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Family-based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome

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14. ABSTRACT

Physical activity (PA) holds strong potential as a non-pharmacological therapeutic approach to complement the management of Prader-Willi Syndrome (PWS). Since childhood obesity is an escalating problem associated with multiple maladies, providing alternative PA intervention strategies is vital. We have developed an at-home PA intervention titled Active Play at Home. We expect improvements in motor coordination, sensory integration, body composition, and quality of life in participants. We have enrolled 62 families at CS UF as of 9/ 14/2012. Our preliminary findings demonstrate high compliance (~88%) with the intervention in most participants. We have also demonstrated that the Bruininks-Oseretsky Test of Motor Proficiency is a re liable instrument to evaluate motor function in individuals with PWS. We have been able to id entify seven distinct nutritional phases in individuals with PWS. We have also shown that hyperghrelinemia begins in early infancy in PWS and decreases as the individual gets older. Therefore, ghrelin is unlikely to be the "key player" in the increased appetite found in individuals with PWS.

15. SUBJECT TERMS

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INTRODUCTION

Prader-Willi Syndrome (PWS) is now the most commonly recognized genetic cause of early onset childhood obesity. Features include infantile hypotonia, mental retardation, short stature, hypogonadotrophic hypogonadism, and hyperphagia with resultant obesity if uncontrolled. Typically youth with PWS display growth hormone deficiency, leading to decreased energy expenditure, increased adiposity, and decreased lean mass (Cassidy and Driscoll, 2009). Childhood obesity in PWS is the result of an imbalance between energy intake and energy expenditure due to hyperphagia, decreased physical activity, and a reduced metabolic rate.

Nutritional Aspects (extension from previous award W81XWH-08-1-0025)

PWS is the most frequently diagnosed and best characterized genetic cause of obesity, making PWS a valuable tool in the scientific study of obesity. It is also typically diagnosed before the onset of obesity (Cassidy and Driscoll, 2009). Classical definitions typically divide PWS into two distinct nutritional stages. We propose to perform a longitudinal study to carefully investigate the following hypotheses: 1) There are 7 (not 2) distinct nutritional phases in PWS characterized by specific abnormalities in metabolism and hormonal levels and 2) Normal weight control, early-onset morbid obese (EMO), and PWS subjects have endocrine and metabolic differences.

Physical activity in youth with PWS

Physical activity (PA) is one way of intervening to prevent or ameliorate obesity in childhood. Not only is PA vital to sustained health, it has a positive impact on weight control, self-efficacy, and quality of life. The low prevalence of PWS (1 in 8,000 to 1 in 20,000 people) increases the difficulty of doing group-based PA interventions, specifically with youth. The emotional characteristics (age-inappropriate temper tantrums, stubbornness, skin picking, impulsivity, ritualistic and repetitive behaviors), as well as less evolved motor patterns prevalent in youth with PWS compared to youth of similar age without the syndrome also argues for tailored interventions. We will test different exercise strategies such as interactive games and goal-oriented and age-appropriate activities that can be utilized in a *24-week* home-based intervention to increase PA levels in youth with PWS *(n=40)* and without the syndrome *(n=40)*. We will determine if the intervention is effective in improving motor, physical, and psychological parameters. Providing alternative PA choices for PWS youth is of utmost importance, as PA is a key component in the management of this syndrome. In addition, demonstrating that tailored activities are successful in engaging individuals with this syndrome is necessary as well as useful for the PWS community, which could use the strategies developed in the current study. Moreover, providing a wide range of home-based activities to obese youth contributes to the national efforts combating childhood obesity.

Parents as facilitators of PA in youth

Parental involvement has been shown to be important in both prevention and treatment of childhood obesity in children without PWS. For youth, parents are the primary mediators of change in adopting a healthy lifestyle and reducing sedentary behaviors. It has been recognized that parents make most of the decisions regarding lifestyle choices for their youth, particularly for youth who are mentally and physically challenged, including youth with PWS. Although parents certainly want their youth to be healthier, there are many concerns that call for their energy and time. Deciding to commit to a PA program is a matter of managing priorities. Therefore, one of the components of our study is a decision-reporting intervention for parents/guardians of youth with PWS. This intervention attempts to demonstrate that asking parents/guardians to document their daily decisions regarding their youth's PA serves to increase that activity. We expect that youth whose parents/guardians report their daily decisions will exhibit higher PA levels than those youth whose parents/guardians do not. We will provide parents with personal digital assistants (PDA's) to record their daily decisions in detail.

BODY

Nutritional Aspects of Prader-Willi Syndrome and Childhood Obesity (University of Florida Progress Report, Revised on January 24th 2013)

This study is a continuation of award W81XWH-08-1-0025. Description of procedures: Subjects with PWS have been recruited from various age groups and compared to two age-matched groups: 1) obese individuals who have early-onset morbid obesity (EMO) and 2) a normal weight sibling of PWS control group (Sib.C). All subjects (PWS, EMO and Sib.C) are admitted to the Clinical Research Center (CRC) at the University of Florida for 2 days of intensive study. Testing begins with a thorough history and physical, including a nutritional assessment. Fasting blood work is obtained for a standard chemistry panel, lipid profile, uric acid, insulin and thyroid function tests. A two-hour oral glucose tolerance test is routinely performed. Serum and plasma are obtained in order to measure various appetite regulating hormones and cytokines. Basal metabolic rate and body composition (via DXA) are also assessed. Subjects are followed annually or biennially in the CRC depending upon their age.

The body of this part of the report was reorganized to address each of the aims of this project.

Aim #1: Establish age range for the various nutritional phases in PWS.

Aim #2: Determine if there are particular hormonal and other biochemical changes associated with each phase.

Aim #3: Establish if there are changes in basal metabolic rate (BMR), body fat measurements and caloric input associated with each phase.

Aim #4: Compare the PWS patients to normal weight sibling controls and EMO patients with respect to chemistries, hormonal levels, BMR, body fat and caloric input.

We initially proposed the following milestones for this project.

Project Milestones (March 1, 2011 to August 31, 2012)

Subject recruitment and data collection	*0 - 12 months*
Biochemical assays	*12 - 13 months*
Nutritional phases data analyses	*14 - 15 months*
Manuscript preparation:	*16 - 17 months*
Manuscript submission	*18 months*

Aims #1 and #3 have been completed – see manuscript (Miller et al, 2011 – Appendix A). We are currently completing aims #2 to #4. Since the last reporting period, we assayed serum ghrelin and plasma leptin levels of individuals with PWS, EMO, and Sib.C between the ages of 0.1 – 21 years. In addition, we have analyzed three analytes using the Luminex system: C-reactive protein, interleukin-6 and tumor-necrosis factor alpha. In addition, we anticipate that we will complete all aims of this project by February 28, 2013. We have written a draft of a manuscript reporting our results on ghrelin and leptin, which we will circulate shortly to our co-authors before submitting to a journal. It is anticipated that a manuscript on the other analytes will be submitted in 2013.

Results

Preliminary findings (as of September 29, 2012 but revised as of January 24, 2013)

Aims #1 and #3

Nutritional Phases

We have identified 7 distinct nutritional phases, with 5 major phases and sub-phases of phases 1 and 2 in individuals with PWS (Miller et al., 2011). The initial phase, phase 0, occurs *in utero*, with decreased fetal movements, birth weight and length. In phase 1 the infant is hypotonic and not obese, with sub-phase 1a characterized by difficulty feeding (often requiring feeding via a gastric tube or nasogastric tube) with or without failure to thrive. This phase is followed by sub-phase 1b when the infant begins to feed better and grows steadily along a growth curve with weight increasing at a normal rate. Phase 2 is associated with weight increase. Sub-phase 2a occurs when the child has an increase in weight without a significant change in appetite or caloric intake, while in sub-phase 2b the child experiences continuing weight increase with an increased interest in food. Phase 3 is characterized by the development of hyperphagia, typically accompanied by food-seeking and lack of satiety. Phase 4 occurs when an individual who was previously in phase 3 no longer has an insatiable appetite and can feel full. This last phase has only been observed in adulthood. It should be noted that not all individuals necessarily go through all the phases and sub-phases delineated above, but most do.

More adult subjects are needed to adequately assess phase 4. With respect to genotype-phenotype correlations we found that the PWS subjects with maternal uniparental disomy of chromosome 15 (UPD) tended to have later completion times for the nutritional phase versus those with paternal 15q11.2 deletions for stages 1b and 2a, but earlier completion times for 2b. However, there were no significant differences between these 2 molecular classes. The change in status from “no growth hormone” to “growth hormone” treatment showed a tendency to accelerate the changes in phases, but this only reached significance for phase 1a ($p<.05$).

A comparison of the adjacent nutritional phases revealed significant differences in fasting IGF-1, glucose, and insulin as well as the BMI z-score, mean resting energy expenditure and percent body fat by DXA (Miller et al., 2011). A significant difference was not found in resting triglycerides and mean respiratory quotient.

Aims #2 and #4

Participant Frequencies and Demographics

During this reporting period, we have enrolled 51 additional participants (PWS: n=24; EMO: n=4, and Sib.C: n=23). Total visits to the CRC during this reporting period were 141 (51 first visits, 89 second visits, and 1 third visit).

We have been enrolling research subjects in this study since obtaining our IRB approval from the University of Florida on October 15, 2008. To date 205 subjects (295 visits) have been admitted to the CRC from the 3 different groups: 1) PWS: 90 subjects; 134 visits; 2) EMO: 15 subjects; 22 visits) and 3) Sib.C: 100 subjects; 141 visits.

Study characteristics for individuals in each group are shown in Table 1. Results for ghrelin and leptin hormonal assays, BMI z-score and body fat percentage (%) through DXA are shown in Table 2.

After adjusting for age, the mean ghrelin level for PWS individuals was 975 pg/ml higher than Sib.C (SE=237, $p<0.001$) and 1147 pg/mL higher than EMO (SE=316, $p<0.001$) individuals. Ghrelin levels decreased on average 76 pg/mL per year of age (SE=15, $p<0.001$) with no statistical evidence of an age by group (PWS vs.

EMO vs. Sib.C) interaction (*p=0.16*). In other words, the rate at which ghrelin decreased with age showed no significant difference amongst the three groups. Ghrelin levels also decreased in PWS subjects, albeit non-significantly, on average by 143 pg/mL per unit increase in BMI z-scores (SE=123, *p=0.25*) after adjusting for age.

Table 1. Characteristics of study participants.

	PWS	**Sib.C**	**EMO**
0 - 1.99 years			
Subjects	18 (9M, 9F)	12 (7M, 5F)	N/A
Observations	25 (15M, 10F)	14 (8M, 6F)	N/A
Age (years)	1.1 ± 0.5	0.91 ± 0.5	N/A
Mol. Class (Del/UPD/ID)	14/9/2	N/A	N/A
GH treatment (Yes/No)	14/11	N/A	N/A
2 - 4.99 years			
Subjects	41 (22M, 19F)	26 (11M, 15F)	9 (6M, 3F)
Observations	53 (27M, 26F)	28 (11M, 17F)	9 (6M, 3F)
Age (years)	3.7 ± 0.7	3.7 ± 0.9	4.1 ± 0.9
Mol. Class (Del/UPD/ID)	33/18/2	N/A	N/A
GH treatment (Yes/No)	49/4	N/A	N/A
5 - 11.99 years			
Subjects	29 (12M, 17F)	54 (25M, 29F)	20 (10M, 10F)
Observations	41 (17M, 24F)	74 (31M, 43F)	28 (16M, 12F)
Age (years)	7.5 ± 1.8	8.0 ± 1.7	8.2 ± 1.8
Mol. Class (Del/UPD/ID)	26/13/2	N/A	N/A
GH treatment (Yes/No)	36/5	N/A	N/A
12 - 20.99 years			
Subjects	12 (7M, 5F)	23 (14M, 9F)	12 (5M, 7F)
Observations	17 (9M, 8F)	31 (18M, 13F)	15 (7M, 8F)
Age (years)	16.2 ± 2.8	15.5 ± 2.0	15.7 ± 2.7
Mol. Class (Del/UPD/ID)	15/2/0	N/A	N/A
GH treatment (Yes/No)	14/3	N/A	N/A

Table 2: Clinical and hormonal data.

	PWS	**Sib.C**	**EMO**	***p1***	***p2***	***p3***
0 – 1.99 years						
Ghrelin (pg/ml)	5521 ± 3696	2883 ± 1172	N/A	0.016* (0.0087**)	N/A	N/A
Leptin (pg/ml)	272 ± 231	216 ± 145	N/A	0.48 (0.39)	N/A	N/A
Weight-for-length (%)	25.07 ± 28.17	57.41 ± 37.50	N/A	0.025* (0.015*)	N/A	N/A
2 – 4.99 years						
Ghrelin (pg/ml)	3113 ± 1898	2556 ± 927	3430 ± 2320	0.12 (0.041*)	0.71 (1.0)	0.30 (0.18)
Leptin (pg/ml)	1389 ± 1785	150 ± 99	2248 ± 1107	<0.001** (0.0040*)	0.098 (0.15)	<0.001** (<0.001**)
BMI *z*-score	0.93 ± 1.55	0.32 ± 1.19	4.29 ± 0.79	0.074 (0.083)	<0.001** (<0.001**)	<0.001** (<0.001**)
Body fat %	24.98 ± 10.47	18.61 ± 6.39	44.04 ± 5.78	0.005** (0.0066)	<0.001** (<0.001**)	<0.001** (<0.001**)
5 – 11.99 years						
Ghrelin (pg/ml)	2476 ± 1332	2111 ± 1013	1645 ± 983	0.21 (0.044*)	0.021* (0.016*)	0.10 (0.55)
Leptin (pg/ml)	2107 ± 1572	397 ± 720	2408 ± 1569	<0.001** (<0.001**)	0.56 (0.25)	<0.001** (<0.001**)
BMI *z*-score	1.62 ± 1.17	0.35 ± 0.92	2.72 ± 0.22	<0.001** (<0.001**)	<0.001** (<0.001**)	<0.001** (<0.001**)
Body fat %	35.08 ± 12.75	20.39 ± 8.05	45.83 ± 4.97	<0.001** (<0.001**)	<0.001** (<0.001**)	<0.001** (<0.001**)
12 – 20.99 Years						
Ghrelin (pg/ml)	2086 ± 885	1233 ± 509	1053 ± 847	0.011* (<0.001**)	0.0085 ** (0.0056**)	0.49 (0.21)
Leptin (pg/ml)	2837 ± 1839	1138 ± 1485	5459 ± 2289	0.0094** (0.0090**)	0.0060** (<0.001**)	<0.001** (<0.001**)
BMI *z*-score	2.10 ± 0.66	0.50 ± 1.08	2.73 ± 0.34	<0.001** (<0.001**)	0.0051** (0.051)	<0.001** (<0.001**)
Body fat %	47.95 ± 8.72	26.00 ± 10.79	54.28 ± 6.09	<0.001** (<0.001**)	0.049* (0.090)	<0.001** (<0.001**)

Note(s): All data are expressed as Mean ± SD. P-values are first given by Mean of Means and then by (Mixed Model).
{* = *P*-value less than 0.05; ** = *P*-value less than 0.01}
p1 = *P*-value for comparison of PWS vs Sib.C
p2 = *P*-value for comparison of PWS vs EMO
p3 = *P*-value for comparison of Sib.C vs EMO

Children less than 2 years old

PWS children less than 2 years old had significantly higher ghrelin levels than their normal counterparts of same age as analyzed by both the mixed model and mean of means (Table 2; Figure 1). The average weight-for-length percentile of normal control children was significantly higher than that for PWS children, however the body fat % of PWS children as measured by DXA was not significantly different from that of the normal control children (Table 2). There was no significant correlation between ghrelin and weight-for-length or body fat in either PWS or normal children less than 2 years old.

Serum leptin levels did not differ significantly between PWS and normal children below the age of 2 years (Table 2). Leptin levels in PWS children correlated significantly with weight-for-length, but not with body fat % or ghrelin. There were no significant leptin correlations observed in the normal children.

Children 2-4 years old

Serum ghrelin levels in PWS children 2 – 4 years old was significantly elevated relative to normal control siblings (Sib.C) by the mixed model but not by mean of means (Table 2). Ghrelin levels in EMO children did not differ significantly from ghrelin levels in PWS and Sib.C in both statistical models (Table 2).

Serum leptin was significantly elevated in PWS relative to Sib.C. EMO children also had significantly higher leptin levels than Sib.C, however, their leptin level was not significantly different from that in PWS children (Table 2).

Body fat % of PWS children as measured by DXA was significantly more than body fat % of Sib.C children, however, average BMI z-scores did not differ significantly between the two groups (Table 2). EMO children had significantly more body fat % than both PWS and Sib.C children. The average BMI z-score of EMO children was also significantly higher than that of PWS and Sib.C children (Table 2).

Children 5-11 years old

PWS children 5 – 11 years old had significantly elevated ghrelin levels relative to their Sib.C counterparts by the mixed model *(p=0.044)* but not by mean of means *(p=0.21).* However, their ghrelin level was significantly elevated relative to their EMO counterparts by both statistical models (Table 2). There was no significant difference between ghrelin levels in Sib.C and EMO children (Table 2).

EMO children had significantly higher leptin, body fat % and BMI z-scores than PWS children. Both PWS and EMO children had significantly higher leptin, BMI z-scores and body fat % than their Sib.C counterparts (Table 2).

Teenagers and young adults 12-20 years old

The ghrelin levels of PWS teenagers and young adults 12 – 20 years old was significantly elevated relative to their Sib.C and EMO counterparts (Table 2). There was no significant difference between ghrelin levels in Sib.C and EMO (Table 2).

Plasma leptin, body fat % and BMI z-scores of PWS and EMO subjects were significantly higher relative to their Sib.C counterparts (Table 2). EMO subjects had significantly higher plasma leptin, body fat % and BMI z-scores than PWS subjects (Table 2).

Ghrelin and PWS nutritional phases

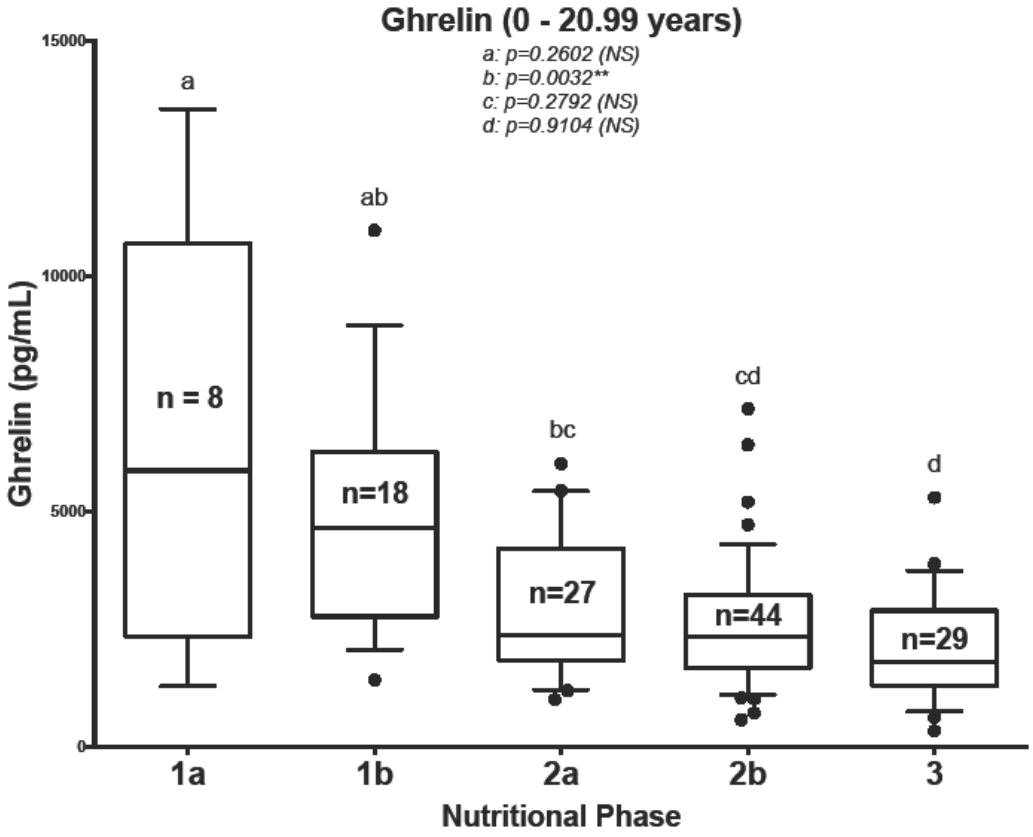


Figure 1. Ghrelin levels at the various nutritional phases in PWS.

PWS subjects in nutritional phase 1a had the highest ghrelin levels measured (Figure 1). Nutritional phase 1a and 1b together have significantly higher ghrelin levels than phases 2a, 2b, 3, 4, and no further partition of the adjacent phases was significant. We estimate the mean ghrelin levels for combined phases 1a and 1b are 3075 pg/mL higher than the other phases (SE=480, $p<0.001$). After adjusting for nutritional phase, there was no significant association between age and ghrelin levels in PWS subjects. However, nutritional phase is highly prognostic of ghrelin levels in PWS individuals after adjusting for age.

Progression from the early nutritional phases (1a, 1b) to the later phases (2a, 2b, 3) correlated with a significant decrease in ghrelin levels in PWS children between the ages of 0 – 5 years (6043 pg/ml, SE=818 vs 2921 pg/ml, SE=210; $p<0.001$). PWS children 0 – 5 years old in nutritional phase 1a and 1b had significantly higher ghrelin levels than Sib.C children of similar age (6043 pg/ml, SE=818 vs 2656 pg/ml, SE=184; $p<0.001$). However, the ghrelin levels of Sib.C children 0 – 5 years old did not differ significantly from their PWS counterparts in nutritional phase 2a, 2b, and 3 of similar age (2656 pg/ml, SE=184 vs 2921 pg/ml, SE=210; $p=0.3574$).

Ghrelin and growth hormone therapy

We analyzed average ghrelin levels in PWS individuals not on growth hormone therapy relative to PWS individuals on growth hormone therapy. Growth hormone treatment was associated with a mean decrease of 1202 pg/mL (SE=535; $p=0.043$) in PWS ghrelin levels after adjusting for age.

Ghrelin and PWS molecular classes

There were no significant differences in ghrelin levels between PWS patients born with Type 1 and Type 2 deletion. Patients born with UPD (Uniparental Disomy) and ID (imprinting defect) tended to have lower ghrelin levels but it was not significantly different from subjects with deletions.

Inflammatory markers (also reported in the February 2012 progress report for Award W81XWH-08-1-0025)

We have assayed a total of 325 plasma samples derived from 206 subjects using the Luminex system.This system is based on an antibody coated, poly-bead microspheres technology allowing for multi-analyte detection (multi-plexing) within a single well of a 96-well micro-plate.

The data from these analyses has been normalized using 4 control samples run on every assay. Many subjects assayed had samples collected at different time points during the course of the study explaining the discrepancy between sample number and subject number. Samples from all three groups (PWS, EMO and Sib. C) were evaluated, controlling for age, gender, body mass index (BMI) standard deviation (Z) scores. Some samples gave spurious values and these were eliminated from the final analyses.

