT, but not SAT, is maintained within the reference range in PWS patients receiving nutritional intervention. Consistently, circulating levels of AST, ALT, TC and HbA1c remained within a reference range in both groups (Table 3).

PWS children who had never received nutritional intervention or GH treatment showed increased VAT and SAT associated with early development of diabetes mellitus

Finally, we evaluated the fat distribution in PWS patients who had never received nutritional intervention or GH treatment. Retrospective analysis of medical records identified three subjects who met these criteria and the clinical characteristics of each subject are shown in Table 5. Case 1 and Case 2 were already obese when they first visited to our department at the age of 6 and 5 years old, respectively. Regular nutritional intervention was not successful in these subjects because strict caloric restriction was not tolerable to them due to the extreme

Table 3 Parameters of DXA analysis

	GH-treated (<i>n</i> = 8)	GH-untreated (<i>n</i> = 9)
Body weight (kg)	18.2 (14.9–21.6)	20.7 (15.2–26.2)
Fat mass (FM) (kg)	4.45 (3.67–5.06)	7.59 (5.60–10.03)
% fat mass (FM) (%)	25.7 (20.0–28.8)	35.8 (32.5–38.1)
LBM (kg)	13.85 (11.77–15.75)	15.07 (10.09–16.69)
% LBM (%)	76.8 (71.3–80.2)	64.1 (60.5–67.7)
Muscle mass (MM) (kg)	13.16 (11.19–14.91)	14.3 (9.60–15.79)
% muscle mass (MM) (%)	73.1 (67.4–76.7)	60.6 (57.1–64.6)
BMC of lumbar spine (g)	13.9 (11.6–14.9)	13.5 (10.2–15.6)
BMD of lumbar spine (g/cm ²)	0.496 (0.476–0.541)	0.52 (0.48–0.63)
BMAD of lumbar spine	0.095 (0.085–0.108)	0.120 (0.091–0.130)
AST (IU/L)	30.5 (27.2–32.5)	28.0 (24.5–32)
ALT (IU/L)	14.0 (11–24.6)	16.0 (14–21)
TC (mg/dL)	186 (157–211.5)	182 (177–193)
HbA1c (%)	5.5 (5.0–5.6)	5.5 (5.2–5.9)

LBM, lean body mass; BMC, bone mineral content; BMD, lumbar spine bone mineral density; BMAD, bone mineral apparent density; TC, total cholesterol

Table 4 Fat distribution by CT analysis

	Male			Female			Combined	
	GH-treated (<i>n</i> = 3)	GH-untreated (<i>n</i> = 4)	Reference (<i>n</i> = 20)	GH-treated (<i>n</i> = 5)	GH-untreated (<i>n</i> = 5)	Reference (<i>n</i> = 16)	GH-treated (<i>n</i> = 8)	GH-untreated (<i>n</i> = 9)
VAT (cm ²)	10.0 ± 3.2	14.8 ± 4.7	12.1 ± 3.7	6.7 ± 0.9	7.5 ± 2.2	15.2 ± 5.5	7.9 ± 2.6	10.8 ± 5.1
SAT (cm ²)	38.4 ± 9.9	75.2 ± 38.3*	31.6 ± 20.6	22.5 ± 11.8	70.6 ± 10.1**	44.9 ± 30.0	28.4 ± 13.5	72.6 ± 26.7

The values are expressed as mean ± SD to maintain consistency with the reference data (ref 30).

VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue

*: *p* < 0.05 vs. age-matched reference, **: *p* = 0.06 vs. age-matched reference

Table 5 Clinical characteristics of subjects who had never received nutritional intervention or GH treatment

Case	Age (years)	Sex	Genotype	HT-SDS	BW-SDS	BMI (kg/m ²)	BMI-SDS	VAT (cm ²)	SAT (cm ²)	AST (IU/L)	ALT (IU/L)	TC (mg/dL)	HbA1c (%)
1	15.9	Female	deletion	−1.56	1.52	29.3	2.05	68.9	342.4	69	108	236	6.4
2	14.9	Male	deletion	−2.10	1.35	30.4	2.13	84.9	283.9	67	106	233	10.7
3	12.1	Male	deletion	−0.90	1.91	32.5	2.36	228.5	391	29	49	243	14.1

HT, height; BW, body weight; BMI, body mass index; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; TC, total cholesterol

difficulty in changing their eating habit. In Case 3, his first visit was 1 years and 8 months old, but the parents refused to receive nutritional support. Despite their regular visit to our department, all three subjects progressively developed obesity. CT analysis revealed that all the subjects showed increased VAT and SAT compared to the age- and sex-matched standard values of the Japanese population (11–15 years, VAT: 21.0 ± 9.3 cm² in boys, 19.1 ± 7.9 cm² in girls, SAT: 45.5 ± 25.7 cm² in

boys, 92.3 ± 34.8 cm² in girls) [32]. Circulating levels of AST, ALT, TC and HbA1c in these subjects were shown in Table 5. All subjects developed diabetes mellitus when they were 17, 13, and 8 years old in Case 1, 2, and 3, respectively. These cases may imply the importance of early introduction of nutritional intervention for preventing the development of obesity.

Discussion

Nutritional intervention and GH treatment have long been central to the management of PWS. Although the beneficial effects of GH treatment have been documented by multiple RCTs and meta-analyses, concrete evidence for the effects of nutritional intervention has been limited partly because performing RCTs to investigate the effects of nutritional intervention are not ethically permitted. Based on the clinical observational studies, 10 kcal/cm/day of nutritional restriction has been a widely used strategy for nutritional management in PWS patients [33, 34]. By usage of this strategy, the reduction in body weight and BMI has been observed in childhood and adolescent PWS [20, 21]. These findings indicate that nutritional intervention is beneficial in maintaining BW and BMI in PWS children.

As mentioned above, the effectiveness of nutritional management has been evaluated based on its effects on reducing BW and/or BMI. Since there is evidence that metabolic complications in PWS are positively associated with VAT [23], it is important to evaluate fat distribution in addition to BW and BMI; however, the effects of nutritional intervention on fat distribution have not been evaluated so far. In the current study, we investigated the effects of nutritional intervention on fat distribution and found the beneficial effect on fat distribution such that the amount of VAT is well managed within the reference range. This may be due to the early introduction of nutritional intervention, because alterations in body composition in PWS occur before the onset of hyperphagia [35]. Indeed, early introduction of nutritional management before 18 months of age has been shown to result in decreased BMI at 4 and 10 years of age compared to those who had never been nutritionally supported [36, 37]. In addition, early introduction of nutritional intervention was usually accompanied by the commencement of educational programs for the parents. This helps parents understand the nutritional and behavioral phases of PWS children, and allows them to control their child's appetite using strict supervision of daily food. Indeed, parents who know the characteristic feeding behavior of a PWS patient were shown to be more successful in arranging a suitable dietary environment [33]. Thus, these findings point to the importance of the early introduction of nutritional intervention with concomitant commencement of educational programs for controlling body weight and VAT in PWS children. In contrast to VAT, SAT was elevated in GH-untreated subjects compared to reference values whereas it was not in GH-treated group, which raised the possibility that nutritional intervention was not sufficient to maintain SAT within a reference range.

In the current study, we were not able to evaluate the effect of GH treatment on body composition because GH treatment is only approved in PWS children with short stature in Japan, which probably causes a selection bias. Previous studies have reported the effects of GH on fat distribution in GH-deficient children and found that VAT was reduced by GH replacement therapy [38], which was partly caused by its stimulatory function on lipolysis in adipose tissue [39]. As shown in Table 4, VAT was resided in the reference range both in GH-treated and GH-untreated groups, suggesting that nutritional intervention alone may be sufficient to keep VAT within a reference range during childhood. In contrast, in adult PWS subjects, there is evidence to show that when GH treatment was discontinued VAT increased in a short period, indicating the beneficial role for GH treatment on VAT [40, 41]. The distinct finding of GH on VAT between childhood and adult PWS may be caused by the fact that nutritional management by caregivers is more strictly controlled during childhood than adulthood; therefore, in the condition of appropriate nutritional intervention especially during the childhood, the effect of GH treatment on VAT may be minimized.

GH treatment has been effective in increasing LBM in PWS children, which is of clinical importance because the amount of LBM was negatively associated with metabolic complications [42]. Numerous RCTs have indicated the positive effects of GH treatment on LBM [10, 12-15, 17, 43-45]. In the present study, as mentioned above, we were not able to compare LBM between GH-treated and GH-untreated group, but in line with the previous reports median value of percent LBM in GH-treated group was higher than that of GH-untreated group. Interestingly, median value of bone-size corrected BMAD was higher in GH-untreated group. Given the evidence showing the lack of effects of GH treatment in increasing BMD in PWS children [46], the increase in BMAD in GH-untreated group may be a consequence increased gravity load on skeleton by heavier BW.