Since some individuals with PWS have good weight control and others have poor weight control we compared individuals with PWS in good weight control (BMI z-score < 2.0) with the sibling control group and individuals with PWS in poor weight control (BMI z-score > 2.0) with the EMO group. In addition, in the first 2 years of life individuals with PWS typically have a poor appetite and need to be tube fed to prevent failure-to-thrive. We therefore compared the young individuals with PWS with the sibling control group.

To date we have been able to analyze three analytes from the Luminex data: C-reactive protein, interleukin-6 and tumor-necrosis factor. These analytes have previously been found to be elevated in obesity and as a marker of systemic inflammation. A discussion of these analytes and our results follow.

C-reactive protein

C-reactive protein (CRP) is a marker of systemic inflammation related to cardiovascular disease and higher levels areseen in obese individuals. Many studies have reported a significant correlation between levels of leptin and CRP but it remains unclear whether the two proteins interact. However, increased concentrations of both CRP and leptin confer the highest risk for cardiovascular disease.

We analyzed plasma CRP levels in PWS infants less than 2 years of age relative to normal control infants of same age and found no significant difference ($p=0.2579$) (Figure 2a). We next analyzed plasma CRP levels in PWS and normal controls older than 2 years of age, but with BMI z-scores less than 2. PWS plasma CRP levels were significantly elevated ($p=0.0084$) relative to normal controls of similar age and BMI-Z scores (Figure 2b). Finally, we analyzed plasma CRP levels in PWS and EMO individuals older than 2 years of age and with BMI-Z scores greater than 2. PWS CRP levels in this age and BMI z-score range were significantly lower ($p=0.0338$) than CRP levels in EMO individuals of similar age and BMI z-scores (Figure 2c). No significant differences were found between the nutritional phases for CRP (Figure 2d).

Fig. 2a

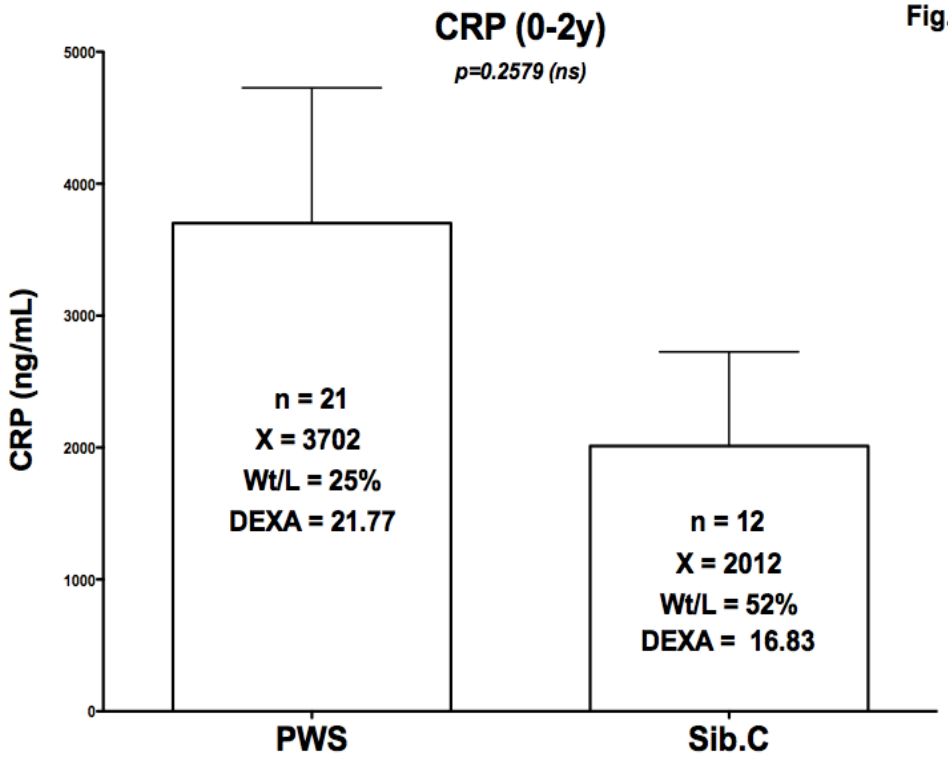
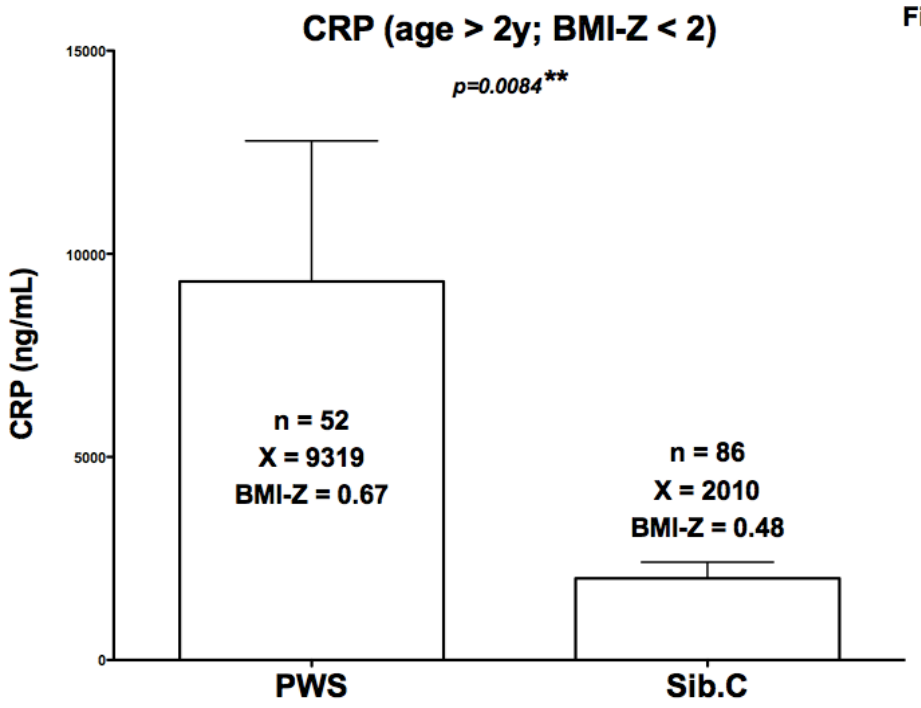

CRP (0-2y)
p=0.2579 (ns)
CRP (ng/mL)
5000
4000
3000
2000
1000
0
n = 21
X = 3702
Wt/L = 25%
DEXA = 21.77
n = 12
X = 2012
Wt/L = 52%
DEXA = 16.83
PWS
Sib.C

Fig. 2b


CRP (age > 2y; BMI-Z < 2)
p=0.0084**
CRP (ng/mL)
15000
10000
5000
0
n = 52
X = 9319
BMI-Z = 0.67
n = 86
X = 2010
BMI-Z = 0.48
PWS
Sib.C

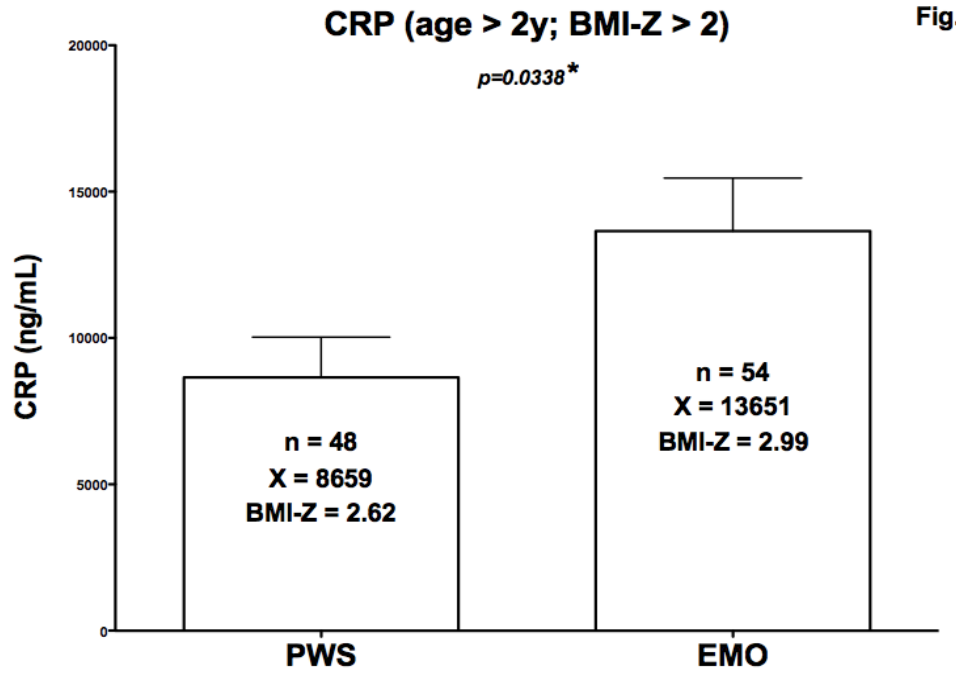

CRP (age > 2y; BMI-Z > 2)
p=0.0338*
CRP (ng/mL)
20000
15000
10000
5000
0
n = 48
X = 8659
BMI-Z = 2.62
n = 54
X = 13651
BMI-Z = 2.99
PWS
EMO

Fig. 2c

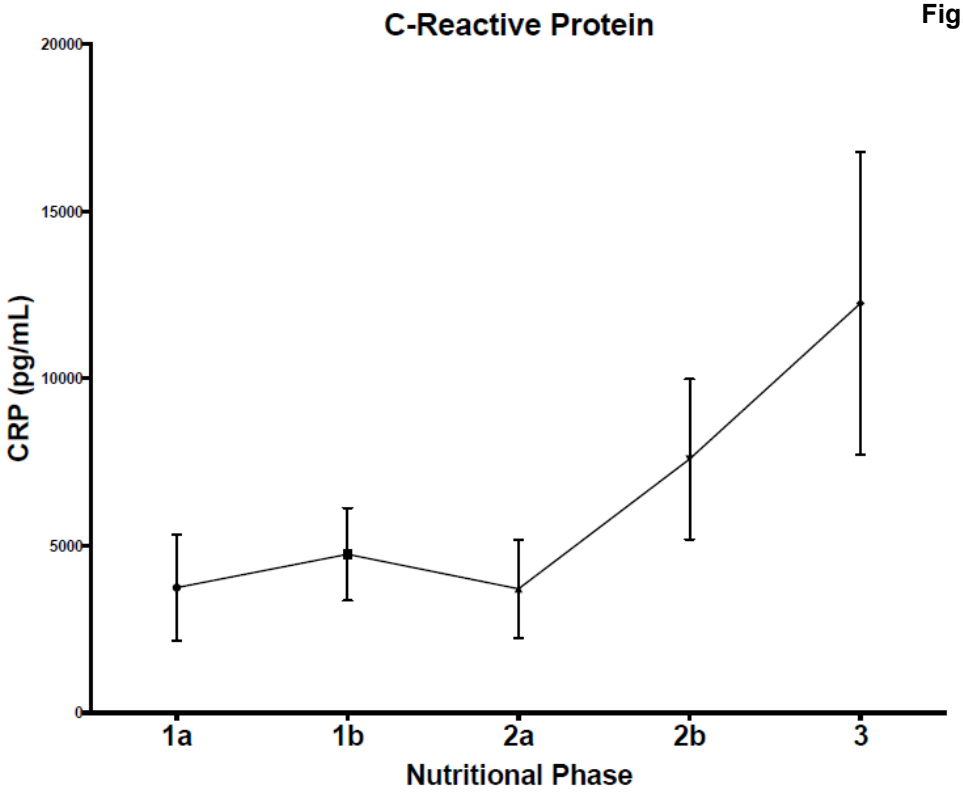

C-Reactive Protein
CRP (pg/mL)
20000
15000
10000
5000
0
1a
1b
2a
2b
3
Nutritional Phase

Fig. 2d

Interleukin-6 and Tumor-necrosis factor

Increased levels of Interleukin-6 (IL-6) and tumor-necrosis factor alpha (TNF-α) are strongly associated with cardiovascular events such as congestive heart failure (CHF), stroke, and coronary heart disease (CHD) and show high predictive value even in those with a low cardiovascular disease (CVD) profile. Patients with high IL-6, TNF-α and CRP levels have a two- to threefold increase in CHD and CHF. IL-6 and TNF-α increase early in inflammation whereas CRP increases later in the process. Thus IL-6 and TNF-α could serve as early warning signs for onset of CVD. We measured plasma IL-6 (Figures 3a, 3b, 3c) and plasma TNF-α (Figures 4a, 4b, 4c) in PWS, EMO and normal control individuals. Plasma levels for each of the nutritional phases were plotted for IL-6 (Figure 3d) and TNF-α (Figure 4d). No significant differences were found between the nutritional phases for IL-6, but multiple comparisons were significant for TNF-α (Figure 4d).

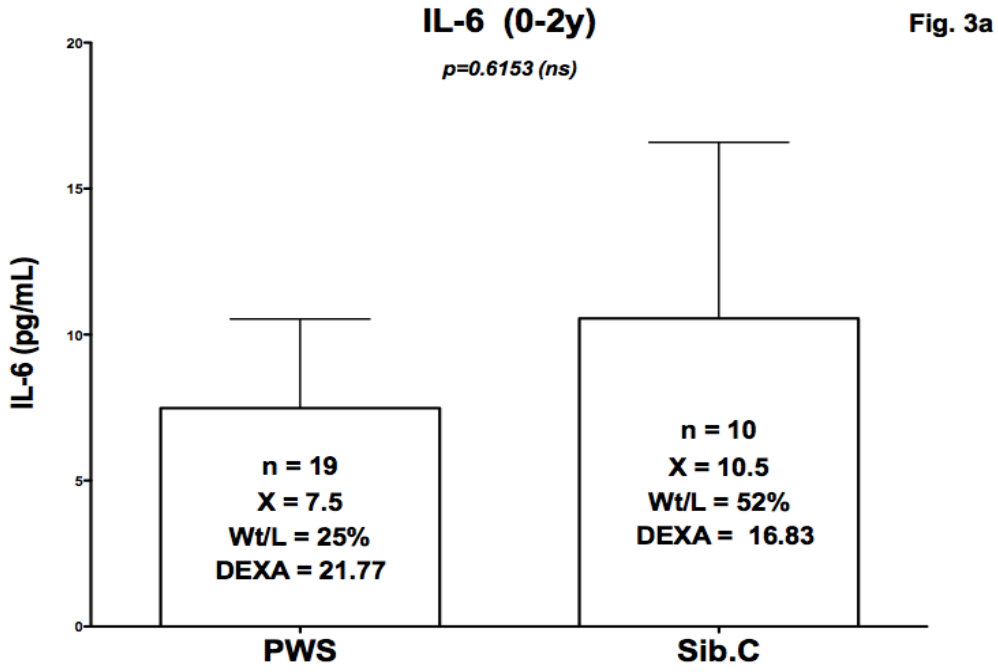

IL-6 (0-2y)
p=0.6153 (ns)
IL-6 (pg/mL)
20
15
10
5
0
n = 19
X = 7.5
Wt/L = 25%
DEXA = 21.77
n = 10
X = 10.5
Wt/L = 52%
DEXA = 16.83
PWS
Sib.C

Fig. 3a

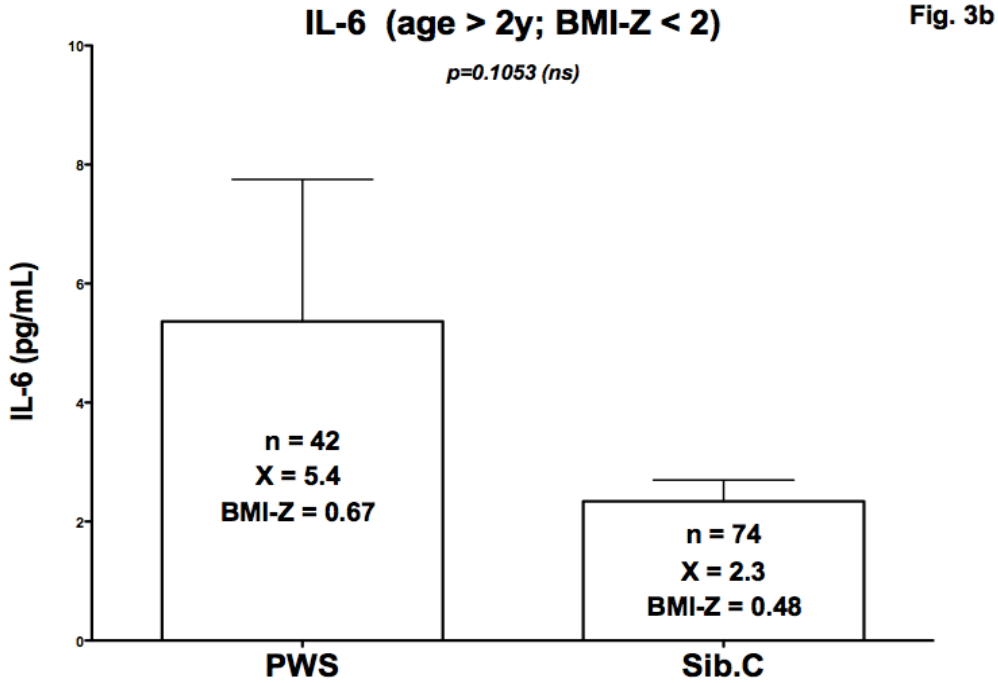

IL-6 (age > 2y; BMI-Z < 2)
p=0.1053 (ns)
IL-6 (pg/mL)
10
8
6
4
2
0
n = 42
X = 5.4
BMI-Z = 0.67
n = 74
X = 2.3
BMI-Z = 0.48
PWS
Sib.C

Fig. 3b

Fig. 3c

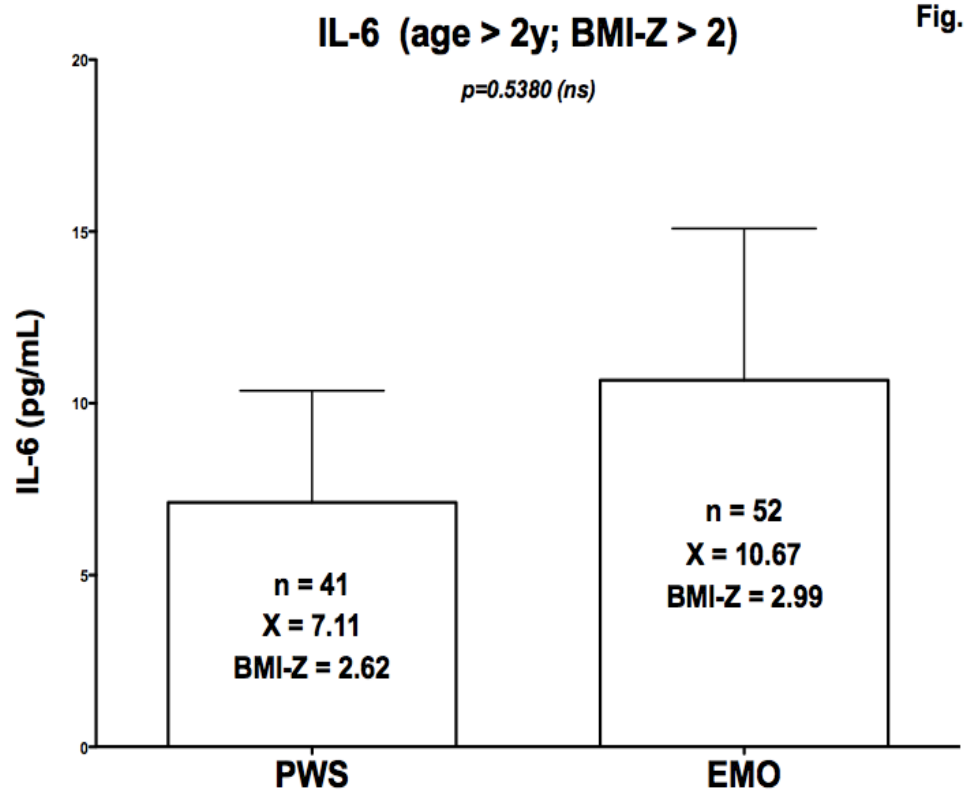
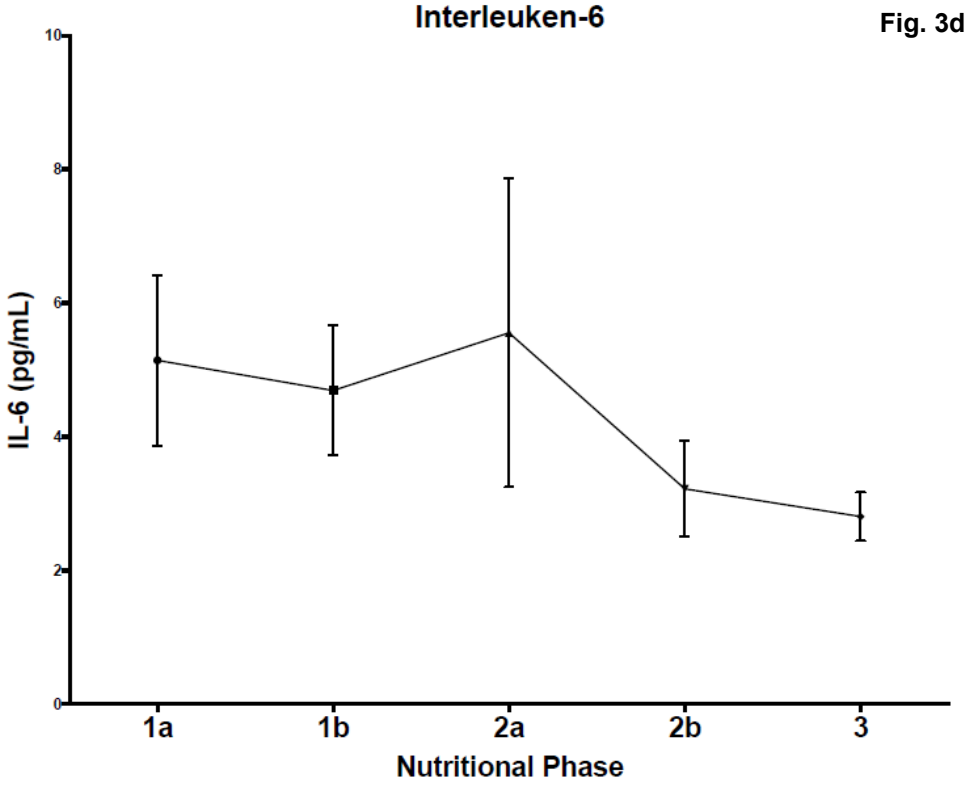

IL-6 (age > 2y; BMI-Z > 2)
p=0.5380 (ns)
IL-6 (pg/mL)
20
15
10
5
0
n = 41
X = 7.11
BMI-Z = 2.62
n = 52
X = 10.67
BMI-Z = 2.99
PWS
EMO

Fig. 3d


Interleuken-6
IL-6 (pg/mL)
10
8
6
4
2
0
1a
1b
2a
2b
3
Nutritional Phase

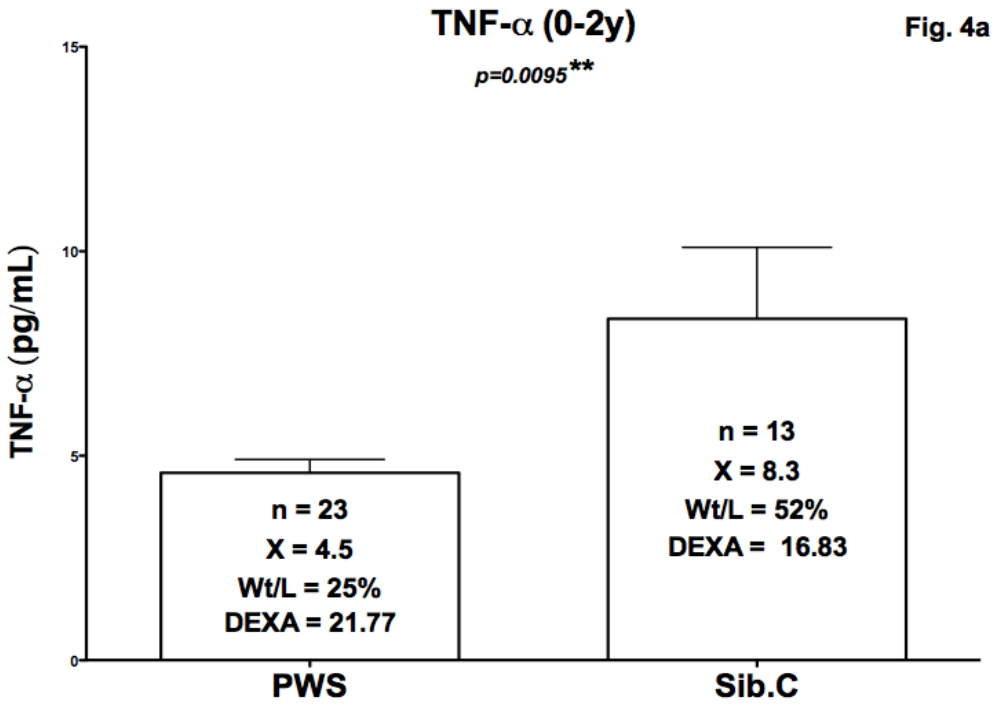

TNF-α (0-2y)
p=0.0095**
TNF-α (pg/mL)
15
10
5
0
n = 23
X = 4.5
Wt/L = 25%
DEXA = 21.77
n = 13
X = 8.3
Wt/L = 52%
DEXA = 16.83
PWS
Sib.C

Fig. 4a

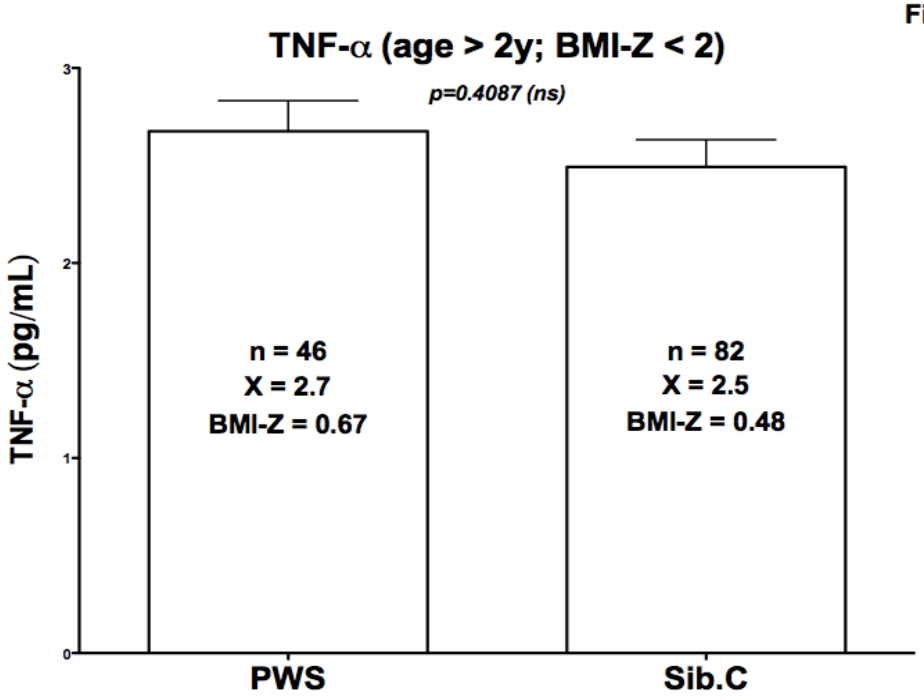

TNF-α (age > 2y; BMI-Z < 2)
p=0.4087 (ns)
TNF-α (pg/mL)
3
2
1
0
n = 46
X = 2.7
BMI-Z = 0.67
n = 82
X = 2.5
BMI-Z = 0.48
PWS
Sib.C

Fig. 4b

Fig. 4c

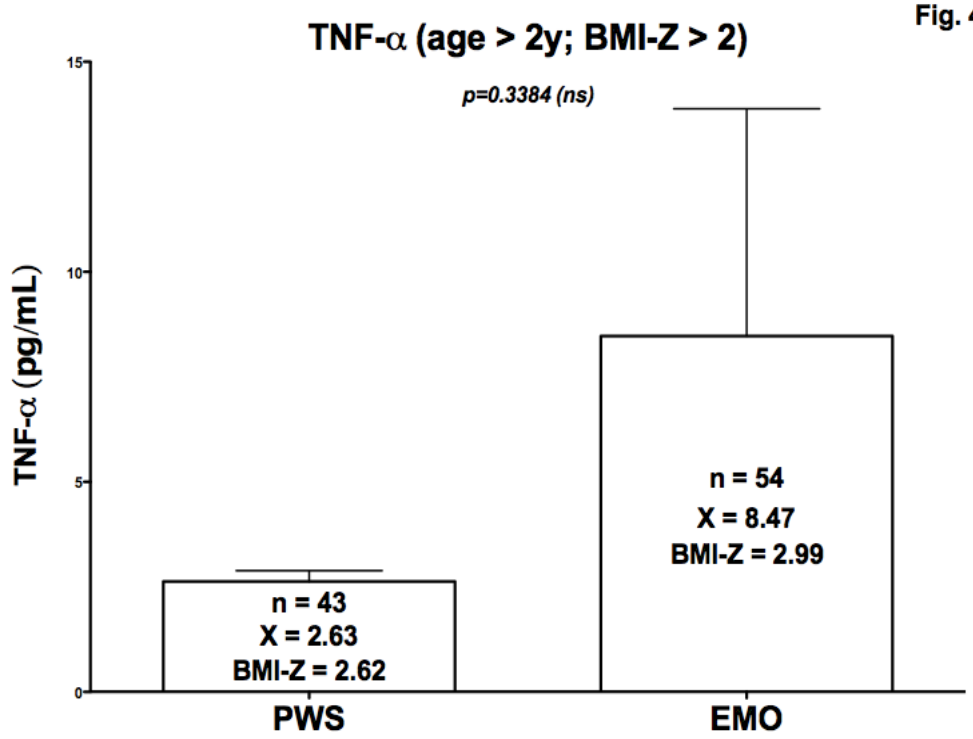
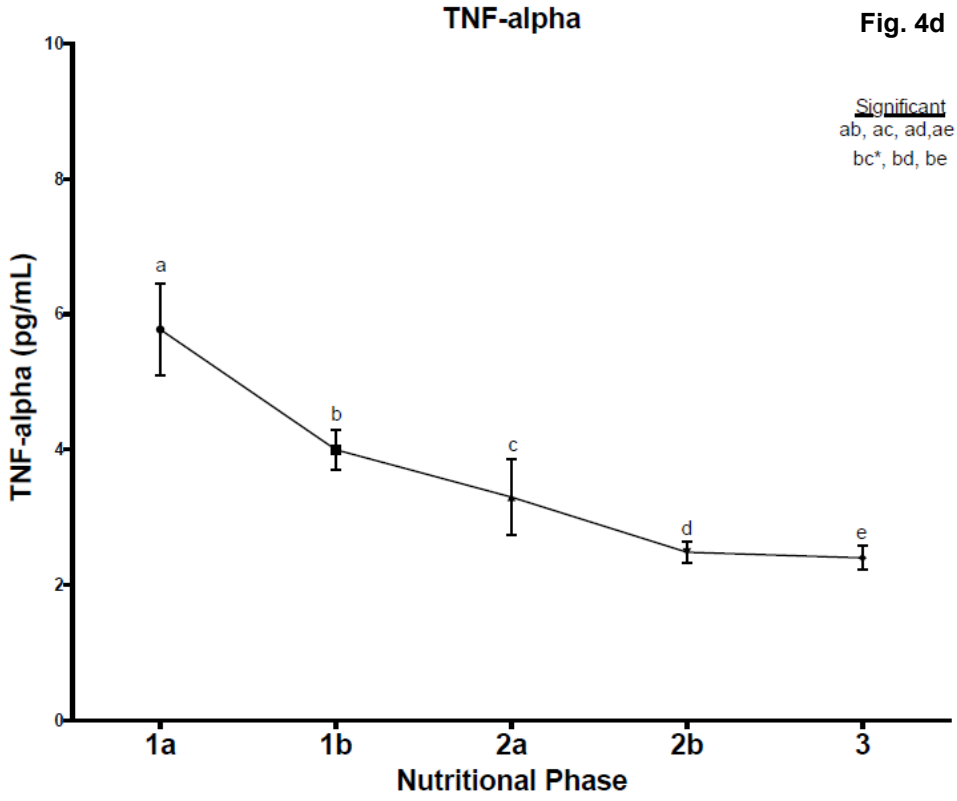

TNF-α (age > 2y; BMI-Z > 2)
p=0.3384 (ns)
TNF-α (pg/mL)
15
10
5
0
n = 43
X = 2.63
BMI-Z = 2.62
n = 54
X = 8.47
BMI-Z = 2.99
PWS
EMO

Fig. 4d


TNF-alpha
Significant
ab, ac, ad,ae
bc*, bd, be
TNF-alpha (pg/mL)
10
8
6
4
2
0
a
b
c
d
e
1a
1b
2a
2b
3
Nutritional Phase

Discussion

We have demonstrated that serum ghrelin is elevated early on in young PWS children long before the onset of obesity and hyperphagia, confirming the results of a previous study done by the French group (Feigerlova et al, 2008). PWS infants in nutritional phase 1a and 1b had significantly elevated ghrelin levels relative to normal infants. Given that ghrelin levels were the highest in PWS children with poor appetite (Phase 1a), it seems unlikely that elevated ghrelin levels are responsible for the switch to the hyperphagic phases of PWS. However, it has been demonstrated in mice that ghrelin can act to increase fat mass independent of its effect on appetite (Perez-Tilve et al., 2011). It is therefore likely that the elevated ghrelin levels are causing the increased fat mass seen in infants with PWS compared to normal infants with similar body mass indices (BMI). This may explain why PWS infants with lower weight-for-length percentile have similar amounts of body fat with normal infants.

Our results show that nutritional phase is highly prognostic of ghrelin levels in PWS and that progression to the hyperphagic nutritional phases correlates with a decrease in ghrelin levels. PWS children in nutritional phase 2a, 2b and 3 had normal ghrelin levels relative to normal control children of similar age, while PWS children in nutritional phase 1a and 1b had significantly elevated ghrelin levels relative to normal control children of similar age. Given that the age of onset of each nutritional phase varies amongst PWS subjects, analysis of their ghrelin levels by age alone may be erroneous and misleading. Thus it is possible that the inconsistencies in the literature on ghrelin levels in PWS children is a result of reliance on age alone as the major delineating factor.

Our data also suggests that growth hormone therapy may affect ghrelin levels in PWS. Individuals with PWS who were on growth hormone therapy had lower ghrelin levels than those who were off the therapy by as much as 1202 pg/ml. It is possible some of the actions of growth hormone in promoting lean body mass in young PWS subjects lie in its ability to significantly decrease ghrelin levels in early childhood.

With respect to the inflammatory factors, it is interesting to note that plasma levels for CRP, IL-6 and TNF-α were all lower in the obese children (> 2 years) with PWS than in the non-PWS EMO group. This suggests that there may be something relatively "protective" (against inflammatory factors) about being obese with PWS versus the non-PWS individuals. With respect to nutritional phases only the TNF- α had significant differences between the various phases. These observations warrant further investigation.

Summary of adverse events, unanticipated problems

None.

Summary of deviations/violations

One minor deviation: Basal Metabolic Rate (BMR) not obtained on a subject February 3, 2011 since the BMR cart was not operational.

Summary of complaints

No complaints. The research subjects and their families have been enthusiastic about the study.

Family-based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome (California State University, Fullerton [CSUF] and University of Florida, Gainesville [UFG], September 14, 2012)

We organized the body of this report to address each of the aims of this project.

Aim #1: To develop and implement a *24-week* PA intervention in youth with PWS using different methods of delivery (i.e., interactive console-based games and a PA toolkit).

Aim #2: To evaluate the effectiveness of the intervention in youth by comparing measurements at baseline, midway through the intervention (*12 weeks)*, and post-intervention (*24 weeks)*, with respect to primary (PA levels) and secondary outcomes (i.e., motor proficiency, central sensory reception and integration, health-related outcomes, quality of life, self-efficacy).

Aim #3: To determine the associations between the parent decision-making process and the level of effort in supporting regular participation in PA among youth with PWS using the hierarchical Behavioral Decision Making model as a theoretical framework.

We initially proposed the following milestones for this project.

Project Milestones (September 1, 2009 to August 31, 2013)

Intervention preparation, refinement, and pilot testing	*0 - 12 months*
Baseline assessment and intervention	*13 - 36 months*
Post intervention 24-week assessment	*19 - 37 months*
Data entry, analysis, paper writing	*20 – 48 months*

We have made substantial progress in data collection and are currently working on achieving our second and third milestones. We anticipate the completion of the third milestone will occur by July 2013 (46 months) in which the 24-week assessment will occur in the last cohort of children recruited at CSUF.

UFG has not started data collection at their site so if their recruitment of 15 children with PWS occurs in a reasonable time frame, it can be expected that milestone #3 will be achieved for the entire project no later than December 2013.

Home-based PA Intervention (Active Play at Home)

Protocol Revisions

Physical Activity Curriculum

A small change done to the protocol is the removal of the Dance Dance Revolution (DDR) game as part of the physical activity program. This removal was due to multiple reasons: 1) the version of the game chosen (DDR Hottest Party 3) was no longer manufactured; 2) the DDR dance pad was no longer compatible with the new version of the Nintendo Wii console; and 3) based on the checklists of children who have completed the program, the children played the DDR game only 18.3% of the time compared to Just Dance 2 (63.5%). Because of this change, families will play Just Dance 2 one day per week for the first 12 weeks of the program. Just Dance 3 will replace Just Dance 2 during the last 12 weeks of the program. This change was approved by the CSUF IRB on May 11, 2012 and the ORP on June 26, 2012.

Preliminary findings (as of September 14, 2012)

Participant Frequencies and Demographics

During this reporting period, we have enrolled 26 additional families, totaling 62 families in the study (Table 3). Seven of the 62 families withdrew from participation during the intervention (~10%).

Table 3. Baseline recruited participant frequencies by group (PWS and OB), sex, and ethnicity. Ethnicity is reported using the Center for Disease Control's race code database, utilized by the GE Prodigy software from the GE Lunar dual x-ray absorptiometry machine.

Group			Ethnicity					Total
			Asian	Black	Hispanic	White	Other	
PWS	Sex	Male	1	0	4	4	0	9
		Female	2	1	3	5	0	11
		Total	**3**	**1**	**7**	**9**	**0**	**20**
OB	Sex	Male	3	0	14	3	2	22
		Female	2	0	12	4	2	20
		Total	**5**	**0**	**26**	**7**	**4**	**42**
Overall Totals			**8**	**1**	**33**	**16**	**4**	**62**

Table 4. Frequency of assessments completed by group and site visit.

	PWS				**OB**		
	Baseline 1 (n=20)	**Baseline 2* (n=18)**	**Midway (n=13)**	**Post (n=14)**	**Baseline (n=42)**	**Midway (n=33)**	**Post (n=26)**
Anthropometrics	20		13	14	42	33	26
DXA Scan	20		13	14	42	33	26
BOTMP-2™	20	14	13	13	42	32	26
SOT	17	16	12	13	42	33	25
Self-efficacy Survey	20	13	10	13	42	33	26
PedsQL™ Survey	20	13	10	13	42	33	26
Accelerometer	20		13	14	40	33	26
Youth PA Log	20		11	12	37	32	26
Food Record Log	20		12	12	40	32	26

**Youth with PWS completed test-retest for the Bruininks-Oseretsky Test of Motor Proficiency (BOTMP-2™), Sensory Organization Test (SOT), self-efficacy, and Pediatric Quality of Life (PedsQL™) surveys during the Baseline 2 visit. Two participants with PWS did not complete a Baseline 2 visit.*

Results

The data presented below is descriptive only – no statistical analyses were conducted.

Baseline

Table 5. Baseline characteristics of all enrolled participants including dropouts, presented as mean ± SD.

	PWS		**OB**	
	Intervention (n=13)	**Control (n=7)**	**Intervention (n=28)**	**Control (n=14)**
Age (y)	11.2 ± 2.1	10.3 ± 2.5	10.1 ± 1.1	9.2 ± 1.2
Height (cm)	146.3 ± 13.5	135.2 ± 12.0	148.9 ± 9.1	140.7 ± 7.9
Body Mass (kg)	62.96 ± 26.60	58.31 ± 37.28	62.64 ± 18.10	54.79 ± 7.78
Waist Circumference (cm)	93.8 ± 22.8	92.4 ± 28.3	94.0 ± 14.1	90.0 ± 5.3
Body Mass Index ($kg \cdot m^{-2}$)	29.52 ± 12.40	30.33 ± 15.16	27.75 ± 5.41	27.56 ± 2.45
Body Fat (%)	47.6 ± 8.7	46.0 ± 8.3	43.9 ± 6.1	44.9 ± 4.2
Trunk Fat (%)	47.9 ± 9.1	46.0 ± 9.7	46.3 ± 6.4	46.7 ± 4.5
Lean Mass (kg)	30.53 ± 11.33	28.17 ± 14.81	32.92 ± 7.68	28.88 ± 4.76
Dual Femur BMD ($g \cdot cm^{-2}$)	0.87 ± 0.18	0.76 ± 0.16	0.92 ± 0.12	0.88 ± 0.11
Spine BMD ($g \cdot cm^{-2}$)	0.94 ± 0.20	0.90 ± 0.09	0.90 ± 0.15	0.84 ± 0.09
Total BMD ($g \cdot cm^{-2}$)	1.01 ± 0.13	0.97 ± 0.15	0.98 ± 0.09	0.96 ± 0.06
Resting HR (bpm)	80 ± 12	80 ± 10	76 ± 11	81 ± 11
Resting SBP (mm Hg)	104 ± 7	98 ± 16	103 ± 12	104 ± 7
Resting DBP (mm Hg)	70 ± 7	68 ± 12	66 ± 8	65 ± 4

PA Program Compliance

During the initial six weeks following visits to CSUF (i.e., Weeks 1-6 and Weeks 13-18) participants are more compliant with the provided curriculum (please see Table 6). Participation compliance goes down during the last six weeks between visits (i.e., Weeks 7-12 and Weeks 13-24). However, the overall compliance percentage remains good (~88%).

Table 6. PA Curriculum compliance percentage by 6-week time point, presented as mean ± SD, for all enrolled intervention group participants.

	OB (n=17)	**PWS (n=10)**	**TOTAL**
Weeks 1-6 (%)	91.9 ± 15.9	93.3 ± 6.0	92.4 ± 13.0
Weeks 7-12 (%)	93.8 ± 16.4	82.1 ± 25.5	89.4 ± 20.6
Weeks 13-18 (%)	88.2 ± 22.0	85.8 ± 18.8	87.3 ± 20.5
Weeks 19-24 (%)	83.3 ± 32.7	84.6 ± 24.4	83.8 ± 29.4

Physiological effects of the PA Program

Twenty-three obese children without PWS and 12 youth with PWS have completed the 24-week Active Play at Home program. Sixteen obese children without PWS and nine youth with PWS were assigned to the intervention group; seven obese children without PWS and three youth with PWS were assigned to the control group and completed the intervention after serving as controls (Table 7).

Table 7. Intervention and control group characteristics, presented as mean ± SD, at baseline and following 24 weeks.

	PWS				**OB**			
	Intervention (n=9)		**Control (n=3)**		**Intervention (n=16)**		**Control (n=7)**	
	Baseline	**W24-PI**	**Baseline**	**W24-NI**	**Baseline**	**W24-PI**	**Baseline**	**W24-NI**
Age (years)	11.6 ± 2.3	12.1 ± 2.3	11.0 ± 3.0	11.7 ± 2.5	10.1 ± 1.0	10.6 ± 0.9	9.4 ± 1.3	9.9 ± 1.2
Height (cm)	148.5 ± 15.5	150.9 ± 15.2	133.0 ± 6.1	134.9 ± 4.3	149.5 ± 8.7	152.8 ± 8.7	139.0 ± 8.4	142.6 ± 8.3
Body Mass (kg)	66.64 ± 29.64	67.46 ± 28.17	61.00 ± 38.89	61.37 ± 34.67	61.08 ± 13.06	64.19 ± 12.87	54.09 ± 8.65	57.19 ± 9.91
Waist Circumference (cm)	95.6 ± 21.4	96.3 ± 20.5	97.2 ±29.3	94.5 ± 25.6	93.0 ± 11.2	92.9 ± 9.9	89.2 ± 6.3	92.1 ± 11.1
Body Mass Index ($kg \cdot m^{-2}$)	30.13 ± 13.07	29.57 ± 11.75	33.86 ± 19.64	33.66 ±18.72	27.00 ± 3.56	27.23 ± 3.41	27.88 ± 2.94	28.08 ± 4.06
Body Fat (%)	47.6 ± 7.3	46.9 ± 7.7	48.5 ± 5.0	48.5 ± 4.7	43.0 ± 6.0	42.1 ± 6.2	46.1 ± 5.3	46.0 ±7.1
Trunk Fat (%)	48.2 ± 8.0	47.3 ± 8.8	48.5 ± 4.2	48.4 ± 4.4	45.6 ± 6.5	44.6 ± 6.7	47.5 ±4.9	48.6 ± 7.3
Lean Mass (kg)	32.64 ± 13.17	33.80 ± 11.26	29.24 ± 16.20	30.47 ± 15.96	32.81 ± 5.26	35.10 ± 5.79	27.79 ± 4.74	29.31 ± 5.41
Dual Femur BMD ($g \cdot cm^{-2}$)	0.89 ± 0.21	0.92 ± 0.23	0.80 ± 0.17	0.80 ± 0.15	0.93 ± 0.10	0.95 ± 0.12	0.90 ± 0.13	0.96 ± 0.13
Spine BMD ($g \cdot cm^{-2}$)	0.97 ± 0.23	1.00 ± 0.24	0.83 ± 0.04	0.87 ± 0.05	0.89 ± 0.11	0.92 ± 0.15	0.84 ± 0.09	0.87 ± 0.11
Total BMD ($g \cdot cm^{-2}$)	1.03 ± 0.15	1.05 ± 0.16	0.96 ± 0.16	0.96 ± 0.15	0.97 ± 0.07	0.99 ± 0.08	0.98 ± 0.07	1.00 ± 0.08
Resting HR (bpm)	81 ± 12	79 ± 13	76 ± 5	95 ± 19	73 ± 11	75 ± 11	86 ± 12	76 ± 8
Resting SBP (mm Hg)	104 ± 5	101 ± 8	99 ± 13	112 ± 24	100 ± 7	99 ± 9	104 ± 5	100 ± 5
Resting DBP (mm Hg)	68 ± 6	68 ± 8	69 ± 13	71 ± 17	67 ± 9	61 ± 6	65 ± 4	63 ± 4

Note(s): "W24-PI" = Week 24 post-intervention for intervention group; "W24-NI" = Week 24 post-no intervention for control group

Bruininks-Oseretsky Test of Motor Proficiency

The BOTMP-2™ assesses fine motor and gross motor proficiency. We assessed fine motor (e.g., Subtests 1-3) and gross motor (e.g., Subtests 4-8) proficiency in all participants during the first visit (baseline) at CSUF. In addition, those with PWS completed a second BOTMP-2™ during visit 2. This was done to establish the reliability of this instrument in Prader-Willi Syndrome. Gross motor proficiency was then assessed midway (Week 12) and following the intervention (Week 24). Participants assigned to the control group completed only the gross motor test items during the visit prior to starting the intervention (Week 24 post-no intervention visit) as these are the items of interest for change because of participation in the intervention. The purpose of collecting fine and gross motor data at baseline was to compare PWS with children without PWS to help characterize motor proficiency in PWS. Therefore, three different aspects of the motor proficiency test are presented below: 1) the reliability of the BOTMP-2™ as an instrument to measure motor proficiency in PWS (Table 8), 2) the difference in motor proficiency in children with PWS versus those without PWS but who are obese (Table 9), and 3) the change in gross motor proficiency in the children completing the intervention and those assigned to the control group (Table 10).