Of note in this study was that HT-SDS significantly decreased during nutritional intervention in GH-untreated group. Since growth curves for Japanese PWS patients indicated that HT-SDS was maintained during childhood [47], this finding indicates the possibility that nutritional intervention may compromise linear growth due to excessive calorie restriction. Consistent with this, IGF-1 SDS, one of the markers for nutritional status, was also declined during the follow-up. Although we were not able to exclude the possibility of accompanied GH deficiency in GH-untreated group (only 1 out of 9 subjects in GH-untreated group received GH provocation test with normal GH response being observed), we need to strictly monitor nutritional status during nutritional

intervention to avoid the nutritional intervention-associated growth restriction. Indeed, dietary restriction has been shown to result in reduced height gain during early childhood [37], suggesting that the most appropriate strategy for nutritional intervention needs further clarification and should be context-specific.

Our study has the following limitations. First, this study was performed in a single center in a retrospective manner; therefore, the sample size was relatively small. Second, energy intake was not monitored in a quantitative manner. Third, statistical analysis between GH-treated and GH-untreated groups was not performed. Since GH treatment is only approved in PWS children with short stature in Japan, there may be a selection bias between GH-treated and GH-untreated groups; therefore statistical analysis between these two groups may lead to the misunderstanding of the data. Fourth, we did not have enough number of age-matched PWS subjects who had never received nutritional intervention or GH treatment. Further prospective analysis is required to better understand the role of nutritional intervention on body composition and fat distribution.

In conclusion, we herein demonstrated the beneficial effects of nutritional intervention for controlling body

composition in PWS children with VAT being maintained within the reference range. The finding that HT-SDS declined during nutritional intervention in GH-untreated group indicated the potential unfavorable effect of nutritional intervention on linear growth. Although we have to strictly monitor linear growth that may be impaired by excessive nutritional intervention, the current results provide evidence that supports the beneficial effects of nutritional intervention for controlling body fat in PWS children.

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Disclosure

None of the authors have any potential conflicts of interest associated with this research.

References

- Cassidy SB, Schwartz S, Miller JL, Driscoll DJ (2012) Prader-Willi syndrome. *Genet Med* 14: 10–26.
- Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold JA, *et al.* (2011) Nutritional phases in Prader-Willi syndrome. *Am J Med Genet A* 155A: 1040–1049.
- Khan MJ, Gerasimidis K, Edwards CA, Shaikh MG (2018) Mechanisms of obesity in Prader-Willi syndrome. *Pediatr Obes* 13: 3–13.
- Grugni G, Crino A, Bedogni G, Cappa M, Sartorio A, *et al.* (2013) Metabolic syndrome in adult patients with Prader-Willi syndrome. *Nutr Metab Cardiovasc Dis* 23: 1134–1140.
- Nagai T, Obata K, Tonoki H, Temma S, Murakami N, *et al.* (2005) Cause of sudden, unexpected death of Prader-Willi syndrome patients with or without growth hormone treatment. *Am J Med Genet A* 136: 45–48.
- Whittington JE, Holland AJ, Webb T, Butler J, Clarke D, *et al.* (2001) Population prevalence and estimated birth incidence and mortality rate for people with Prader-Willi syndrome in one UK Health Region. *J Med Genet* 38: 792–798.
- Sanchez-Ortiga R, Klibanski A, Tritos NA (2012) Effects of recombinant human growth hormone therapy in adults with Prader-Willi syndrome: a meta-analysis. *Clin Endocrinol (Oxf)* 77: 86–93.
- Deal CL, Tony M, Hoybye C, Allen DB, Tauber M, *et al.* (2013) Growth Hormone Research Society workshop summary: consensus guidelines for recombinant human growth hormone therapy in Prader-Willi syndrome. *J Clin Endocrinol Metab* 98: E1072–E1087.
- Bakker NE, Siemensma EP, van Rijn M, Festen DA, Hokken-Koelega AC (2015) Beneficial effect of growth hormone treatment on health-related quality of life in children with Prader-Willi syndrome: a randomized controlled trial and longitudinal study. *Horm Res Paediatr* 84: 231–239.
- Bakker NE, Siemensma EP, Koopman C, Hokken-Koelega AC (2015) Dietary energy intake, body composition and resting energy expenditure in prepubertal children with Prader-Willi syndrome before and during growth hormone treatment: a randomized controlled trial. *Horm Res Paediatr* 83: 321–331.
- de Lind van Wijngaarden RF, de Klerk LW, Festen DA, Duivendoorn HJ, Otten BJ, *et al.* (2009) Randomized controlled trial to investigate the effects of growth hormone treatment on scoliosis in children with Prader-Willi syndrome. *J Clin Endocrinol Metab* 94: 1274–1280.
- Festen DA, de Lind van Wijngaarden R, van Eekelen M, Otten BJ, Wit JM, *et al.* (2008) Randomized controlled GH trial: effects on anthropometry, body composition and body proportions in a large group of children with Prader-Willi syndrome. *Clin Endocrinol (Oxf)* 69: 443–451.
- Carrel AL, Moerchen V, Myers SE, Bekx MT, Whitman BY, *et al.* (2004) Growth hormone improves mobility and