Reliability of the BOTMP-2™

Data on the BOTMP-2™ have been obtained in 14 youth with PWS to evaluate test-retest reliability. The data was presented at the Southwest Chapter of the American College of Sports Medicine Annual Meeting in October 2011 and at the American College of Sports Medicine Annual Meeting in June 2012 (see Appendices B and E).

Table 8. BOTMP-2™ test-retest score, presented as mean ± SD, intra-class correlation, Pearson-r correlation, and significance.

	Test	Retest	ICC	Correlation (p)
Total motor composite				
Standard Score	26.8 ± 5.2	27.1 ± 5.4	0.98	0.99 (0.000)
Composite 1: Fine manual control				
Scale Score	16.4 ± 7.4	15.9 ± 7.8	0.90	0.90 (0.000)
Standard Score	34.6 ± 8.4	33.6 ± 8.3	0.89	0.89 (0.000)
Subtest 1: Fine motor precision				
Total Score	27.0 ± 8.6	26.8 ± 7.7	0.91	0.91 (0.000)
Scale Score	7.1 ± 3.9	6.9 ± 3.8	0.89	0.88 (0.000)
Subtest 2: Fine motor integration				
Total Score	27.8 ± 10.5	27.5 ± 11.9	0.96	0.96 (0.000)
Scale Score	9.4 ± 6.1	9.0 ± 5.5	0.92	0.92 (0.000)
Composite 2: Manual coordination				
Scale Score	11.6 ± 5.7	12.6 ± 5.2	0.84	0.86 (0.000)
Standard Score	28.9 ± 5.5	29.8 ± 5.4	0.85	0.86 (0.000)
Subtest 3: Manual dexterity				
Total Score	20.2 ± 5.5	20.9 ± 6.4	0.86	0.87 (0.000)
Scale Score	6.9 ± 3.6	7.4 ± 3.7	0.85	0.85 (0.000)
Subtest 7: Upper limb coordination				
Total Score	14.7 ± 13.1	17.4 ± 11.5	0.89	0.91 (0.000)
Scale Score	4.6 ± 3.8	5.1 ± 2.8	0.84	0.87 (0.000)
Composite 3: Body coordination				
Scale Score	9.4 ± 3.3	9.6 ± 4.9	0.83	0.89 (0.000)
Standard Score	27.0 ± 3.1	27.1 ± 5.0	0.82	0.90 (0.000)
Subtest 4: Bilateral coordination				
Total Score	9.8 ± 5.7	9.8 ± 7.3	0.83	0.84 (0.000)
Scale Score	4.7 ± 2.3	4.8 ± 3.2	0.70	0.72 (0.003)
Subtest 5: Balance				
Total Score	18.8 ± 6.6	18.6 ± 8.5	0.85	0.86 (0.000)
Scale Score	4.7 ± 1.5	4.8 ± 2.3	0.75	0.79 (0.001)
Composite 4: Strength and agility				
Scale Score	8.9 ± 4.3	9.9 ± 4.9	0.85	0.87 (0.000)
Standard Score	27.4 ± 5.3	28.5 ± 5.8	0.91	0.93 (0.000)
Subtest 6: Running speed and agility				
Total Score	15.6 ± 9.3	18.1 ± 9.6	0.91	0.94 (0.000)
Scale Score	4.9 ± 3.2	5.6 ± 3.1	0.91	0.93 (0.000)
Subtest 8: Strength				
Total Score	8.7 ± 5.8	9.0 ± 6.6	0.82	0.81 (0.000)
Scale Score	4.0 ± 1.6	4.3 ± 2.0	0.34	0.33 (0.261)

Table 9. Baseline BOTMP-2™ scores, presented as mean ± SD, and descriptive categories by group for all enrolled participants including dropouts. Subtest scores are presented as sum point scores. Composite scores are also presented as sum scale scores. Total composite score is presented as standard score.

		PWS		**OB**	
		Intervention (n=13)	**Control (n=7)**	**Intervention (n=28)**	**Control (n=14)**
Subtest 1: Fine motor precision	Score	28.1 ± 8.4	26.6 ± 9.0	37.6 ± 3.1	34.9 ± 4.2
	Descriptive	<	<	=	=
Subtest 2: Fine motor integration	Score	29.0 ± 10.0	26.0 ± 10.5	37.8 ± 2.0	37.3 ± 3.2
	Descriptive	<	<	=	=
Subtest 3: Manual dexterity	Score	20.3 ± 5.8	16.1 ± 3.7	31.1 ± 3.2	27.3 ± 4.6
	Descriptive	<	<	=	=
Subtest 4: Bilateral coordination	Score	9.8 ± 5.6	9.3 ± 3.9	21.3 ± 5.6	18.7 ± 3.7
	Descriptive	<	<<	=	=
Subtest 5: Balance	Score	19.9 ± 5..6	16.6 ± 7.0	31.2 ± 2.9	29.1 ± 3.8
	Descriptive	<<	<<	=	=
Subtest 6: Running speed and agility	Score	16.7 ± 9.7	13.0 ± 9.7	33.9 ± 4.3	30.8 ± 6.4
	Descriptive	<<	<<	=	=
Subtest 7: Upper limb coordination	Score	15.7 ± 12.7	12.9 ± 9.4	34.4 ± 4.6	30.8 ± 6.7
	Descriptive	<	<<	=	=
Subtest 8: Strength	Score	9.5 ± 5.6	8.9 ± 4.6	21.8 ± 5.1	18.1 ± 6.2
	Descriptive	<<	<<	=	<
Composite 1: Fine Manual Control	Score	17.1 ± 7.4	15.9 ± 7.6	30.7 ± 6.9	30.8 ± 7.8
	Descriptive	<	<	=	=
Composite 2: Manual Coordination	Score	11.5 ± 5.9	9.4 ± 3.5	30.7 ± 7.0	28.5 ± 9
	Descriptive	<<	<<	=	=
Composite 3: Body Coordination	Score	9.5 ± 3.4	8.3 ± 2.8	25.4 ± 6.8	22.4 ± 6.6
	Descriptive	<<	<<	=	<
Composite 4: Strength and Agility	Score	9.6 ± 4.4	9.3 ± 5.5	26.8 ± 6.6	24.4 ± 8.4
	Descriptive	<<	<<	=	=
Total Composite Score	Score	27.4 ± 5.0	25.1 ± 5.1	46.3 ± 8.9	44.5 ± 7.8
	Descriptive	<<	<<	=	=

Note(s): "<<" = well-below average; "<" = below average; "=" = average; ">" = above average; ">>" well-above average

Table 10. Intervention and control group BOTMP-2™ scores, presented as mean ± SD, at baseline and following 24 weeks. Subtest scores are presented as sum point scores. Composite scores are presented as sum scale scores.

	PWS				**OB**			
	Intervention (n=9)		**Control (n=3)**		**Intervention (n=16)**		**Control (n=7)**	
	Baseline	**W24-PI**	**Baseline**	**W24-NI**	**Baseline**	**W24-PI**	**Baseline**	**W24-NI**
Subtest 4: Bilateral coordination	10.6 ± 6.1	13.9 ± 7.1	9.7 ± 2.1	10.0 ± 9.8	21.4 ± 2.6	22.6 ± 1.8	20.3 ± 2.4	20.1 ± 3.4
Subtest 5: Balance	19.1 ± 5.5	20.8 ± 5.4	16.7 ± 2.5	13.7 ± 2.5	31.6 ± 2.6	32.0 ± 2.2	30.4 ± 3.6	32.6 ± 2.0
Subtest 6: Running speed and agility	17.8 ± 10.6	23.1 ± 9.4	12.3 ± 10.7	19.7 ± 10.7	33.8 ± 4.9	37.0 ± 2.4	31.7 ± 8.1	31.7 ± 7.3
Subtest 7: Upper limb coordination	18.6 ± 14.1	20.1 ± 12.8	15.3 ± 5.7	15.0 ± 5.2	35.1 ± 4.4	37.8 ± 1.6	31.6 ± 7.6	33.3 ± 7.1
Subtest 8: Strength	9.1 ± 4.5	10.6 ± 5.3	10.3 ± 2.1	9.7 ± 2.5	22.7 ± 5.4	24.8 ± 4.6	19.7 ± 7.3	17.8 ± 7.2
Composite 3: Body Coordination	9.6 ± 3.8	12.3 ± 6.3	7.3 ± 2.5	7.0 ± 4.6	26.1 ± 6.0	28.3 ± 4.6	24.9 ± 5.4	26.3 ± 5.1
Composite 4: Strength and Agility	10.1 ± 4.9	12.3 ± 5.6	8.3 ± 3.8	11.7 ± 4.0	27.1 ± 7.1	30.8 ± 6.0	26.1 ± 9.0	24.0 ± 9.0

Note(s): "W24-PI" = Week 24 post-intervention for intervention group; "W24-NI" = Week 24 post-no intervention for control group

Discussion

Participants are responding very well to the Active Play at Home program, particularly they have been highly compliant with the intervention. From the preliminary analyses in the test-retest data collected using the Bruininks-Oseretsky Test of Motor Proficiency this test is reliable to assess different domains of motor proficiency such as: fine motor precision and integration, manual dexterity, bilateral and upper limb coordination, balance, and strength. It appears that participants in the intervention are improving their gross motor skills because of participation in the Active Play at Home study. Parents have also been very receptive to the program. They have provided input as we went forward in how to improve different aspects. The common response of participating parents is that providing the equipment, a very well organized curriculum and the regular phone calls to check upon them makes the carry out of the program at home doable. If invited they would likely participate again.

Adverse Events, SAE's, and Unanticipated Problems

No adverse events, serious adverse events, or unanticipated problems occurred during this reporting period.

Deviations/Violations

During this reporting period, two participants with PWS (Subjects PA-1029 and PA-1038) and four participants with obesity (Subjects PA-1028, PA-1034, PA-1036, and PA-1039) withdrew from the study. PA-1029 and PA-1034 withdrew midway through the program and PA-1028, PA-1036, and PA-1039 withdrew at the end of the program due to an inability to comply with the protocol. PA-1038 withdrew prior to beginning the program (control group). Because of the nature of the Sensory Organization Test (child stands on a force plate under different conditions: eyes open or closed, floor moves or stays fixed, surrounding walls move or are fixed) we have not collected representative data in some participants with PWS (see Table 4). Further, some youth with PWS did not understand the youth questionnaires and the test administrator deemed the results invalid and therefore, will not be used (see Table 4).

Complaints

No complaints were reported for this reporting period.

Decision-reporting intervention for parents/guardians of youth with PWS

Preliminary findings (as of September 14, 2012)

Participant Frequencies and Demographics

Results

The data presented below is descriptive only – no statistical analyses were conducted.

Subject Characteristics

Thirty-two parents have completed the baseline and post-intervention survey to date. Demographic information about this group is as follows: 85.7% are females with a mean age of 42 years old. 68.6% identified English as their main language, while 31.4% identified Spanish as their main language. In terms of employment, 54.3% of participants reported that they currently work while 45.7% are unemployed. In terms of marriage, 71.4% of participants are married, while 20% are divorced or separated, 5.7% are single, and 2.9% are widowed.

PDA Compliance

Eleven participants completed the decision reporting intervention. The average compliance rate for their PDA responses was 81.4% (Table 11). One participant is currently enrolled in the PDA intervention.

Table 11. Mean PDA response compliance percentage by 6-week time point.

ID	Weeks 1-6 (%)	Weeks 7-12 (%)	Weeks 13-18 (%)	Weeks 19-24 (%)	Participant Total (%)
PDA-2001	100.0	97.0	78.0	100.0	94.0
PDA-2002	93.0	93.0	96.0	100.0	96.0
PDA-2004	100.0	100.0	10.0	100.0	100.0
PDA-2006	84.0	92.0	93.0	100.0	92.3
PDA-2007	82.0	85.0	90.0	87.0	86.0
PDA-2009	71.0	71.0	78.0	87.0	76.8
PDA-2010	73.0	64.0	75.0	80.0	73.0
PDA-2012	84.0	15.0	26.0	70.0	48.8
PDA-2013	82.0	76.0	92.0	92.0	85.0
PDA-2014	70.0	72.0	97.0	91.0	82.5
PDA-2015	68.0	75.0	44.0	56.0	60.8
Total (%)	82.5	76.4	79.0	87.6	81.4

Discussion

We have almost reached our goal in terms of numbers of participants that we proposed to collect data from. PDA data collected thus far has been coded and inputted in the SPSS dataset. Participating parents were motivated and took the intervention seriously, exemplified by high compliance.

Adverse Events, SAE's, and Unanticipated Problems

No adverse events, serious adverse events, or unanticipated problems occurred during this reporting period.

Deviations/Violations

During this reporting period, three additional parents withdrew from the study, totaling four parents overall. PDA-2003, PDA-2008, and PDA-2011 withdrew midway through the intervention and PDA-2005 withdrew at the end of the program due to an inability to comply with the protocol.

Complaints

No complaints were reported for this reporting period.

KEY RESEARCH ACCOMPLISHMENTS

Nutritional Aspects of Prader-Willi Syndrome and Childhood Obesity: We have been able to identify seven distinct nutritional phases in individuals with PWS. This knowledge should provide a solid foundation for future investigations of the hormonal and metabolic factors associated with these changes. An improved understanding of the various nutritional phases of PWS will not only benefit the treatment and management of individuals with PWS, but also provide valuable insights into the pathophysiology of obesity in general.

We have also shown that hyperghrelinemia begins in early infancy in PWS and decreases as the individual gets older, but is still significantly higher than the sibling control and EMO groups at any age. Therefore, ghrelin is unlikely to be the "key player" in the increased appetite found in individuals with PWS, but is likely responsible for the increased fat mass and decreased lean mass found in PWS.

We are currently exploring whether high ghrelin levels early in life in individuals with PWS correlate with other factors later in life like obesity, appetite drive, behavioral issues and IQ.

Family-based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome: We have made substantial progress in data collection during this reporting period. The compliance rate among participants is good (88%). It appears that all children improve gross motor proficiency (with and without PWS) as a result of participating in the intervention. It has been demonstrated that the Bruininks-Oseretsky Test of Motor Proficiency is a reliable assessment to determine aspects of motor proficiency in youth with PWS ages 8 to 16 years (please see Table 8).

REPORTABLE OUTCOMES

Publications:

Miller J.L., C.H. Lynn, D.C. Driscoll, et al. (2011). "Nutritional Phases in Prader-Willi Syndrome." American Journal of Medical Genetics A. **155**:1040-9. (Appendix A)

A manuscript on ghrelin and leptin levels in PWS has been submitted to a medical journal and will be presented by Dr. Driscoll at the 2nd annual Hyperphagia meeting in Baton Rouge, LA in October 2012.

Presentations:

White, E.W., Schroeder, L., Wright, P., Rubin, D.A., Rose, D.J., & Wiersma, L. (October 2011). Reliability of the Bruininks-Oserestky Test of Motor Proficiency in children and adolescents with Prader-Willi Syndrome. Poster Presentation at Southwest Chapter of the American College of Sports Medicine Annual Meeting in Reno, NV. (Appendix B)

Wright, P., Rubin, D.A., Castner, D.M., & Judelson, D.A. (October 2011). Body fat patterning in congenital obesity caused by Prader-Willi Syndrome. Poster Presentation at Southwest Chapter of the American College of Sports Medicine Annual Meeting in Reno, NV. (Appendix C)

Kweh, F.A., Miller, J.L., Sulsona, C.R., & Driscoll, D.J. (November 2011). Hyperghrelinemia begins early in Prader-Willi Syndrome. Poster session at the Prader-Willi Syndrome Association (USA) Scientific Meeting in Orlando, FL. (Appendix D)

Rubin, D.A., Wright, P., Haqq, A.M., Castner, D.M., Judelson, D.A. (February 2012). Body Composition in Children with Prader-Willi Syndrome. Poster session at the Keystone Symposia on Molecular and Cellular Biology Genetic and Molecular Basis of Obesity and Body Weight Regulation Meeting in Santa Fe, NM. (Appendix E)

Dr. Driscoll presented data generated by this grant at an invited lecture at Columbia University School of Medicine in New York City, April 2012.

White, E.W., Schroeder, L., Wright, P., Rubin, D.A., Rose, D.J., Wiersma, L. (June 2012). Reliability of the Bruininks-Oseretsky Test of Motor Proficiency in Children and Adolescents with Prader-Willi Syndrome. Poster Presentation at the American College of Sports Medicine Annual Meeting in San Francisco, CA. (Appendix F)

Wright, P. M., Rubin, D.A., Castner, D.M., Judelson, D.A. (June 2012). Evaluation of Body Fat Patterning in Children with Non-Syndromal and Syndromal Pediatric Obesity. Poster Presentation at the American College of Sports Medicine Annual Meeting in San Francisco, CA. (Appendix G)

Rubin, D.A. (June 2012) Exercise in Children with Congenital Obesity (Prader-Willi Syndrome) and Non-Congenital Obesity. Special event presentation at American College of Sports Medicine Annual Meeting in San Francisco, CA. (Appendix H)

Other:

Grant funding supported stipends to two research assistants (Lindsay Shroeder and Pamela Wright) who earned Master's of Science degrees in the Department of Kinesiology at CSUF. Both students graduated in August 2012.

CONCLUSION

Nutritional Aspects of Prader-Willi Syndrome and Childhood Obesity: Obesity is now a worldwide problem and has reached "epidemic" proportions. Obesity is the major cause of morbidity and mortality in PWS By comparing analytes from individuals with PWS at various ages and in different nutritional stages with obese and lean controls, we will better understand how individuals with PWS progress from being initially "failure-to-thrive" with a poor appetite to morbidly obese (if untreated) with an insatiable appetite. An improved understanding of the metabolic factors associated with the various nutritional phases of PWS will not only benefit the treatment and management of PWS, but also provide valuable insights into obesity in general. A recent report (*Mission Readiness: Still Too Fat of Fight*) by a group of retired military leaders found that 25% of young Americans cannot join the military because they are overweight and that this is an issue that needs to be dealt with aggressively.

Family-based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome: Preliminary results on compliance demonstrate that the intervention is feasible to be implemented in the home environment of people with and without PWS. Moreover, compliance rate is good (88%). Preliminary data analysis show that participation in the intervention leads to improvement in motor proficiency. More data must be collected to have enough statistical power in other main outcomes. UFG has received approval from their local IRB to begin testing at their site.

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Miller J.L., C.H. Lynn, D.C. Driscoll, et al. (2011). “Nutritional Phases in Prader-Willi Syndrome.” American Journal of Medical Genetics A. **155**:1040-9.

Perez-Tilve, D., Heppner, K., et al. (2011). “Ghrelin-induced adiposity is independent of orexigenic effects.” FASEB J. **25**(8): 1-9.

LIST OF APPENDICES

A Manuscript: Miller J.L., C.H. Lynn, D.C. Driscoll, et al. (2011). "Nutritional Phases in Prader-Willi Syndrome." American Journal of Medical Genetics A. **155**:1040-9.

B Poster (White): Southwest Chapter of American College of Sports Medicine Annual Meeting in Reno, NV, October 2011.

C Poster (Wright): Southwest Chapter of American College of Sports Medicine Annual Meeting in Reno, NV, October 2011.

D Poster: Prader-Willi Syndrome Association (USA) Scientific Meeting in Orlando, FL, November 2011.

E Poster: Keystone Symposia on Molecular and Cellular Biology Genetic and Molecular Basis of Obesity and Body Weight Regulation Meeting in Santa Fe, NM, February 2012.

F Poster (White): American College of Sports Medicine Annual Meeting in San Francisco, CA, June 2012.

G Poster (Wright): American College of Sports Medicine Annual Meeting in San Francisco, CA, June 2012.

H Oral Presentation: American College of Sports Medicine Annual Meeting in San Francisco, CA, June 2012.

I CSUF IRB Approval: Cover letter, PA Intervention Informed Assent/Consent, & PDA Intervention Informed Consent

APPENDIX A

Manuscript: Miller J.L., C.H. Lynn, D.C. Driscoll, et al. (2011). “Nutritional Phases in Prader-Willi Syndrome.” American Journal of Medical Genetics A. **155**:1040-9.

RESEARCH ARTICLE

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AMERICAN JOURNAL OF medical genetics PART A

Nutritional Phases in Prader–Willi Syndrome

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Prader–Willi syndrome (PWS) is a complex neurobehavioral condition which has been classically described as having two nutritional stages: poor feeding, frequently with failure to thrive (FTT) in infancy (Stage 1), followed by hyperphagia leading to obesity in later childhood (Stage 2). We have longitudinally followed the feeding behaviors of individuals with PWS and found a much more gradual and complex progression of the nutritional phases than the traditional two stages described in the literature. Therefore, this study characterizes the growth, metabolic, and laboratory changes associated with the various nutritional phases of PWS in a large cohort of subjects. We have identified a total of seven different nutritional phases, with five main phases and sub-phases in phases 1 and 2. Phase 0 occurs *in utero*, with decreased fetal movements and growth restriction compared to unaffected siblings. In phase 1 the infant is hypotonic and not obese, with sub-phase 1a characterized by difficulty feeding with or without FTT (ages birth—15 months; median age at completion: 9 months). This phase is followed by sub-phase 1b when the infant grows steadily along a growth curve and weight is increasing at a normal rate (median age of onset: 9 months; age quartiles 5–15 months). Phase 2 is associated with weight gain—in sub-phase 2a the weight increases without a significant change in appetite or caloric intake (median age of onset 2.08 years; age quartiles 20–31 months;), while in sub-phase 2b the weight gain is associated with a concomitant increased interest in food (median age of onset: 4.5 years; quartiles 3–5.25 years). Phase 3 is characterized by hyperphagia, typically accompanied by food-seeking and lack of satiety (median age of onset: 8 years; quartiles 5–13 years). Some adults progress to phase 4 which is when an individual who was previously in phase 3 no longer has an insatiable appetite and is able to feel full. Therefore, the progression of the nutritional phases in PWS is much more complex than previously recognized. Awareness of the various phases will aid researchers in unraveling the pathophysiology of each phase and provide a foundation for developing rational therapies. Counseling parents of newly diagnosed infants with PWS as to what to expect with regard to these nutritional phases may help prevent or slow the early-onset of obesity in this syndrome.