- body composition in infants and toddlers with Prader-Willi syndrome. *J Pediatr* 145: 744–749.
14. Whitman B, Carrel A, Bekx T, Weber C, Allen D, *et al.* (2004) Growth hormone improves body composition and motor development in infants with Prader-Willi syndrome after six months. *J Pediatr Endocrinol Metab* 17: 591–600.
 15. Myers SE, Carrel AL, Whitman BY, Allen DB (2000) Sustained benefit after 2 years of growth hormone on body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome. *J Pediatr* 137: 42–49.
 16. Lindgren AC, Hagenas L, Muller J, Blichfeldt S, Rosenborg M, *et al.* (1998) Growth hormone treatment of children with Prader-Willi syndrome affects linear growth and body composition favourably. *Acta Paediatr* 87: 28–31.
 17. Haqq AM, Stadler DD, Jackson RH, Rosenfeld RG, Purnell JQ, *et al.* (2003) Effects of growth hormone on pulmonary function, sleep quality, behavior, cognition, growth velocity, body composition, and resting energy expenditure in Prader-Willi syndrome. *J Clin Endocrinol Metab* 88: 2206–2212.
 18. Kuppens RJ, Bakker NE, Siemensma EP, Tummers-de Lind van Wijngaarden RF, Donze SH, *et al.* (2016) Beneficial effects of GH in young adults with Prader-Willi syndrome: a 2-year crossover trial. *J Clin Endocrinol Metab* 101: 4110–4116.
 19. Kuppens RJ, Bakker NE, Siemensma EP, Donze SH, Stijnen T, *et al.* (2017) Metabolic health profile in young adults with Prader-Willi syndrome: results of a 2-year randomized, placebo-controlled, crossover GH trial. *Clin Endocrinol (Oxf)* 86: 297–304.
 20. Bonfig W, Dokoupil K, Schmidt H (2009) A special, strict, fat-reduced, and carbohydrate-modified diet leads to marked weight reduction even in overweight adolescents with Prader-Willi syndrome (PWS). *ScientificWorldJournal* 9: 934–939.
 21. Lima VP, Emerich DR, Mesquita ML, Paternez AC, Carreiro LR, *et al.* (2016) Nutritional intervention with hypocaloric diet for weight control in children and adolescents with Prader-Willi syndrome. *Eat Behav* 21: 189–192.
 22. Tang L, Zhang F, Tong N (2016) The association of visceral adipose tissue and subcutaneous adipose tissue with metabolic risk factors in a large population of Chinese adults. *Clin Endocrinol (Oxf)* 85: 46–53.
 23. Talebizadeh Z, Butler MG (2005) Insulin resistance and obesity-related factors in Prader-Willi syndrome: comparison with obese subjects. *Clin Genet* 67: 230–239.
 24. Tanaka Y, Abe Y, Oto Y, Itabashi H, Shiraishi M, *et al.* (2013) Characterization of fat distribution in Prader-Willi syndrome: relationships with adipocytokines and influence of growth hormone treatment. *Am J Med Genet A* 161A: 27–33.
 25. Goldstone AP, Thomas EL, Brynes AE, Bell JD, Frost G, *et al.* (2001) Visceral adipose tissue and metabolic complications of obesity are reduced in Prader-Willi syndrome female adults: evidence for novel influences on body fat distribution. *J Clin Endocrinol Metab* 86: 4330–4338.
 26. Isojima T, Kato N, Ito Y, Kanzaki S, Murata M (2016) Growth standard charts for Japanese children with mean and standard deviation (SD) values based on the year 2000 national survey. *Clin Pediatr Endocrinol* 25: 71–76.
 27. Suwa S, Tachibana K (1993) Standard growth charts for height and weight of Japanese children from birth to 17 years based on a cross-sectional survey of national data. *Clin Pediatr Endocrinol* 2: 87–97.
 28. Kato N, Takimoto H, Sudo N (2011) The cubic functions for spline smoothed L, S and M values for BMI reference data of Japanese children. *Clin Pediatr Endocrinol* 20: 47–49.
 29. Carter DR, Bouxsein ML, Marcus R (1992) New approaches for interpreting projected bone densitometry data. *J Bone Miner Res* 7: 137–145.
 30. Isojima T, Shimatsu A, Yokoya S, Chihara K, Tanaka T, *et al.* (2012) Standardized centile curves and reference intervals of serum insulin-like growth factor-I (IGF-I) levels in a normal Japanese population using the LMS method. *Endocr J* 59: 771–780.
 31. Kashiwagi A, Kasuga M, Araki E, Oka Y, Hanafusa T, *et al.* (2012) International clinical harmonization of glycated hemoglobin in Japan: From Japan Diabetes Society to National Glycohemoglobin Standardization Program values. *J Diabetes Investig* 3: 39–40.
 32. Satake E, Nakagawa Y, Kubota A, Saegusa H, Sano S, *et al.* (2010) Age and sex differences in fat distribution in non-obese Japanese children. *J Pediatr Endocrinol Metab* 23: 873–878.
 33. Holm VA, Pipes PL (1976) Food and children with Prader-Willi syndrome. *Am J Dis Child* 130: 1063–1067.
 34. Hoffman CJ, Aultman D, Pipes P (1992) A nutrition survey of and recommendations for individuals with Prader-Willi syndrome who live in group homes. *J Am Diet Assoc* 92: 823–830, 833.
 35. Eiholzer U, Blum WF, Molinari L (1999) Body fat determined by skinfold measurements is elevated despite underweight in infants with Prader-Labhart-Willi syndrome. *J Pediatr* 134: 222–225.
 36. Schmidt H, Pozza SB, Bonfig W, Schwarz HP, Dokoupil K (2008) Successful early dietary intervention avoids obesity in patients with Prader-Willi syndrome: a ten-year follow-up. *J Pediatr Endocrinol Metab* 21: 651–655.
 37. Schmidt H, Schwarz HP, Enders A (2001) Dietary intervention in the first four years prevents abnormal weight gain but negatively affects height development in Prader-Willi syndrome. *Acta Paediatr* 90: 468–469.
 38. Roemmich JN, Huerta MG, Sundaresan SM, Rogol AD (2001) Alterations in body composition and fat distribution in growth hormone-deficient prepubertal children during growth hormone therapy. *Metabolism* 50: 537–547.
 39. Chaves VE, Junior FM, Bertolini GL (2013) The metabolic effects of growth hormone in adipose tissue. *Endocrine* 44: 293–302.

40. Koizumi M, Ida S, Shoji Y, Nishimoto Y, Etani Y, *et al.* (2018) Visceral adipose tissue increases shortly after the cessation of GH therapy in adults with Prader-Willi syndrome. *Endocr J* 65: 1127–1137.
41. Oto Y, Tanaka Y, Abe Y, Obata K, Tsuchiya T, *et al.* (2014) Exacerbation of BMI after cessation of growth hormone therapy in patients with Prader-Willi syndrome. *Am J Med Genet A* 164A: 671–675.
42. Khazem S, Itani L, Kreidieh D, El Masri D, Tannir H, *et al.* (2018) Reduced lean body mass and cardiometabolic diseases in adult males with overweight and obesity: a pilot study. *Int J Environ Res Public Health* 15: 2754.
43. Eiholzer U, L'Allemand D, Schlumpf M, Rousson V, Gasser T, *et al.* (2004) Growth hormone and body composition in children younger than 2 years with Prader-Willi syndrome. *J Pediatr* 144: 753–758.
44. Carrel AL, Myers SE, Whitman BY, Allen DB (1999) Growth hormone improves body composition, fat utilization, physical strength and agility, and growth in Prader-Willi syndrome: a controlled study. *J Pediatr* 134: 215–221.
45. de Lind van Wijngaarden RF, Cianflone K, Gao Y, Leunissen RW, Hokken-Koelega AC (2010) Cardiovascular and metabolic risk profile and acylation-stimulating protein levels in children with Prader-Willi syndrome and effects of growth hormone treatment. *J Clin Endocrinol Metab* 95: 1758–1766.
46. Jorgensen AP, Ueland T, Sode-Carlsen R, Schreiner T, Rabben KF, *et al.* (2013) Two years of growth hormone treatment in adults with Prader-Willi syndrome do not improve the low BMD. *J Clin Endocrinol Metab* 98: E753–E760.
47. Nagai T, Matsuo N, Kayanuma Y, Tonoki H, Fukushima Y, *et al.* (2000) Standard growth curves for Japanese patients with Prader-Willi syndrome. *Am J Med Genet* 95: 130–134.