How to Cite this Article:
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Am J Med Genet Part A 155:1040–1049.

Key words: Prader–Willi; nutrition; appetite; weight gain

Abbreviations: PWS, Prader–Willi syndrome; BMI, body mass index; Del, deletion in the paternally inherited chromosome 15q11-q13 region; FDA, Food and Drug Administration; FTT, failure to thrive; GH, growth hormone; ID, imprinting defect; NIH, National Institutes of Health; RDA, recommended dietary allowance; RDCRN, Rare Disease Clinical Research Network; REE, resting energy expenditure; RQ, respiratory quotient; UPD, maternal uniparental disomy of chromosome 15.

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INTRODUCTION

Prader–Willi syndrome (PWS) is a complex neurobehavioral disorder which is due to the absence of normally active paternally expressed genes from the chromosome 15q11-q13 region. PWS is an imprinted condition with 70–75% of the cases due to a *de novo* deletion in the paternally inherited chromosome 15 11-q13 region, 20–30% from maternal uniparental disomy 15 (UPD), and the remaining 2–5% from either microdeletions or epimutations of the imprinting center (i.e., imprinting defects; IDs) [Bittel and Butler, 2005; Cassidy and Driscoll, 2009]. Clinical features of PWS include hypotonia and poor feeding in infancy which almost always requires some type of assisted feeding for a period of time. Obesity typically begins around age 2 years if the diet is not restricted. Behavioral problems and neuroendocrine abnormalities are also characteristic of PWS [Goldstone, 2004; Davies et al., 2008; Cassidy and Driscoll, 2009].

PWS is classically described as having two distinct nutritional stages: Stage 1, in which the individual exhibits poor feeding and hypotonia, often with failure to thrive (FTT); and Stage 2, which is characterized by "hyperphagia leading to obesity" [Gunay-Aygun et al., 2001; Goldstone, 2004; Butler et al., 2006]. Preoccupation with food, food-foraging, food obsessions and compulsions, and persistent hunger are reported to lead to the obesity that occurs in this syndrome [Gunay-Aygun et al., 2001; Eiholzer et al., 2003; Butler et al., 2006]. The etiology of the switch from poor feeding/FTT to obesity/hyperphagia has yet to be elucidated, but is thought to be associated with abnormalities in the hypothalamic circuitry or peripheral satiety signals [Eiholzer et al., 2003; Goldstone, 2004]. Individuals with PWS have differences in various gut hormones, including high levels of obestatin (an anorexogenic hormone) in infancy, with markedly elevated levels of ghrelin (an orexogenic hormone) in childhood and adulthood. These shifts in gut hormones may possibly correspond to the change between the poor feeding and FTT stage and the hyperphagia and obesity stage of PWS [Eiholzer et al., 2003; Butler et al., 2004; Goldstone, 2004; Bittel et al., 2005; Haqq et al., 2008; Bizzarri et al., 2010]. Individuals with PWS have also been shown to have structural brain abnormalities which may contribute to appetite aberrations [Miller et al., 2007a; Iughetti et al., 2008]. Functional MRI studies indicate that these individuals have an increased reward value to food and have increased activation of the limbic and paralimbic areas of the brain that drive eating behaviors, even post-meal, indicating that brain abnormalities likely also play a role in the appetite in this syndrome [Shapira et al., 2005; Holsen et al., 2006, 2009; Miller et al., 2007b; Dimitropoulos and Schultz, 2008; Hinton et al., 2010].

Animal studies suggest a link between body fatness and appetite, as adipokines produced in adipose tissue play a role in regulating food intake [Stofkova et al., 2009]. When growth hormone (GH) therapy was Food and Drug Administration (FDA) approved for use in individuals with PWS, there was hope that the decrease in fat mass, increase in lean muscle mass, increased metabolic rate, and resting energy expenditure (REE) conferred by GH would result in a decreased appetite in hyperphagic individuals in with PWS [Lee, 2002; Butler et al., 2007]. The effect of GH treatment on the appetite stages in PWS has not yet been reported.

The literature suggests that there is a "switch" between poor feeding and hyperphagia that occurs at approximately 18–36 months of life in individuals with PWS [Eiholzer et al., 2003; Goldstone, 2004; Butler et al., 2006; Haqq et al., 2008; Bizzarri et al., 2010]. However, we have carefully been following the natural history of the feeding behaviors of individuals with PWS for the last 10 years at the University of Florida and for the past 4 years under the auspices of the multicenter Rare Disease Clinical Research Network (RDCRN). We have observed that the changes in appetite and weight gain in PWS are much more gradual and complex than what has been traditionally described. Our group first reported in 2005 our observation that individuals with PWS began to gain excessive weight before the increased appetite develops [McCune and Driscoll, 2005]. We subsequently presented our updated clinical description of the various nutritional phases at the 2006 Second Expert Meeting of the Comprehensive Care of Patients with PWS [Goldstone et al., 2008].

In this study we have investigated our clinical impressions of these more nuanced phases in three different ways. Specifically, we have: (1) carefully characterized and described the nutritional phases of PWS; (2) correlated these phases with objective growth, metabolic, and laboratory data; and (3) examined the effect of GH therapy on the natural history of these nutritional phases.

METHODS

Participants

Families of children and adults with PWS have been enrolled in a natural history study conducted at the University of Florida over the last 10 years. In 2006 this natural history study became part of the Rare Disease Clinical Research Network. Birth measurements were available for 79 individuals with PWS and 84 of their siblings. Complete and accurate growth records and nutritional histories were available on 58 individuals with genetically confirmed PWS, which were used to calculate the onset and duration of the various nutritional phases. In addition we were able to collect laboratory data and concomitantly assign a nutritional phase associated with that data, to 82 individuals with PWS. Many of these individuals had multiple return visits. Fifty-eight percent were male, 90% were white (5% black, 5% Hispanic), and they ranged from 3 months at the time of the first visit to 35 years of age. Thirty-five individuals with PWS had a *de novo* paternal deletion of the chromosomal 15q11-q13 region, 22 had UPD, and 1 had an ID. These individuals came from 16 different states across the United States and three different provinces in Canada. This study was approved by the University of Florida Institutional Review Board, and all adult participants or guardians provided written informed consent and, where appropriate, participants provided assent.

Individuals with PWS were classified into the appropriate genetic molecular classification (i.e., deletion, UPD, or ID) by standard genetic techniques [Cassidy and Driscoll, 2009]. Subjects in the deletion class were further characterized by deletion subtype using the methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) assay [Bittel et al., 2007; Dikow et al., 2007]. MS-MLPA was done using a commercial MS-MLPA version A1 kit for Prader–Willi/Angelman syndrome (MRC-Holland, Amsterdam, the Netherlands) which contains 25 probes specific for sequence in 15q11-q13. We identified 21% with a Type 1

deletion (i.e., deletion between breakpoints 1 and 3), 34% with a Type 2 deletion (i.e., deleted between breakpoints 2 and 3), and 5% with a unique or atypical deletion.

Metabolic Rate and Body Fat Measurements

REE and respiratory quotient (RQ) were measured on all 82 participants following an overnight fast in the General Clinical Research Center at the University of Florida using a metabolic cart (Parvomedics, Sandy, UT). REE is a calculation of the basal metabolism of an individual, while RQ is a measure of the ratio of the volume of carbon dioxide (V_c) produced by an organism to the volume of oxygen consumed (V_o) [Gropper et al., 2009]. Measurement of RQ provides information about which foods are being used as an energy source. Individuals eating a "standard American diet" have an average RQ of 0.85 indicating that they are utilizing the fat, protein, and carbohydrates they are consuming for energy production. When an individual is being underfed, which promotes use of endogenous fat stores for energy, the RQ is low and is typically closer to 0.7. Overfeeding, however, which results in lipogenesis, increases in the RQ typically to greater than 0.95, indicating that the excess carbohydrates and fats being eaten are being converted into adipose tissue [Gropper et al., 2009]. Only those data points obtained during a steady state (when oxygen consumption and carbon dioxide excretion were stable) were used for data analysis. Body fat was measured using a DEXA (dual energy X-ray absorptiometry; General Electric, Chalfont St. Giles, UK) scanner.

Nutritional Phase Assessment

Nutritional phases were assessed for each individual by two physicians (DJD and JLM) and a dietician (CHL) who have considerable expertise in PWS. Assessments were based on growth charts and nutritional/dietary records, as well as with parental recall. Judgments were made independently and then discussed with the other members of the team. Subjects were excluded if we lacked information to make an adequate assessment of the nutritional phases.

Statistical Analysis

Estimated times (medians and quartiles) to the completion of a nutritional phase (which is reported in Table II as the beginning of the next phase) were assessed by fitting Kaplan–Meier curves. Those individuals who had not completed a phase at last follow-up were censored. Birth parameters (Table III) were compared for subgroups by two-sample *t*-tests. All two group comparisons were two-sided. For descriptive purposes, $P < 0.05$ was labeled as significant. McNemars test for matched proportions was used to compare *in utero* fetal movements between subjects with PWS and their sibling controls.

The major analyses contrasted phases 1a, 1b, 2a, 2b, and 3. Sufficient data in phase 4 were lacking for analysis. Because we had repeated measures, both within and between stages, our primary analysis utilized a mixed model approach, with these five phases/sub-phases as fixed categorical independent variables and subjects as random independent variables. We employed a model with a compound symmetric covariance matrix to describe the within-subject associations. There were four analyses where the SAS program Proc Mixed failed to converge, and for those we utilized a fixed repeated measures analysis. These are identified in Table IVb. The following eight dependent variables were utilized: serum IGF-1 measurements, BMI Z-score, glucose, insulin, triglycerides, mean RQ, mean REE, and percentage of body fat by DEXA scan. The analytic strategy was to conduct a five-way analysis first (1a vs. 1b vs. 2a vs. 2b vs. 3) for each variable as a control of studywise error. Whether or not significant at $P < 0.05$, we contrasted the adjacent phases by a similar two-way analysis, but report *P*-values only if the 5-way analysis was significant at $P < 0.05$. Quantitative estimates for mean differences between adjacent phases are reported in Table IVb as the most important descriptive statistics. For descriptive purposes, we also report means and standard deviations for these phases in Table IVa, but ignore the repeated measures aspects.

RESULTS

We identified seven distinct nutritional phases, with five major phases and sub-phases of phases 1 and 2 in individuals with PWS. The initial phase, phase 0, occurs *in utero*, with decreased fetal movements, birth weight and length. In phase 1 the infant is hypotonic and not obese, with sub-phase 1a characterized by difficulty feeding (often requiring feeding via a gastric tube or nasogastric tube) with or without FTT. This phase is followed by sub-phase 1b when the infant begins to feed better and grows steadily along a growth curve with weight increasing at a normal rate. Phase 2 is associated with weight increase. Sub-phase 2a occurs when the child has an increase in weight without a significant change in appetite or caloric intake, while in sub-phase 2b the child experiences continuing weight increase with an increased interest in food. Phase 3 is characterized by the development of hyperphagia, typically accompanied by food-seeking and lack of satiety. Phase 4 occurs when an individual who was previously in phase 3 no longer has an insatiable appetite and can feel full. This last phase has only been observed in adulthood. The clinical characteristics of each nutritional phase and sub-phase are delineated in Table I.

Actuarial Ages for Nutritional Phases

While not every single subject experienced every phase, the vast majority of individuals went through each of the phases up to phase 3. Only two of the participants in this study entered phase 4, both during their early 20s. Table II shows estimated actuarial age in years at the onset of each phase. The majority of those who entered phase 3 have remained in this phase during the course of our ongoing natural history study.

Since phase 0 occurs *in utero* we compared length of gestation and fetal movements, in addition to birth weight, length, and BMI for individuals with PWS versus their unaffected siblings. Fetal movements were decreased in 85% of the newborns with PWS compared to 0% of the siblings ($P < 0.001$) (Table III). Birth weight, length, and BMI were also significantly lower in individuals with PWS versus their siblings (Table III). In addition, mean gestational age for individuals with PWS was significantly different than that of their siblings (38.2 ± 3.0 weeks vs. 39.2 ± 1.6 weeks; $P < 0.001$ by

TABLE I. Clinical Characteristics of the Nutritional Phases

Phase 0	**Decreased fetal movements and lower birth weight**
	Full-term birth weight and BMI are about 15–20% less than the siblings
	Typically normal gestational age
	85% have decreased fetal movements
Phase 1a	**Hypotonia with difficulty feeding (0–9 months)**
	Weak, uncoordinated suck. Usually cannot breastfeed
	Needs assistance with feeding either through feeding tubes (nasal/oral gastric tube or gastrostomy tube) or orally with special, widened nipples. Many would die without assisted feeding
	Oral feeds are very slow
	Severely decreased appetite. Shows little or no evidence of being hungry
	Does not cry for food or get excited at feeding time
	If feeding just occurred when baby "acted hungry" then would have severe "failure-to-thrive"
	Weak cry
Phase 1b	**No difficulty feeding and growing appropriately on growth curve (9–25 months)**
	No longer needs assisted feeding
	Growing steadily along growth curve with normal feeding
	Normal appetite
Phase 2a	**Weight increasing without an increase in appetite or excessive calories (2.1–4.5 years)**
	Infant starts crossing growth curve centile lines
	No increase in appetite
	Appetite appropriate for age
	Will become obese if given the recommended daily allowance (RDA) for calories or if eating a "typical" toddler diet of 70% carbohydrates
	Typically needs to be restricted to 60–80% of RDA to prevent obesity
Phase 2b	**Weight increasing with an increase in appetite (4.5–8 years)**
	Increased interest in food. Frequently asking "food related" questions
	Preoccupied with food. Very concerned about the next meal/snack (e.g., "Did you remember to pack my lunch?")
	Increased appetite
	Will eat more food than a typical child if allowed
	Will eat food within their line of sight if unattended
	Will become obese if allowed to eat what they want
	Can be fairly easily redirected about food
	Can feel full
	Will stop eating voluntarily
Phase 3	**Hyperphagic, rarely feels full (8 years adulthood)**
	Constantly thinking about food
	While eating one meal they are already thinking about the next meal
	Will awaken from sleep early thinking about food
	Will continue eating if portion size is not limited
	Rarely (truly) feels full
	Will steal food or money to pay for food
	Can eat food from garbage and other unsavory/inedible sources (e.g., dog food, frozen food, crayons, etc.)
	Typically are not truthful about what they have eaten (i.e. amount and types of food)
	Will gain considerable amount of weight over a short period of time if not supervised (e.g., some individuals are known to have gained up to 20 pounds in one weekend)
	Food typically needs to be locked up. Frequently the child will ask the parent to lock the food if the parent has forgotten
	Will break into neighbors' houses for food
	Temper tantrums and "meltdowns" frequently related to food
	Needs to be placed on a diet that is approximately 50–70% of the RDA to maintain a healthy weight
Phase 4	**Appetite is no longer insatiable (adulthood)**
	Appetite may still be increased or may be normal or less than normal
	Previously in phase 3, but now a noticeable improvement in their appetite control
	Can feel full
	Appetite can fluctuate in this phase, but the key component is noticeable improvement in control of appetite compared to when they were younger
	Not as preoccupied with food
	Absence of major temper tantrums and "meltdowns" related to food
	Onset in adulthood. Could be as early as 20s or as late as 40–50s
	Most adults have not gone into this phase and maybe some (most?) never will

TABLE II. Estimated Actuarial Ages* at Onset of Nutritional Phase

Nutritional phase	25%-ile	50th%-ile (median)	75th%-ile
1a	Birth	Birth	Birth
1b	0.42	0.75	1.25
2a	1.67	2.08	2.58
2b	3.00	4.50	5.25
3	5.00	8.00	13.00

*Ages given in years.

matched pair *t*-test). When only full-term pregnancies (gestational age $\geq$37 weeks) were compared, individuals with PWS still had a significantly lower birth weight than their siblings (3.0 kg vs. 3.5 kg; $P < 0.01$).

Every individual with PWS experienced some difficulty feeding after birth, and thus, were identified as being in phase 1a. Phase 1a lasted until a median age of 9 months (quartiles 5 and 15 months) (Table II). Nine of the 58 individuals we had complete growth records and nutritional data for had severe, prolonged FTT despite receiving what was thought to be adequate calories (i.e., >100 kcal/kg/day) during phase 1a. No associations were found between genetic subtype and prolonged FTT, as seven of these patients had deletion-positive PWS, while two had UPD. There were no significant differences amongst the deletion patients with severe FTT between type 1 and type 2 deletions (three type 1 deletions, four type 2 deletions).

Phase 1b (taking adequate nutrition) lasted to a median age of 25 months (quartiles 20 and 31 months). The end of phase 2a occurred at a median age of 4.5 years (quartiles 3 and 5.25 years). Phase 2b ended (and phase 3 began) at a median age of 8 years (quartiles 5 and 13 years). All but two of the individuals who had entered phase 3 at any age were in this phase when evaluated, with an excessive appetite and lack of satiety.

Deletion Versus UPD

There were no significant differences in length of gestation, birth weight, length, or BMI between infants born with deletion and UPD. Consistent with previous findings, those with UPD had an older maternal age than those with deletion (35.4 years vs. 30.6 years; $P < 0.001$; Table III). There were no differences in the median age of completion of phases between individuals with deletion and those with UPD.

Age at Start of Growth Hormone Therapy

All of the subjects who first enrolled in the study as infants were started on GH therapy. This allowed us to analyze whether starting GH in infancy, as opposed to starting GH later in childhood, made any difference in the tempo or natural history of these nutritional phases. Starting GH in infancy accelerated the pace of phase 1a ($P = 0.039$), thus allowing the infants to enter phase 1b earlier. The age of starting GH did not have any significant effect on the pace or timing of any of the other nutritional phases.

RQ, Body Fat, and Metabolic Changes

Phase 1. Infants in phase 1a who were being fed via nasogastric or gastric tube had a RQ within the normal range from 0.8 to 0.9 (mean 0.89) (Table IVa). However, those infants who were exclusively bottle fed (either with breast milk or formula) had an RQ consistent with underfeeding (0.5–0.7). Percentage body fat was extremely variable amongst infants in this phase but the mean was 22 $\pm$ 9.44% fat (Table IVa and Fig. 1b). Fasting serum insulin levels and insulin-like growth factor levels (IGF-1) ranged from undetectable to the low end of the normal range, while fasting blood glucose levels were normal (Table IVa and Fig. 1c–e). When infants entered phase 1b their percentage body fat did not change significantly, nor did their REE for weight and length, RQ, serum fasting insulin/IGF-1 levels, or blood glucose values (Tables IVa and IVb). BMI Z-scores were not available in phase 1a

TABLE III. Birth Information of Individuals With PWS and Their Siblings (Means and Standard Deviations)

	Type 1 deletion (T1D)	Type 2 deletion (T2D)	Uniparental disomy (UPD)	Siblings	*P* values
Mean gestational age (weeks)	38.1 $\pm$ 3.5 (n = 16)	38.1 $\pm$ 3.3 (n = 28) All PWS 38.2 $\pm$ 3.0	38.1 $\pm$ 2.8 (n = 28)	39.2 $\pm$ 1.6 (n = 84)	$P = 0.97$ T1D vs. T2D; $P = 0.76$ Del vs. UPD; $P = <0.001$ PWS vs. sibs
Birth weight (kg) [SD]	2.7 $\pm$ 0.56 (n = 16)	2.9 $\pm$ 0.62 (n = 28)	2.7 $\pm$ 0.51 (n = 28)	3.46 $\pm$ 0.50 (n = 83)	$P = 0.29$ T1D vs. T2D; $P = 0.40$ Del vs. UPD; $P < 0.001$ PWS vs. sibs
Birth length (cm) [SD]	48.7 $\pm$ 3.98 (n = 14)	50.2 $\pm$ 3.94 (n = 22)	48.7 $\pm$ 3.0 (n = 24)	51.6 $\pm$ 3.0 (n = 58)	$P = 0.30$ T1D vs. T2D; $P = 0.29$ Del vs. UPD; $P < 0.001$ PWS vs. sibs
BMI	11.2 $\pm$ 1.65 (n = 14)	11.5 $\pm$ 1.53 (n = 22)	11.2 $\pm$ 1.8 (n = 24)	13.5 $\pm$ 2.0 (n = 58)	$P = 0.51$ T1D vs. T2D; $P = 0.66$ Del vs. UPD; $P < 0.001$ PWS vs. sibs
Maternal age at delivery (years)	30.6 $\pm$ 5.4		35.4 $\pm$ 5.0	31.2 $\pm$ 5.4	$P < 0.001$ Del vs. UPD; $P = 0.016$ UPD vs. sibs; $P = 0.13$ PWS vs. sibs

TABLE IVa. Laboratory and Metabolic Parameters of Nutritional Phases of PWS

	1a, N = 11	1b, N = 22	2a, N = 30	2b, N = 54	3, N = 49	4, N = 2
Mean age (median age)	0.72 ± 0.4 (0.78)	1.92 ± 0.8 (1.77)	4.46 ± 2.6 (3.82)	7.89 ± 6.3 (5.57)	17.1 ± 9.9 (15.8)	27.9 ± 4.6 (26.59)
Weight/length	17%	24%[a] (n = 15)	n/a	n/a	n/a	n/a
BMI Z-score	n/a	−0.7 ± 0.98[a] (n = 7)	0.81 ± 1.37	1.5 ± 1.16	2.1 ± 0.91	1.58 ± 0.84
% Body fat by DEXA	22.0 ± 9.44	19.3 ± 6.8	26.4 ± 13.5	34.0 ± 12.4	45.2 ± 9.9	45.5 ± 10.1
Respiratory quotient	0.89 ± 0.17	0.84 ± 0.13	0.88 ± 0.14	0.89 ± 0.12	0.86 ± 0.12	0.89 ± 0.05
REE	399.9 ± 196.3	675.1 ± 169.7	988.4 ± 312.6	1074.2 ± 367.7	1393.9 ± 431.0	1291.9 ± 174.9
Serum IGF-1 level (ng/ml)	40 ± 25.0	122.7 ± 77.3	211 ± 98.2	279 ± 151.3	291.9 ± 193.8	163.3 ± 23.7
Fasting blood glucose (mg/dl)	72 ± 11.5	77 ± 9.0	80 ± 10.1	83 ± 11.3	88 ± 13.6	83 ± 7.1
Fasting insulin level (mcIU/ml)	1.72 ± 1.9	3.28 ± 2.2	6.36 ± 4.0	10.71 ± 8.4	11.89 ± 12.6	4.39 ± 2.1
Fasting triglycerides (mg/dl)	106 ± 71.0	84.7 ± 39.5	85.8 ± 41.1	91.6 ± 48.0	99.9 ± 51.8	74.2 ± 34.4

n/a, not applicable.

[a]BMI Z-scores from CDC are only available for ≥2 years of age. Some of the subjects in phase 1b were <2 years and some >2 years.

and for many of the individuals in phase 1b due to their young age (i.e., <2 years).

Phase 2. Phase 2a is associated with an increase in body weight without a change in appetite or dietary intake. There were no significant differences in fasting insulin and glucose levels between phase 1b and phase 2a, but fasting insulin levels did trend higher in phase 2a (6.26 mIU/L vs. 3.28 mIU/L; $P = 0.08$) (Fig. 1c,d). As children transitioned between phase 1b and phase 2a they had significant increases in serum IGF-1 levels ($P = 0.002$; Fig. 1e; Table IVb), but no significant change in fasting insulin and blood glucose values. Interestingly, although all of the children were on GH treatment (dose range 0.20–0.26 mg/kg/week) at the time of transition into phase 2a, the IGF-1 levels increased while on a stable dose of GH, suggesting a change in the rate of metabolism of GH. As children transitioned from phase 1b to 2a the REE decreased from 62% (63 kcal/kg/day) of the recommended dietary allowance (RDA) for age (102 kcal/kg/day) to 52% (47 kcal/kg/day with RDA for age of 90 kcal/kg/day). There was no significant difference in RQ between phase 1b and 2a (0.85 in phase 1b vs. 0.88 in phase 2a; $P = 0.47$).

However, as the average age at which children with PWS enter into phase 2 is associated with a decrease in BMI in typical children, we compared the RQ of the children with PWS entering phase 2 with that of a group of normal control siblings of similar ages. The average RQ of the controls of the same age was 0.76, indicating lipolysis in the typical children as compared to lipogenesis in the children with PWS. Percentage body fat increased from 19.3% in phase 1b to 26.4% in phase 2a ($P = 0.20$) and the BMI SDS increased from −0.70 in phase 1b to 0.8 in phase 2a ($P = 0.032$) (Tables IVa and IVb; Fig. 1a,b).

As individuals transitioned from phase 2a to 2b, which is associated with an increased interest in food, fasting insulin levels continued to increase. (6.36 mIU/L vs. 10.7 mIU/L; $P = 0.01$), but IGF-1 levels and serum glucose levels did not significantly change

TABLE IVb. Comparison of Adjacent Stages by Mixed Models using Compound Symmetric Covariance

Variable	*P*-value, 5-way	1b–1a, Difference	2a–1b, Difference	2b–2a, Difference	3–2b, Difference
Entries are estimated mean difference (std error) [*P*-value, two-sided]					
IGF-1	<0.001	130 (42) [0.013*]	92.7 (27.8) [0.0022]	65.9 (39.7) [0.10]	12.6 (41.7) [0.76]
BMI Z-score	<0.001	—	1.31 (0.58) [0.032]	0.80 (0.33) [0.018]	0.66 (0.25) [0.0094]
Glucose	<0.001	6.1 (3.8) [0.18]	3.9 (3.1) [0.22]	2.2 (2.9) [0.45]	4.4 (2.8) [0.11]
Insulin	<0.001	0.72 (1.52) [0.64*]	6.2 (3.4) [0.081*]	4.4 (1.6) [0.010]	1.1 (2.3) [0.64]
Triglycerides	0.72	−21.3 (20.0)	2.5 (12.7)	2.3 (11.8)	9.3 (11.3)
Mean RQ	0.85	−0.052 (0.062)	0.033 (0.046)	0.009 (0.033)	−0.017 (0.025)
Mean REE	<0.001	277 (73) [0.0011]	297 (92) [0.0032]	98 (107) [0.36]	373 (109) [0.0012]
DEXA fat (%)	<0.001	5.5 (3.2) [0.12*]	4.7 (3.6) [0.20]	7.2 (3.7) [0.054]	13.2 (3.1) [<0.001]

The four *P*-values with * were actually done by fixed effects, repeated measures, as the mixed model failed to converge.

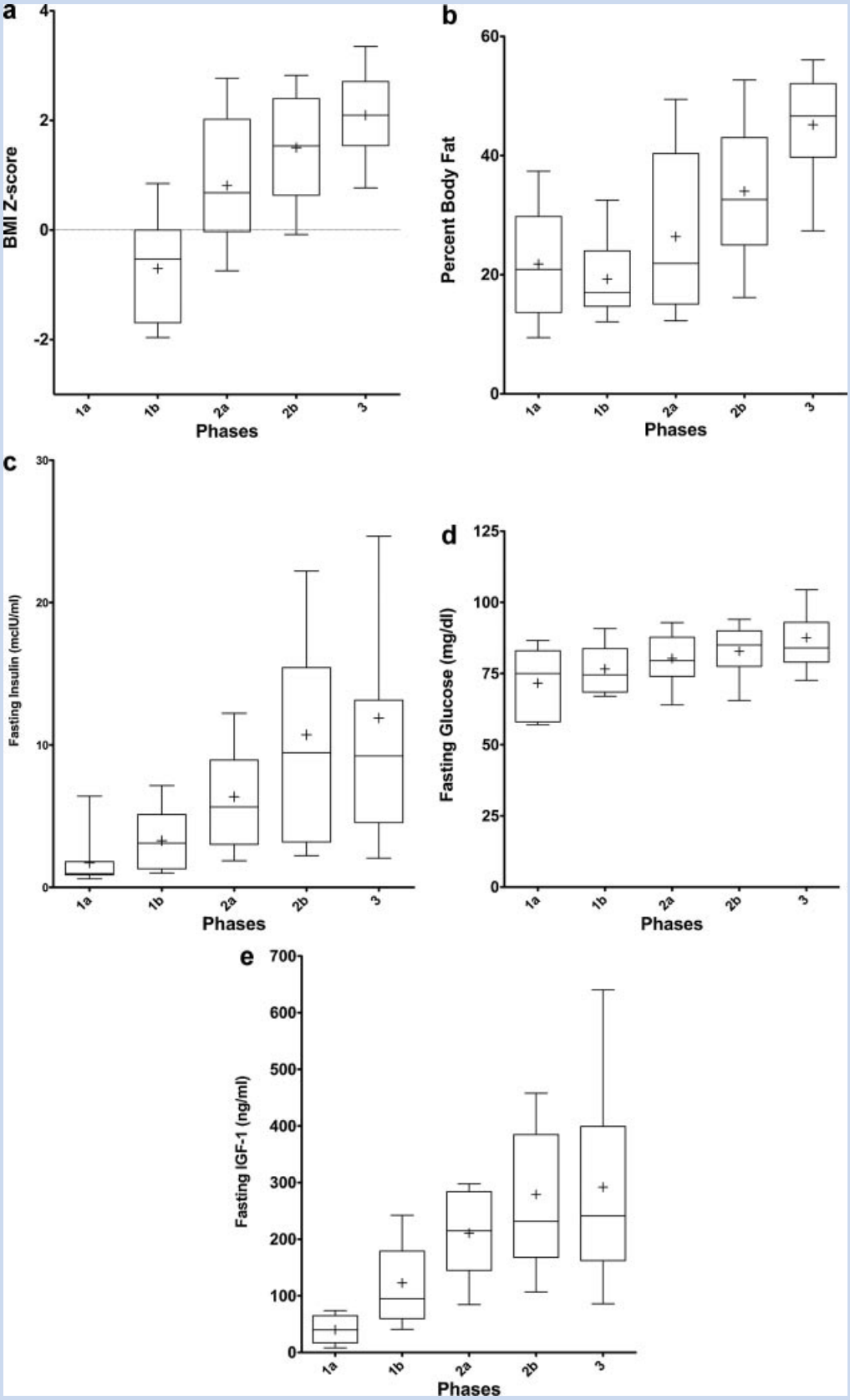

FIG. 1. All figures contain information presented as boxplots. The bottom of the box indicates the 25th centile, the line within the box indicates the median, the cross within the box indicates the mean, and the top of the box indicates the 75th centile. The whiskers above and below the box indicate the 90th and 10th centiles. a: BMI Z-score by phase. (For individuals in phases 1b-3; z-score not available for phase 1a as BMI Z-scores from CDC are only available for $\geq$2 years of age.) b: Percentage body fat by DEXA by phase. c: Fasting insulin levels by phase. d: Fasting blood glucose levels by phase. e: Serum IGF-1 levels by phase.

(Tables IVa and IVb). BMI SDS increased from 0.8 in phase 2a to 1.5 ($P = 0.018$) which was due to an increase in percent body fat from 26.4% to 34.0% in phase 2b ($P = 0.05$; Table IVb and Fig. 1a,b). RQ remained stable during this transition (0.88 vs.0.89; $P = 0.78$), while REE decreased to 31 kcal/kg/day which is 44% of the RDA for age (70 kcal/kg/day).

Phase 3. Individuals in phase 3 have an increased appetite with decreased satiety, but they had no significant changes in their fasting insulin, IGF-1, or blood glucose values as compared to individuals in phase 2b. However, BMI SDS increased to 2.10 ($P = 0.0094$ vs. phase 2b) and percent body fat increased to 45.2% ($P < 0.001$ vs. phase 2b) (Fig. 1a,b). RQ remained stable in this phase.

Phase 4. Only two adults in this study had transitioned to phase 4. Additional research is needed with more adults to identify changes in RQ, hormonal levels, or body fat associated with this phase.

DISCUSSION

In contrast to the long-held view that people with PWS go through just two nutritional phases, this study found compelling evidence for five major nutritional phases. Data also point to sub-phases within the first two phases, which further highlights the complexities of the nutritional phases and transitions in individuals with PWS.

Although in the literature, phase 1 begins in infancy with poor feeding and FTT, abnormalities in nutrition in PWS actually begin *in utero*. Here, we propose a phase 0 to reflect these abnormalities and to call attention to the importance of the prenatal environment in subsequent development. In our study the mean birth weights and BMIs of PWS probands was about 15% and 20% less, respectively, than their siblings. Similar reduced birth weights in infants with PWS have also been reported by our group and others [Butler et al., 2009, 2010].

There were 9 of 58 individuals who had severe FTT despite adequate caloric intake during phase 1a. We hypothesize that these individuals had a higher metabolic rate than their peers who did not have difficulty gaining weight. Support for this hypothesis comes from the PWS mouse model with a deletion of the snoRNA *Snord116* gene [Ding et al., 2008]. These mice have an increased appetite and caloric intake, but remain lean due to their increased metabolic rates compared to their wild-type littermates [Ding et al., 2008]. Unfortunately, most of the individuals in our study with severe FTT did not have their metabolic rate measured until well after their FTT had resolved. Alternatively, the FTT in these individuals could be due to decreased absorption of nutrients. This subset of individuals will need to be prospectively studied in the future. Future studies need to identify the metabolic rates and nutrient absorption in this high-risk subset of infants, and how, or if, their longer periods of FTT impact their subsequent development.

Interestingly, we found that phase 1b ended at a median age of 2.1 years, which is often cited as the beginning of Stage 2 (i.e., increased appetite and obesity) in the traditional nomenclature [Eiholzer et al., 2003; Haqq et al., 2008; Bizzarri et al., 2010]. However, we found that when individuals enter phase 2a they began to gain weight without any change in appetite or calories [McCune and Driscoll, 2005; Goldstone et al., 2008]. This observation has also recently been independently confirmed by researchers in the United Kingdom [Butler et al., 2010]. The age of onset of increased interest in food (i.e., phase 2b) in our study was not until a median of 4.5 years. However, the onset of the classically described "insatiable appetite" phase did not begin until a median age of 8 years, which is much older than what has traditionally been thought.

Because PWS is now typically diagnosed in infancy we are better positioned to offer parents prospective advice on these nutritional phases. While we do not yet know what triggers transitions between phases, we hypothesize that there is likely a decrease in metabolic rate and/or an increase in the absorption of calories and nutrients from the diet as children enter phase 2a, which then worsens in subsequent nutritional phases. In these children the REE decreased from approximately 60% of the RDA for age in phases 1a and 1b to 52% of the RDA in phase 2a. The REE then continued to decrease compared to the RDA for age as the children progressed through the nutritional phases. Based on these data, we recommend that parents have their children's length and weight measured monthly. When increasing weight gain without a change in calories is noted, we typically need to recommend that the parents decrease the caloric intake to about 50–80% of the RDA for age as we continue to follow the growth parameters closely for each individual. In so doing, it is important to ensure that the diet remains well balanced with 30% fat, 45% carbohydrates, and 25% protein. If children with PWS remained on a typical American toddler diet which can be composed of 60–70% carbohydrates, their obesity would be even worse as their increased RQ compared to typically developing toddlers suggests that they are prone to convert extra carbohydrates into adipose tissue.

Although parental counseling and caloric restriction have not changed the tempo or timing of the phases, we have been able to achieve great success with many of our infants and young children in keeping the weight for height normal before the child enters phase 2b. When we retrospectively reviewed growth charts of our older individuals with PWS who were typically not diagnosed until 8–12 years of age, we found that they were already obese when they entered phase 2b, so the increased interest in food served to worsen their existing obesity. Parents of our patients diagnosed in infancy thus have the opportunity to institute food-related modifications and healthy eating habits well before the child's appetite or interest in food increases. As a result, when phase 3 begins it is often less severe in those families who have implemented early intervention measures versus what has been traditionally described in the literature.

Best practice in early intervention in PWS also now includes recommendations for GH therapy. GH therapy decreases fat mass and increases muscle mass. Preliminary data also suggest that it may have a beneficial effect on weight gain, and possibly appetite, in individuals with PWS [Myers et al., 2000; Burman et al., 2001]. The present study found that GH therapy in infancy significantly shortened phase 1a, allowing infants to spend more time in phase 1b, during which time they gain weight appropriately. Although at this point GH therapy did not significantly affect any of the other nutritional phases, the majority of participants who started GH treatment in early infancy are not yet old enough to have progressed

through phases 2b, 3, or 4. Follow-up data on these children are needed before drawing conclusions about the efficacy of infantile GH therapy on the progression or timing of the later nutritional phases.

Although this study identified novel ways of conceptualizing nutritional phases in PWS, it also had certain limitations. First, some of the data on older individuals is retrospective and based on analysis of growth charts and parents' memory. However, we have excellent historical data on a number of our older patients (many of whom have been followed by our group for 10–20 years and who were diagnosed in early infancy) which documents the progression of these individuals through the various nutritional phases which we have described. Further prospective work is clearly needed on the life course of the nutritional phases. A second weakness is that the study did not include measurements of appetite-regulating hormones and neurotransmitters as participants progressed through the various stages. Even so, this study provides a critical step in describing and verifying these various nutritional phases and setting the stage for future collaborative rare disease consortium studies on shifts in hormones and neurotransmitters as individuals transition through various nutritional phases. Data are especially needed on transitions between phase 3 and 4, and mechanisms that explain why some adults have a lessening of their hyperphagia while others do not. Although there were only two individuals in this study who had entered phase 4, we have seen several adults in clinic who have entered this phase, but we do not have research measurements on them at this time.

In summary, we have been able to identify seven distinct nutritional phases in individuals with PWS. This knowledge should provide a solid foundation for future investigations of the hormonal and metabolic factors associated with these changes. An improved understanding of the various nutritional phases of PWS will not only benefit the treatment and management of individuals with PWS, but also provide valuable insights into the pathophysiology of obesity in general.

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APPENDIX B

Poster (White): Southwest Chapter of the American College of Sports Medicine Annual Meeting, Reno, NV, October 2011.

Reliability of the Bruininks-Oseretsky Test of Motor Proficiency in Children and Adolescents with Prader-Willi Syndrome

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ABSTRACT

Individuals with Prader-Willi Syndrome (PWS) present with overall motor deficiency, but the specific areas have yet to be identified in youth. To determine specific areas of deficiency, a reliable instrument must be used. **Purpose:** To determine if the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) is a reliable instrument for assessing motor proficiency in children and adolescents with PWS. **Methods:** 10 children with PWS (5 girls/5 boys, mean age 11.1±1.7 yrs) participated in this study. Participants completed the test on two separate morning visits, one week apart. The BOT-2 test evaluates seven items related to motor proficiency: Fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, running speed and agility, upper limb coordination, and strength. The test provides subtest item scores and a total composite score (TCS). **Results:** The Pearson product correlation coefficients between visits ranged from r=.712 to r=.965, with a total composite test score r=.989 (all significant at $p<0.021$). **Discussion:** The total composite test score and the majority of subtest item scores showed moderate-to- high test-retest correlation coefficients. In conclusion, based on **these** pilot data, the BOT appears to be a reliable test to assess motor proficiency in children and adolescents with PWS ages 8 to 15 years old.

INTRODUCTION

In general, individuals with Prader-Willi Syndrome demonstrate poor motor coordination, balance, and stamina, but most characterizations of the syndrome have been limited to toddlers and adults.[3] In order to characterize these aspects of motor proficiency test-administrators must use a reliable instrument. The Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) characterizes motor performance, specifically in four main areas: fine manual control, manual coordination, body coordination, and strength and agility.[2] While the reliability of the BOT-2 has been established with other special populations it has yet to be established with youth with PWS. [4] A previous study conducted in PWS has used a few items from the BOT-2, [1] but no previous study has used the BOT-2 in its full entirety.

PURPOSE

To determine if the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) is a reliable instrument for assessing motor proficiency in general, and in specific areas with children and adolescents with PWS.

METHODS

Ten children/adolescents with PWS participated in this study. Important participant characteristics are summarized in Table 1. Each participant completed the BOT-2 on two separate occasions, one week apart and at approximately the same time of day. Three trained testers conducted the test, but the same test administrator administered the test across both sessions. The BOT-2 test evaluates eight subset categories of motor proficiency (see Table 2).

Table 1: Participant Characteristics

Descriptive characteristics	Mean ± SD or frequency
Age	11.1±1.7
Sex (M/F)	5/5
Height (cm)	144.5±10.7
Body mass (kg)	62.0±27.7
Body fat mass percentage	48.0±9.5
On growth hormone therapy (yes/no)	9/1
Received physical therapy (yes/no)	8/2

Table 2: BOT-2 Subset Categories, Sample Test items, & Number

Subset Category	Sample Test Items	Total # of items
Fine motor precision	•Filling in Shapes •Drawing lines through path	7
Fine motor integration	•Copying a wavy line •Copying a star	8
Manual dexterity	•Making dots in circles •Sorting cards	5
Bilateral coordination	•Jumping jacks •Tapping feet and fingers	7
Balance	•Standing on one leg on a line •Walking forward heel-to-toe on a line	9
Running speed & agility	•Shuttle run •Two legged side hop	5
Upper limb coordination	•Dropping and catching a ball with both hands •Throwing ball at target	7
Strength	•Standing long jump •Wall sit	5

RESULTS

High correlation values **(>.90)** were obtained for the total composite score as well as the specific areas of fine motor integration, manual dexterity (Table 3 below). Good correlations **(>.80)** were evident for the specific areas of fine motor precision, running speed and agility, upper limb coordination, and strength while only moderate correlations **(> .70)** were observed for the specific areas of bilateral coordination, and balance.

Table 3: Mean Performance Scores and Pearson Correlation Coefficients between Visits

Category	Visit 1 Mean ± SD	Visit 2 Mean ± SD	Correlation Coefficient	P-Value
Fine motor precision	30.4±6.0	28.5±7.0	.809	**.005**
Fine motor integration	32.3±6.8	32.8±6.1	.965	**.000**
Manual dexterity	22.3±5.4	24.0±4.4	.930	**.000**
Bilateral coordination	12.0±4.8	12.3±7.1	.712	**.021**
Balance	22.0±4.9	22.0±6.7	.731	**.016**
Running speed & agility	17.4±8.9	18.8±8.4	.866	**.001**
Upper limb coordination	16.0±12.2	20.2±9.4	.891	**.001**
Strength	9.3±5.1	9.3±6.5	.805	**.005**
Total Composite Score	28.8±4.8	29.0±5.1	.989	**.000**

Values in bold type are significant at $p<0.05$.

Acknowledgements

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DISCUSSION

Overall the BOT-2 appears to be a reliable test that can be used to assess motor proficiency in children and adolescents with PWS, between 8 and 15 years old. Only two Subset Categories (#4 and #5) showed moderate correlation coefficients. Multiple factors may have influenced the test-retest reliability in these two categories: Test administrator expertise; participant characteristics (e.g., attention, motivation, cognitive ability); environmental factors (weather conditions, indoor/outdoor testing location). Despite the moderate correlation coefficients for two subtest categories, our results suggest that this instrument is both a reliable measure of overall motor proficiency and specific subcategories in children and adolescents ages 8-15 years with PWS. This test has the potential to identify motor deficiencies in PWS as well as to monitor progress over time in response to an intervention.

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APPENDIX C

Poster (Wright): Southwest Chapter of the American College of Sports Medicine Annual Meeting, Reno, NV, October 2011.

CALIFORNIA STATE UNIVERSITY FULLERTON

Body fat patterning in congenital obesity caused by Prader-Willi Syndrome

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ABSTRACT

Prader-Willi Syndrome (PWS) is a genetic disorder resulting in excessive adiposity and reduced lean mass. Adults with PWS present differences in fat patterning (increased fat mass in the limbs) compared to non-syndromal obese adults who have increased fat mass in the trunk. In children, there is paucity of data. **Purpose:** To describe fat patterning in children with PWS as it compares to obese children without PWS. **Methods:** Eleven children with PWS and 42 obese (OB=body fat >95th percentile) children ages 8-11 y participated. Children underwent body mass, stature, waist circumference (WC) measurements, and a total body dual x-ray absorptiometry scan. Body fat % was measured for total, trunk, gynoid, and android fat. Body mass index (BMI) was calculated. **Results:** Independent t-tests showed that PWS and OB had similar BMI (PWS: 24.7 ± 6.3 kg/m^2; OB: 27.8 ± 9.4 kg/m^2), and waist circumference (PWS: 80.4 ± 14.0 cm; OB: 88.6 ± 11.0 cm) (p>.05). Also, no significant differences were observed for total body fat % (PWS: 43.8 ± 8.0%; OB: 42.1 ± 8.0%), trunk fat (PWS: 43.9 ± 10.4%; OB: 44.1 ± 8.8%), gynoid fat (PWS: 53.2 ± 3.3%; OB: 49.8 ± 6.7%), and android fat (PWS: 53.6± 6.4%; OB: 51.0 ± 8.5%) (p >0.05 for all). **Discussion:** Previously, it has been shown that PWS adult males presented with higher gynoid body fat % than non-syndromal obese males with similar BMI. Our results suggest no differences in body fat patterning, particularly in the abdominal and limb regions, between PWS children and non-syndromal obese children with similar BMI and levels of body fat.

INTRODUCTION

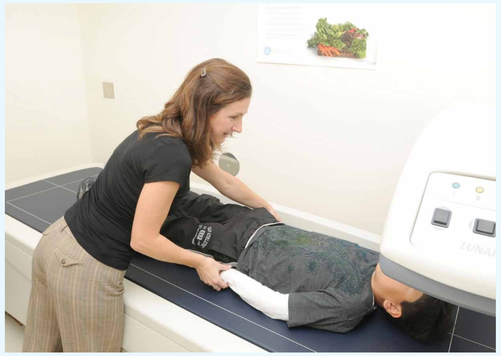
Prader Willi Syndrome (PWS) is a genetic disorder characterized by hypotonia and excessive fat mass (1). Excessively high total adiposity, particularly in the abdominal region (android-type obesity) is a risk factor for heart disease, diabetes, and other chronic conditions (2). In non-syndromal obese children body fat appears to be more commonly distributed in the trunk region of the body (3). In PWS individuals however, adiposity is typically distributed in the hip region of the body (gynoid-type obesity) making them less susceptible to certain chronic conditions, such as insulin resistance (3,4). Studies have shown that when PWS individuals are treated with growth hormone (GH) therapy, they have increased lean mass and decreased fat mass (5,6). Previous reports have also found that adult males with PWS, who have not had GH therapy, have differences in fat patterning compared to non-syndromal adults; namely, increased fat distribution in the legs (7). Because GH treatment changes body composition in PWS, it is possible that the differences in body composition seen in adults are not present in children with PWS because currently, GH is part of the standard of care in children with PWS (1).

PURPOSE

To compare body fat patterning in children with PWS to non-syndromal obese children with similar BMI percentile.

METHODS

Children with PWS and non-syndromal obese children ages 8-11 years old participated in the study. Children without PWS were categorized as obese if their body fat was higher than the 95th percentile for age and sex (8). Children underwent anthropometric measurements following NHANES guidelines: body mass (kg), stature (cm), and waist circumference (WC) also in cm. Body mass index (BMI) was calculated from body weight and stature. Body composition was measured using dual x-ray absorptiometry scan (DXA) and total, trunk, gynoid, and android fat percentage (%) was derived from the DXA results.



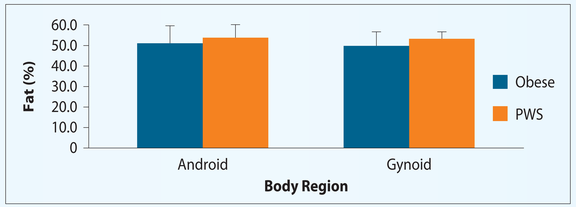
RESULTS

There were 11 children with PWS and 42 non-syndromal obese children. The participant characteristics by group are presented in table 1. There were no significant differences (p > 0.05) in any of the anthropometrics measurements or body fat percentage measurements (Table 2) between the two groups. In particular, there were no differences between android and gynoid fat percentage (Figure 1) between non-syndromal obese children and children with PWS.

Table 1. Participant demographics, anthropometrics, and physiological characteristics (frequencies or mean ± SD)

	Obese	PWS
Frequency	42	11
Age (years)	10±1.0	10 ±1.2
Stature (cm)	143.0 ±13.0	142.0 ±15.0
Sex (F/M)	27/15	6/6
Body Mass (kg)	56.0 ± 13.3	50.5 ± 17.7
BMI (kg/m^2)	27.8 ± 9.4	24.7 ± 6.3
WC (cm)	88.6 ± 11.0	80.4 ± 14.0
GH Therapy (yes/no)	Not applicable	10/1
Total Body Fat %	42.1 ± 8.0%	43.8 ± 8.0%
Trunk Fat %	44.1 ± 8.8	43.9 ± 10.4

Figure 1. Android and gynoid body fat percentage in obese children with and without Prader-Willi Syndrome (PWS)



CONCLUSIONS

As previously mentioned, current literature suggests that adults with PWS present with increased fat mass in the hips compared to non-syndromal obese adults who store fat mass mostly in the trunk (7). In contrast, our results show similar fat distribution in children with PWS compared to those who have non-syndromal obesity. The differences between our findings and previous results in adults are most likely due to the fact that the children in our study were on GH therapy; whereas, adults in previous studies were not. Another possible reason why fat patterning differences were not found between groups could be that all of our participants without PWS were 8-11 years old and hadn't entered puberty yet. During puberty there is a marked increase in testosterone in boys, which plays a role in the android type shape compared to females who don't experience this hormonal change and tend to deposit fat in the hips (9). Based on our findings, the typical fat distribution in PWS may be changing as more individuals are treated with GH, and these changes are making fat patterning more similar to non-syndromal obesity.

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APPENDIX D

Poster: Prader-Willi Syndrome Association (USA) Scientific Meeting in Orlando, FL, November 2011.

Hyperghrelinemia Begins Early in Prader-Willi Syndrome

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Introduction: Obesity is the major cause of morbidity and mortality in Prader-Willi syndrome (PWS) and typically begins between 1-4 years of age. The driving mechanisms behind the development of obesity in PWS are unclear. Ghrelin is an orexigenic hormone that increases food intake while decreasing energy expenditure and fat catabolism. It is significantly elevated in older children and adults with PWS. However ghrelin's role in the obesity and hyperphagia in PWS remains unclear and its level in young children with PWS is controversial. In this study, we measured ghrelin levels in individuals with PWS from early infancy to 36 years of age. Individuals with non-PWS early-onset morbid obesity (EMO) and normal weight sibling controls were used as comparison groups.

Methods: Fasting serum ghrelin was measured using a fluorescent Enzyme-linked Immunosorbent Assay (ELISA) kit from Phoenix Pharmaceuticals, Inc., California, USA. Most of the study subjects (PWS, EMO and siblings) had fasting serum samples collected more than once at different time points during the course of our Rare Disease Natural History study. We analyzed 136 PWS fasting serum samples (73 subjects); 55 EMO samples (40 subjects); and 143 sibling samples (95 subjects) for a total of 334 fasting samples.

Results: Serum ghrelin was significantly elevated in children with PWS between the ages of 0 and 5 years relative to the control siblings ($p=0.0057$), but was not significantly higher than in the EMO group ($p=0.9951$). After 5 years serum ghrelin levels decreased significantly in all groups with the EMO group showing the greatest decrease. PWS serum ghrelin levels were significantly higher than the EMO group in the 5-12 year age range ($p=0.0095$), but not the control sibling group ($p=0.0631$). After 12 years of age there was a significant decrease in serum ghrelin in the sibling group. Teenagers (12-20 years) and adults (20-36 years) with PWS had significantly higher ghrelin levels than their counterparts in the EMO and sibling groups.

We found that serum ghrelin was significantly elevated in PWS children well before the onset of obesity and hyperphagia ($p<0.0001$). In fact, the highest ghrelin levels were seen in the first nutritional phase (1a) of PWS, which is a time of poor feeding and reduced appetite. Interestingly, the most significant decrease in PWS serum ghrelin ($p=0.0032$) coincided with the transition from phase 1b (relatively normal infant growth and appetite) to phase 2a (rapid weight gain with no significant change in appetite or caloric intake).

Discussion: Serum ghrelin levels were measured in individuals with PWS from 2 months to 36 years of age. We found that ghrelin levels were significantly elevated beginning in early infancy in PWS well before the onset of obesity and hyperphagia. Given that ghrelin levels were the highest in PWS in infants still in the poor appetite phase (i.e., 1a) it seems unlikely that elevated ghrelin levels are causing the switch to the hyperphagic phases of PWS. However, it has been shown in mice that ghrelin can also act to increase fat mass independent of its effect on appetite (Perez-Tilve et. al, *FASEB J.* 25:2814, 2011). Therefore, it is likely that the elevated ghrelin levels are causing the increased fat mass seen in infants with PWS compared to normal infants with similar body mass indices (BMI).

APPENDIX E

Poster: Keystone Symposia on Molecular and Cellular Biology Genetic and Molecular Basis of Obesity and Body Weight Regulation Meeting, Santa Fe, NM, February 2012.

Body Composition in Children with Prader-Willi Syndrome

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ABSTRACT

Introduction: Prader-Willi Syndrome (PWS) presents with early-onset obesity, growth hormone deficiency and hypogonadism. Adults with PWS typically experience decreased lean mass and a gynoid deposition of body fat compared to non-syndromal obesity. Growth hormone replacement therapy (GHRT) influences body composition and is currently standard of care in PWS. The aim of this study was to compare body composition in children with PWS on GHRT to obese (OB) controls.

Methods: Nine children (4F/5M) with PWS who had been on GHRT at least two years and nine OB children (4F/5M; body fat % > 95th percentile for age and sex) matched by age (8-11 y) and height were studied. Participants completed anthropometrics (body mass, stature, waist circumference) and a full body dual x-ray absorptiometry scan (Lunar Prodigy, GE Healthcare, Madison, WI). Independent t-tests were used to assess for differences between groups.

Results: Children with PWS had similar body mass and waist circumference as OB but lower body mass index (PWS = 23.6 ± 3.5 vs. OB = 28.0 ± 4.5 kg/m^2; p = 0.03). Children with PWS displayed lower total lean mass (PWS = 24.6 ± 6.1 vs. OB = 31.8 ± 6.4 kg; p = 0.02), as well as lower lean mass in legs and arms than OB (Legs: PWS = 8.0 ± 2.2 vs. OB = 11.0 ± 2.2 kg; Arms: PWS = 2.4 ± 0.7 vs. OB = 3.2 ± 0.8 kg; p = 0.03 and p < 0.01, respectively) There were no significant differences between groups in total, legs, arms, trunk, android, or gynoid percentages of body fat or fat mass (p > 0.05 for all).

Conclusion: These results suggest that some peculiarities in body composition, such as lower lean mass in the arms and legs, persist in PWS children despite GHRT. However, our findings suggest that the gynoid deposition of body fat common in most adults with PWS is not present in children with PWS. The similarity in fat patterning between groups may be related to GHRT in PWS or to the lack of pubertal hormone influences until mid or late puberty.

INTRODUCTION

Prader Willi Syndrome (PWS) is a genetic disorder characterized by hypotonia and excessive fat mass (1). Excessively high adiposity, particularly in the abdominal region (android-type obesity) is a risk factor for heart disease, diabetes, and other chronic conditions in children (2). In adults with PWS, adiposity is typically distributed in the hip and legs region of the body (3,4). Growth hormone replacement therapy (GHRT) is now standard of care in PWS (1); available data show that when individuals with PWS are treated with GH, their lean mass increases and fat mass decreases (5). As GH affects lipid metabolism, it is possible that body fat patterning is changed in those with PWS who received GHRT. This is particularly important in children as adiposity in childhood can be associated with disease in adulthood (2). The purpose of this study was to compare body composition in children with PWS on GHRT to obese controls.

METHODS

Participants were nine children with PWS and nine non-syndromal obese children matched by age and height. Obese children had a body fat percentage higher than the 95th percentile for age and sex (6). Children with PWS received GHRT for at least two years. Participants completed anthropometrics (body mass, stature, waist circumference) and a full body dual x-ray absorptiometry scan (Lunar Prodigy, GE Healthcare, Madison, WI). The DXA scan provided 1) body fat percentages of the total body, arms, legs, trunk, gynoid and android regions, and 2) lean mass of the total body, arms, and legs. Body mass index (BMI) was calculated. Differences between children with PWS and obese controls were determined using independent t-tests.

RESULTS

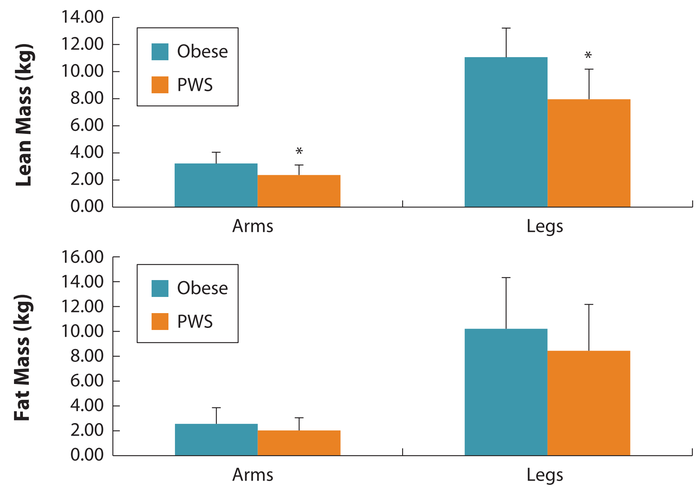
Children with PWS had similar body mass and waist circumference as obese but lower BMI (Table 1). There were no significant differences between groups in total, arms, legs, trunk, android, or gynoid percentages of body fat or fat mass. Children with PWS displayed lower total, arms, and legs lean mass (Figure 1). In contrast, percentage of lean mass in the arms was similar between groups.

Table 1. Participant demographics, anthropometrics, and physiological characteristics (frequencies or mean ± SD)

	Obese (n=9)	PWS (n=9)
Sex (M/F)	5/4	5/4
Age (years)	9.7 ± 1.4	9.7 ± 1.4
Stature (cm)	145.6 ± 10.7	141.7 ± 14.2
Body Mass (kg)	60.13 ± 15.23	48.51 ± 15.93
Body Mass Index (kg/m^2)	**28.0 ± 4.5**	**23.6 ± 3.5***
Waist Circumference (cm)	91.5 ± 11.7	82.6 ± 11.7
Total Body Fat (%)	43.9 ± 6.7	45.6 ± 5.6
Trunk Fat (%)	45.0 ± 7.3	45.7 ± 6.2
Android Fat (%)	52.1 ± 7.1	52.0 ± 6.6
Gynoid Fat (%)	50.7 ± 4.8	53.6 ± 3.6
Arms Fat (%)	9.4 ± 1.9	9.3 ± 1.6
Legs Fat (%)	39.2 ± 3.5	39.0 ± 4.0
Total Lean Mass (kg)	**31.2 ± 6.4**	**24.6 ± 6.1***
Total Lean Mass (%)	54.4 ± 6.4	52.7 ± 5.3
Arms Lean Mass (%)	10.0 ± 1.3	10.0 ± 1.0
Legs Lean Mass (%)	**34.7 ± 1.2**	**32.1 ± 1.9***

Note: * = Values are significant at p < 0.05.

Figure 1. Arms and legs (a) lean mass (kg) and (b) fat mass (kg) in obese children with and without Prader-Willi Syndrome (PWS)



Note: * = Values are significant at p < 0.05.

CONCLUSIONS

Previous studies show that adults with PWS present with increased fat mass in the hips compared to non-syndromal obese adults who store fat mass mostly in the trunk (3,4). Our results in children contradict the findings in adults as they show no difference in fat patterning between PWS and non-syndromal obesity. The differences between our findings and adults' data might be due to our subjects' use of GHRT and/or prepubertal status (5,7). Interestingly, despite the lack of differences in body mass, fat mass, or body fat percentage between groups, children with PWS presented lower total lean mass than obese children. This difference seemed to stem from a reduced lean mass of the arms and legs in children with PWS. Our results suggest that children with PWS who received GHRT present a fat patterning similar to non-syndromal obesity.

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ACKNOWLEDGEMENTS

Supported by USAMRMC Award W81XWH-08-1-0025 & W81XWH-09-1-0682

APPENDIX F

Poster (White): American College of Sports Medicine Annual Meeting, San Francisco, CA, June 2012.

Reliability of the Bruininks-Oseretsky Test of Motor Proficiency in Children and Adolescents with Prader-Willi Syndrome

Elizabeth White B.S., Lindsay Schroeder, B.S., Pamela Wright B.S., Daniela Rubin, Ph.D., Debra J. Rose Ph.D., Lenny Wiersma, Ph.D.

Department of Kinesiology, California State University, Fullerton, Fullerton, CA

ABSTRACT

Individuals with Prader-Willi Syndrome (PWS) present with overall motor deficiency, but the specific areas have yet to be identified in youth. To determine specific areas of deficiency, a reliable instrument must be used. **Purpose:** To determine if the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) is a reliable instrument for assessing motor proficiency in children and adolescents with PWS. **Methods:** 10 children with PWS (5 girls/5 boys, mean age 11.1±1.7 yrs) participated in this study. Participants completed the test on two separate morning visits, one week apart. The BOT-2 test evaluates seven items related to motor proficiency: Fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, running speed and agility, upper limb coordination, and strength. The test provides subtest item scores and a total composite score (TCS). **Results:** The Pearson product correlation coefficients between visits ranged from r=.712 to r=.965, with a total composite test score r=.989 (all significant at $p<0.021$). **Discussion:** The total composite test score and the majority of subtest item scores showed moderate-to- high test-retest correlation coefficients. In conclusion, based on these pilot data, the BOT appears to be a reliable test to assess motor proficiency in children and adolescents with PWS ages 8 to 15 years old.

INTRODUCTION

In general, individuals with Prader-Willi Syndrome demonstrate poor motor coordination and balance, but characterization of the syndrome have been limited to toddlers and adults.[1] To characterize motor proficiency test-administrators must use a reliable instrument. The Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) characterizes motor performance, specifically in four main areas: fine manual control, manual coordination, body coordination, and strength and agility.[2] This instrument is reliable in children with intellectual disabilities and has been used in children with Down Syndrome. [3,4] A previous study conducted in PWS has used a few items from the BOT-2, [5] but no previous study has used the BOT-2 in its full entirety.

PURPOSE

To determine if the Bruininks-Oseretsky Test of Motor Proficiency, Second Edition (BOT-2) is a reliable instrument for assessing motor proficiency in general, and in specific areas with children and adolescents with PWS.

METHODS

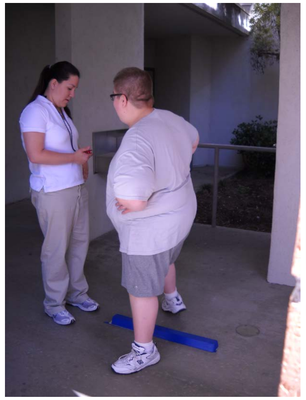
Ten children/adolescents with PWS participated in this study. Important participant characteristics are summarized in Table 1. Each participant completed the BOT-2 on two separate occasions, one week apart and at approximately the same time of day. Three trained testers conducted the test, but the same test administrator administered the test across both sessions. The BOT-2 test evaluates eight subset categories of motor proficiency (see Table 2).

Table 1: Participant Characteristics

Descriptive characteristics	Mean ± SD or frequency
Age	11.1±1.7
Sex (M/F)	5/5
Height (cm)	144.5±10.7
Body mass (kg)	62.0±27.7
Body fat mass percentage	48.0±9.5
On growth hormone therapy (yes/no)	9/1
Received physical therapy (yes/no)	8/2

Table 2: BOT-2 Subset Categories, Sample Test items, & Number

Subset Category	Sample Test Items	Total # of items
Fine motor precision	•Filling in Shapes •Drawing lines through path	7
Fine motor integration	•Copying a wavy line •Copying a star	8
Manual dexterity	•Making dots in circles •Sorting cards	5
Bilateral coordination	•Jumping jacks •Tapping feet and fingers	7
Balance	•Standing on one leg on a line •Walking forward heel-to-toe on a line	9
Running speed & agility	•Shuttle run •Two legged side hop	5
Upper limb coordination	•Dropping and catching a ball with both hands •Throwing ball at target	7
Strength	•Standing long jump •Wall sit	5



RESULTS

High correlation values **(>.90)** were obtained for the total composite score as well as the specific areas of fine motor integration, manual dexterity (Table 3 below). Good correlations **(>.80)** were evident for the specific areas of fine motor precision, running speed and agility, upper limb coordination, and strength while only moderate correlations **(> .70)** were observed for the specific areas of bilateral coordination, and balance.

Table 3: Mean Performance Scores and Pearson Correlation Coefficients between Visits

Category	Visit 1 Mean ± SD	Visit 2 Mean ± SD	Correlation Coefficient	P-Value
Fine motor precision	30.4±6.0	28.5±7.0	.809	**.005**
Fine motor integration	32.3±6.8	32.8±6.1	.965	**.000**
Manual dexterity	22.3±5.4	24.0±4.4	.930	**.000**
Bilateral coordination	12.0±4.8	12.3±7.1	.712	**.021**
Balance	22.0±4.9	22.0±6.7	.731	**.016**
Running speed & agility	17.4±8.9	18.8±8.4	.866	**.001**
Upper limb coordination	16.0±12.2	20.2±9.4	.891	**.001**
Strength	9.3±5.1	9.3±6.5	.805	**.005**
Total Composite Score	28.8±4.8	29.0±5.1	.989	**.000**

Values in bold type are significant at $p<0.05$.

Acknowledgements

Supported by USAMRAA
W81XWH-09-1-0682

DISCUSSION

Overall the BOT-2 appears to be a reliable test that can be used to assess motor proficiency in children and adolescents with PWS, between 8 and 15 years old. Only two Subset Categories (#4 and #5) showed moderate correlation coefficients which were comparable to the correlations reported in the BOT-2 manual (r=0.70 for ages 8-12 years old)[6]. Multiple factors may have influenced the test-retest reliability in these two categories: test administrator expertise; participant characteristics (e.g., attention, fatigue, motivation, cognitive ability); environmental factors (weather conditions, indoor/outdoor testing location). Despite the moderate correlation coefficients for two subtest categories, our results suggest that this instrument is both a reliable measure of overall motor proficiency and specific subcategories in children and adolescents ages 8-15 years with PWS. This test has the potential to identify motor deficiencies in PWS as well as to monitor progress over time in response to an intervention.

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APPENDIX G

Poster (Wright): American College of Sports Medicine Annual Meeting, San Francisco, CA, June 2012.

CALIFORNIA STATE UNIVERSITY FULLERTON

Evaluation of Body Fat Patterning in Children with Non-Syndromal and Syndromal Pediatric Obesity

Pamela Wright ◆ Daniela A. Rubin ◆ Diobel Mendoza-Castner ◆ Daniel A. Judelson

Fitness Assessment Laboratory ◆ Department of Kinesiology ◆ California State University, Fullerton

ABSTRACT

Prader-Willi Syndrome (PWS) is a genetic disorder resulting in excessive adiposity and reduced lean mass. Adults with PWS present differences in fat patterning (increased fat mass in the limbs) compared to non-syndromal obese adults who have increased fat mass in the trunk. In children, there is paucity of data. **Purpose:** To describe fat patterning in children with PWS as it compares to obese children without PWS. **Methods:** Eleven children with PWS and 42 obese (OB=body fat >95th percentile) children ages 8-11 y participated. Children underwent body mass, stature, waist circumference (WC) measurements and a total body dual x-ray absorptiometry scan. Body fat % was measured for total, trunk, gynoid, and android fat. Body mass index (BMI) was calculated. **Results:** Independent t-tests showed that PWS and OB had similar BMI (PWS: 24.7 ± 6.3 kg/m^2; OB: 27.8 ± 9.4 kg/m^2), and waist circumference (PWS: 80.4 ± 14.0 cm; OB: 88.6 ± 11.0 cm) (p>.05). Also, no significant differences were observed for total body fat % (PWS: 43.8 ± 8.0%; OB: 42.1 ± 8.0%), trunk fat (PWS: 43.9 ± 10.4%; OB: 44.1 ± 8.8%), gynoid fat (PWS: 53.2 ± 3.3%; OB: 49.8 ± 6.7%), and android fat (PWS: 53.6± 6.4%; OB: 51.0 ± 8.5%) (p >0.05 for all). **Discussion:** Previously, it has been shown that PWS adult males presented with higher gynoid body fat % than non-syndromal obese males with similar BMI. Our results suggest no differences in body fat patterning, particularly in the abdominal and limb regions, between PWS children and non-syndromal obese children with similar BMI and levels of body fat.

INTRODUCTION

Prader Willi Syndrome (PWS) is a genetic disorder characterized by hypotonia and excessive fat mass (1). Excessively high total adiposity, particularly in the abdominal region (android-type obesity) is a risk factor for heart disease, diabetes, and other chronic conditions (2). In non-syndromal obese children body fat appears to be more commonly distributed in the trunk region of the body (3). In PWS individuals however, adiposity is typically distributed in the hip region of the body (gynoid-type obesity) making them less susceptible to certain chronic conditions, such as insulin resistance (3,4). Studies have shown that when PWS individuals are treated with growth hormone replacement therapy (GHRT), they have increased lean mass and decreased fat mass (5,6). Previous reports have also found that adult males with PWS, who have not had GHRT, have differences in fat patterning compared to non-syndromal adults; namely, increased fat distribution in the legs (7). Because GHRT changes body composition in PWS, it is possible that the differences in body composition seen in adults are not present in children with PWS because currently, GHRT is part of the standard of care in children with PWS (1).

PURPOSE

To compare body fat patterning in children with PWS to non-syndromal obese children with similar BMI percentile.

METHODS

Children with PWS and non-syndromal obese children ages 8-11 years old participated in the study. Children without PWS were categorized as obese if their body fat was higher than the 95th percentile for age and sex (8). Children underwent anthropometric measurements following NHANES guidelines: body mass (kg), stature (cm), and waist circumference (WC) also in cm. Body mass index (BMI) was calculated from body weight and stature. Body composition was measured using dual x-ray absorptiometry scan (DXA) and total, trunk, gynoid, and android fat percentage (%) was derived from the DXA results.

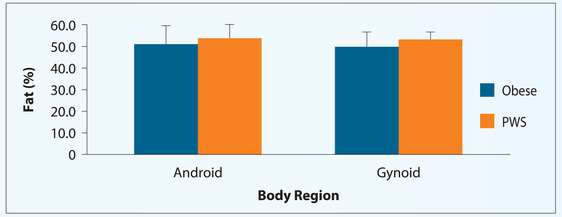
RESULTS

There were 11 children with PWS and 42 non-syndromal obese children. The participant characteristics by group are presented in table 1. There were no significant differences (p > 0.05) in any of the anthropometrics measurements or body fat percentage measurements (Table 2) between the two groups. Particularly there were no differences between android and gynoid fat percentage (Figure 1) between obese children with and without PWS.

Table 1. Participant demographics, anthropometrics, and physiological characteristics (frequencies or mean ± SD))

	Obese	PWS
Frequency	42	11
Age (years)	10 ±1.0	10 ±1.2
Stature (cm)	143.0 ±13.0	142.0 ±15.0
Sex (F/M)	27/15	6/6
Body Mass (kg)	56.0 ± 13.3	50.5 ± 17.7
BMI (kg/m2)	27.8 ± 9.4	24.7 ± 6.3
WC (cm)	88.6 ± 11.0	80.4 ± 14.0
GHRT (yes/no)	Not applicable	10/1
Trunk Fat %	44.1 ± 8.8	43.9 ± 10.4

Figure 1. Android and gynoid body fat percentage in obese children with and without Prader-Willi Syndrome (PWS)



CONCLUSIONS

Previous studies suggest that adults with PWS present with increased fat mass in the hips compared to non-syndromal obese adults who store fat mass mostly in the trunk (7). Also, adults with PWS appear to present a higher body fat percentage compared to obese controls (3). In contrast, our results show similar fat percentage and distribution in children with PWS compared to those who have non-syndromal obesity. The differences between our findings and previous results in adults are most likely due to the majority of children in our study were on GHRT and the potential effect that GHRT may have on decreasing body fat mass (5,6). Another possible reason why fat patterning differences were not found between groups could be that all participants were 8-11 years old and many of them may not have gone through puberty. During puberty there is a marked increase in testosterone in boys, which plays a role in the android type shape compared to females who don't experience this hormonal change and tend to deposit fat in the hips (9). Our findings suggest that body fat percentage and distribution appears to be similar in children with PWS and those with non-syndromal obesity.

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9. Sperling M.A. (2008). *Pediatric Endocrinology Third Edition*. Philadelphia, PA: Springer.

ACKNOWLEDGEMENTS

Supported by USAMRMC Award W81XWH-08-1-0025

APPENDIX H

Oral Presentation: American College of Sports Medicine Annual Meeting, San Francisco, CA, June 2012.

478 **3:45 PM - 4:10 PM**
Exercise in Children with Congenital Obesity (Prader-Willis Syndrome) and Non-Congenital Obesity
Daniela Rubin. *California State University Fullerton, Fullerton, CA.*
(No relationships reported)

479 **4:10 PM - 4:35 PM**
Exercise in Children with Severe Burns
Elisabet Børsheim. *UTMB/Shriners Hospitals for Children, Galveston, TX.*
(No relationships reported)

480 **4:35 PM - 5:00 PM**
Exercise, Inflammation and Oxidative Stress in Children with Diabetes
Pietro Galassetti. *UC-Irvine, Irvine, CA.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-44 Symposium - Behavioral Compensation to Exercise: Do We Eat More and Do Less?

FRIDAY, JUNE 1, 2012 3:15 PM - 5:15 PM
ROOM: 3000

481 **3:15 PM - 3:20 PM**
Chair: Barry Braun, FACSM. *University of Massachusetts, Amherst, MA.*
(No relationships reported)

482 **3:20 PM - 3:45 PM**
Do Sedentary Behavior and Habitual Physical Activity Influence Responsiveness to Exercise Training
Sarah Kozey Keadle. *University of Massachusetts, Amherts, MA.*
(No relationships reported)

483 **3:45 PM - 4:10 PM**
The Effects of Exercise on Non-exercise Activity and Energy Expenditure
Edward Melanson, FACSM. *University of Colorado Denver Anschutz Med Campus, Aurora, CO.*
(No relationships reported)

484 **4:10 PM - 4:35 PM**
The Effects of Exercise on Ad Libitum Energy and Macronutrient Intake
Joseph E. Donnelly, FACSM. *University of Kansas Medical Center, Kansas City, KS.*
(No relationships reported)

485 **4:35 PM - 5:00 PM**
The Interaction Between Exercise and Appetite: Hedonic and Homeostatic Compensatory Responses
Neil King. *Queensland University of Technology, Brisbane, Australia.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-45 Symposium - Exercise Induced Activation of Bioenergetic Pathways in Skeletal Muscle

FRIDAY, JUNE 1, 2012 3:15 PM - 5:15 PM
ROOM: 2001

486 **3:15 PM - 3:20 PM**
Chair: Harry B. Rossiter, FACSM. *University of Leeds, Leeds, United Kingdom.*
(No relationships reported)

487 **3:20 PM - 3:45 PM**
The Dynamics of Skeletal Muscle Bioenergetics
L Bruce Gladden, FACSM. *Auburn University, Auburn, AL.*
(No relationships reported)

488 **3:45 PM - 4:10 PM**
The Sensitisation of Oxidative Metabolism in Whole Muscles and Single Fibers
Harry B. Rossiter, FACSM. *University of Leeds, Leeds, United Kingdom.*
(No relationships reported)

489 **4:10 PM - 4:35 PM**
The Mitochondrial Membrane and Redox Potentials at the Onset of Muscle Contractions
Michael C. Hogan, FACSM. *University of California-San Diego, La Jolla, CA.*
(No relationships reported)

490 **4:35 PM - 5:00 PM**
Exercise-Induced Increases in Mitochondrial Respiratory Sensitivity
P Darrell Neufer. *East Carolina University, Greenville, NC.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-46 Symposium - Skeletal Muscle Blood Flow Studied Sans Metabolism: Implications from Basic Science to Rehabilitative Medicine

FRIDAY, JUNE 1, 2012 3:15 PM - 5:15 PM
ROOM: 2014

491 **3:15 PM - 3:20 PM**
Chair: Russell S. Richardson. *University of Utah, Salt Lake City, UT.*
(No relationships reported)

492 **3:20 PM - 3:45 PM**
Impact of Body Position and Afferent Feedback on Central and Peripheral Hemodynamic Contributions to Movement-Induced Hyperemia: Implications for Rehabilitative Medicine
Joel D. Trinity. *University of Utah, Salt Lake City, UT.*
(No relationships reported)

493 **3:45 PM - 4:10 PM**
Attenuated Exercise Induced Hyperaemia with Age: Mechanistic Insight from Passive Limb Movement
John McDaniel. *Kent State University, Cleveland, OH.*
(No relationships reported)

494 **4:10 PM - 4:35 PM**
Passive Limb Movement: A New Tool for Assessing Nitric-Oxide Mediated Vascular Function
Russell S. Richardson. *University of Utah, Salt Lake City, UT.*
(No relationships reported)

495 **4:35 PM - 5:00 PM**
Passive Movement Training: Impact on Angiogenic Factors and Capillary Growth in Human Skeletal Muscle
Ylva Hellsten. *University of Copenhagen, Copenhagen, WY, Denmark.*
(No relationships reported)

5:00 PM - 5:15 PM Overall Discussion

F-47 Tutorial Lecture - The Relations of Resistance Training and Strength with Morbidity and Mortality

FRIDAY, JUNE 1, 2012 3:15 PM - 4:05 PM
ROOM: 3014

496 **Allen W. Jackson, FACSM.** *University of North Texas, Denton, TX.*
(No relationships reported)

497 **Jakob L. Vingren.** *University of North Texas, Denton, TX.*
(No relationships reported)

APPENDIX I

CSUF IRB Approval: Cover letter, PA Intervention Informed Assent/Consent, & PDA Intervention Informed Consent

CAL STATE FULLERTON
Graduate Programs and Research
Institutional Review Board
(657) 278-7640/ Fax (657) 278-7238

REAPPROVAL NOTICE

From the Institutional Review Board
California State University Fullerton

Date: May 16, 2012

From: Ron Oliver, Chair RO

To: **Dr. Daniela Rubin**
Department: Kinesiology, KHS-121

Re: Use of Human Subjects in Research Project entitled:
Family-based Exercise Intervention for Children and Adolescents with Prader- Willi Syndrome

The forms you submitted to this office requesting continued approval for the use of human subjects in the above-referenced proposal were reviewed by the California State University Fullerton, Institutional Review Board ("CSUF IRB") at its fully convened meeting held on **May 11, 2012**. Your request for continuation of your research protocol has been approved.

The CSUF IRB has not evaluated your proposal for scientific merit, except to weigh the risk to the human participants and the aspects of the proposal related to potential risk and benefit. This approval notice does not replace any departmental or additional approvals which may be required.

If the above-referenced project has not been completed by **May 10, 2013** you must request renewed approval for continuation of the proposal. The regulations allow for approval on a yearly basis and not to exceed 365 days. This notice is based upon receipt of your request for renewal, and the previous expiration date for your study (not the date on this notice). There is no grace period. If you have not completed your project by the above date, you must stop until renewed approval is secured.

It is of utmost importance that you strictly adhere to the guidelines for human participant and that you follow the plan/methodology/procedures described in your research proposal. Any change in protocol or consent form procedure requires resubmission to the CSUF IRB for approval prior to implementation. Additionally, the principal investigator must promptly report, in writing, any unanticipated or adverse events causing risks to research participants or others.

Please be advised that if you are seeking external funding for this proposal, the above-referenced title should match exactly with the title submitted to the funding sponsor. Any change in project title should be submitted to the CSUF IRB prior to implementation.

By copy of this notice, the chairman of your department (and/or co-investigator) is reminded that s/he is responsible for being informed concerning research projects involving human participants in the department, and should review all protocols of such investigations as often as needed to ensure that the project is being conducted in compliance with our institutional policies and with DHHS regulations.

This institution has an Assurance on file with the Office for Human Research Protections.
The Assurance Number is FWA00015384.

Cc: Dr. Jie Weiss
Application No. HSR-12-0211

CALIFORNIA STATE UNIVERSITY, FULLERTON

Assent Form to participate in Research Project

Project title: "Family-Based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome"

You are being asked to be in an exercise project for children with and without Prader-Willi Syndrome. Prader-Willi Syndrome is a condition that affects the health of some children. By participating in this project, you will be trying out different activities, games, and exercises at home for 6 months (24 weeks). The purpose of the project is to give you activities, games, and exercises to increase the time you do physical activity.

If you agree to be in this project, you will be asked to play the games that we will give you at least 3 days a week. In addition, you will come to California State University, Fullerton (CSUF) 5 to 6 times. All visits will last about three and a half hours. During each visit, we will ask you to complete a few exercises with your body like tapping with feet and fingers, walking on a small surface, standing on one foot (also in a small surface with eyes open or closed), jumping, running back and forth, dribbling a tennis ball, and holding still with your eyes open or closed, or even if the floor moves.

In addition, you will take home a special machine, smaller than a cell phone, that tells us how much you move during the day. You will also get a belt so you can wear the machine around your waist. You will wear the machine all the hours that you are awake for eight days. At the end of each day, you will write all the physical activities you have done during the day in a log with the help of your mom, dad, or guardian (the person who looks after you).

We will also ask you to complete a test where you will lay still for a few minutes while a special machine takes a picture of your body (an x-ray). You will also have some questions to answer about how you feel about exercise, your daily activities, and yourself. In addition, you might need to answer some questions about your body.

On one visit to CSUF, you will play some playground games and video games using the Nintendo Wii™ console such as Wii Fit™ Plus, Just Dance 2, and Just Dance 3. The purpose of this is to show you and your mom/dad or guardian the activities we would like you to do at home.

If you want to, you will also participate in a blood draw study. Before your blood draw, we will ask you not to eat any food or drink any liquids other than water for a minimum of eight hours. During the morning of your site visit at CSUF, a nurse will take some blood from your arm. When the nurse takes your blood, it might hurt where you get pinched. You might also get a bruise. The nurses will do everything they can to keep you safe and healthy. Your mom, dad, or guardian will be next to you during the tests if you want them to be.

It is possible that you will get tired because of all the tests you will complete. While you do the exercise tests and play the games, you may feel out of breath (like in gym class or physical education). This is normal and something you have felt before.

You do not have to be in this study if you do not want to. If you start the study you may stop at any time. Nobody will be mad at you for stopping. Also, if some of the questions feel too personal to answer, you

can skip any question you don't feel comfortable answering. Only Dr. Rubin and her helpers from CSUF, and your parents or guardian will see your information. Your name will never be used or seen by anybody else. Your mom, dad, or guardian has said that it is okay to be in this study.

If you do decide to participate, you will get a gift card worth $30 after you finish each visit to CSUF and the eight days of wearing the special machine around your hip (once your mom, dad or guardian returns the special machine to us). Also, you can get a gift card worth $60 every six weeks if you do the activities we have given you at least 3 days a week and you write them in a log that we will give you. Thus, you could get up to $240 by the end of the study for doing the physical activities. If you participate in the blood draw study, you will receive an additional $15 gift card for each completed blood draw.

If you get hurt or sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. Please tell your parents or guardians that you are hurt or sick and they will contact Dr. Rubin.

By writing your name below it means that you have read or somebody has read this form to you and you understand this form. You have asked questions about anything you did not understand. A copy of this form will be given to your mom, dad, or guardian.

I want to be part of this research project: ☐ **Yes** ☐ **No**

_____ _____

My Name (Printed) My Signature

_____

Date

I certify that I have explained to the above individual the nature and purpose of the study, potential benefits, and possible risks associated with participation in this study. I have also answered all the questions that the child has asked.

Printed name of individual obtaining assent: _____

Title: _____ Date: _____

Signature: _____

I have witnessed the explanation of the research project to the participant. The participant was given the opportunity to ask questions, and the participant's questions, if any, were answered.

Printed name of witness: _____ Date: _____

Signature of Witness: _____

CALIFORNIA STATE UNIVERSITY, FULLERTON

Formulario de Asentimiento

Titulo del Proyecto: "Programa Familiar de Ejercicio para Niños y Adolecentes con el Síndrome de Prader-Willi"

Te han pedido que participes en un proyecto de ejercicio para niños con y sin el Síndrome de Prader-Willi. El Síndrome de Prader-Willi es una condición que afecta la salud de algunos niños. Por ser parte de este proyecto, estarás probando varias actividades, juegos y ejercicios en tu casa por 6 meses (como 24 semanas). El propósito de este proyecto es darte actividades, juegos y ejercicios para incrementar el tiempo que pasas haciendo actividad física.

Si decides participar en este proyecto, te pediremos que juegues los juegos que te daremos por lo menos 3 días a la semana. Además, vendrás a la Universidad Estatal de California, Fullerton (CSUF, por sus siglas en Ingles) 5 a 6 veces. Todas las visitas durarán alrededor de 3 horas y media. Durante cada visita, te pediremos que completes varios ejercicios con tu cuerpo como crear un ritmo en una mesa con tus manos y tus pies, caminar en una área pequeña, pararte en un pie (también en una área pequeña con tus ojos abiertos o cerrados), brincar, correr de un lado al otro, botar un pelota de tenis y pararte bien fijo con tus ojos abiertos o cerrados, o también cuando se mueva el piso.

Además, te llevaras a casa una maquinita especial, más chica que un teléfono celular, que nos dice cuanto te mueves durante el día. También recibirás un cinturón para que puedas usar esta maquinita sobre tu cadera. Tendrás que tenerla puesta todas las horas que estés despierto por ocho días. Al final de cada día, escribirás todas las actividades físicas que hiciste durante el día en una hoja de registro con la ayuda de tu mamá, tu papá o tu encargado legal (la persona que te cuida).

También te pediremos que completes una prueba donde te acostarás muy quieto por unos minutos mientras que una maquina especial toma una fotografía de tu cuerpo (como los rayos x). Si eres niña, puede que des un poco de orina para determinar si estas embarazada. También tendrás algunas preguntas que responder sobre cómo te sientes sobre el ejercicio, tus actividades diarias, y de ti misma/o. Además, puede que tengas que responder algunas preguntas sobre tu cuerpo.

En una de las visitas a la Universidad (CSUF), jugaras algunos juegos recreativos y juegos de video usando el Nintendo Wii™ como el Wii Fit™ Plus, Just Dance 2, y Just Dance 3. El propósito de esto es para enseñarte a ti y a tu mamá/papá o encargado legal las actividades que queremos que hagan en la casa.

Si quieres, también participaras en un estudio de sangre. Antes de que te saquemos sangre por la mañana, te pediremos que no comas ninguna comida y no bebas ningún liquido al menos que sea agua. Durante la mañana de tu visita a CSUF, una enfermera te sacara una pequeña muestra de sangre de tu brazo. Cuando la enfermera saque tu sangre, puede que te duela un poquito cuando te pique. Puede que también te salga un morete. La enfermera hará todo lo posible para mantenerte seguro y saludable. Tu mamá, papá o encargado legal estarán a tu lado durante las pruebas si quieres que te acompañen.

Es posible que te canses de todas las pruebas que completarás. Mientras que hagas las pruebas de ejercicio y juegues los juegos, puede que te sientas un poco sin respiración (como en la clase de gimnasia o educación física). Esto es normal y algo que has sentido antes.

Si no quieres, no tienes que participar en este estudio. Si comienzas el estudio, lo puedes abandonar en cualquier momento. Nadie se enojara contigo si abandonas este estudio. También, si algunas preguntas te parecen muy personales, puedes decidir no contestarlas si te sientes incómodo respondiendo. Solo la Dra. Rubin y sus ayudantes de CSUF, y tus papás o encargado legal verán tu información. Tu nombre nunca

será visto ni usado por ninguna persona. Tu mamá, papá o encargado legal ha dicho que está bien que participes en este estudio.

Si tú decides participar, recibirás una tarjeta de regalo que vale $30 después de que termines cada visita a CSUF y los ocho días de usar la maquinita especial en tu cadera (siempre y cuando tu mamá, papá o encargado legal regrese la maquinita especial). También, puedes recibir una tarjeta de regalo que vale $60 cada seis semanas si haces las actividades que te hemos dado por lo menos 3 días a la semana y los escribas en el diario que te daremos. Así, puedes recibir un total de $240 al final del estudio por hacer las actividades físicas. Si participas en el estudio de sangre, recibirás una tarjeta de regalo adicional con un valor de $15 por cada muestra de sangre que completes.

Si te lastimas o te enfermas por estar en el estudio de investigación, puedes recibir atención médica en un hospital o clínica del Ejército sin cobro. Solo serás tratado por las lesiones que fueron directamente causadas por el estudio de investigación. Por favor avísale a tus papas o encargado legal si estas lastimado o enfermo y ellos contactarán a la Dra. Rubin.

Al escribir tu nombre en las líneas de abajo, estás diciendo que has leído o alguien te ha leído este formulario y lo has entendido y que has hecho preguntas acerca de las cosas que no entendiste. Una copia será dada a tu mamá, papá o encargado legal.

Quiero ser parte de este proyecto de investigación: **Si** ☐ **No** ☐

_____ _____

Mi Nombre (En Letra) Mi Firma

_____

Fecha

Certifico que he explicado al individuo firmante la naturaleza y el propósito de este estudio, sus posibles beneficios, y los riesgos asociados con su participación. También he contestado todas las preguntas que el niño/a ha hecho

Nombre del Individuo Obteniendo Asentimiento:_____

Titulo: _____ Fecha:_____

Firma: _____

He sido testigo de la explicación de este proyecto de investigación al participante. El participante ha tenido oportunidad de hacer preguntas, y sus preguntas, si alguna, fueron contestadas.

Nombre del Testigo: _____ Fecha: _____

Firma del Testigo: _____

CALIFORNIA STATE UNIVERSITY, FULLERTON

INFORMED CONSENT TO PARTICIPATE IN RESEARCH

"Family-Based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome"

Invitation to Participate

You and your child have been invited to take part in a research project called "Family-Based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome" that is funded by the United States Department of Defense. Prader-Willi Syndrome (PWS) is a genetic condition that can affect a child's health. The purpose of this project is to test the effectiveness of a home-based physical activity program (Active Play @ Home) aiming to increase physical activity levels and improve motor performance, health, and self-esteem in your child. Youth will be recruited to participate at California State University, Fullerton (CSUF): 25 youth (8-16 years old) who have PWS and 40 children (8-11 years old) who do not have this condition and are obese (have a body mass index >95th percentile for age and sex). This research will be conducted at CSUF by Daniela Rubin, Ph.D., Jie Weiss, Ph.D., Debra Rose, Ph.D., and Lenny Wiersma, D.P.E, Kathleen S Wilson, Ph.D.

Description of Procedures

You and your child are invited to participate in a home-based physical activity program lasting 24 weeks. You and your child will be given video games (i.e., Nintendo Wii™ Fit Plus, Just Dance 2, and Just Dance 3), sports equipment, and a manual with playground activities and games for you and your child to do at home during the 24-week period. If you allow your child to participate, he or she will be asked to engage in physical activity at home using the materials provided for a minimum of 25-45 minutes each session, 3 days a week during the 24 weeks. In addition, your child will visit CSUF five times: 2 visits before the program (1 week apart), after 12 weeks into the program, at the end of the program (week 24) and six months after completing the program. Each visit will last up to 3.5 hours long. If your child is assigned to a control group, he or she will complete 6 visits instead of 5 and will receive the program 6 months after agreeing to participate.

During all visits, the research team may assess: 1) your child's sensory and motor proficiency (e.g., his or her ability to process different kinds of sensory information, motor coordination, muscle strength, balance, and agility), 2) your child's body height, weight, and composition, 3) your child's physical activity, and 4) your child's feelings about physical activity and quality of life (using surveys). You will be required to fill out a medical history form about your child. Your child might also complete a questionnaire that describes his or her physical development. In addition, as the parent/guardian, you will complete a questionnaire that includes questions on: 1) demographic information about yourself and your family, your stress level about parenthood, your perceived family functioning, the general sense of self-efficacy, and your perceived social support; 2) your motivation to join the home-based physical activity program, specific self-efficacy for helping your child be active, your intention to help your child be more physically active, and the influences you use for prompting your child's physical activity; and 3) a set of questions related to your child's quality of life.

Sensory and motor proficiency tests: the sensory organization test requires that your child stands still during 6 different conditions (eyes open, eyes closed, floor still, floor moving and a combination of). At all times your child will be attached to a harness just in case he or she loses balance. We will explain what is going to happen in every situation to your child and will complete all 6 conditions twice, becoming familiar with the tasks. During the motor proficiency test your child will do fine motor activities such as drawing, cutting a circle, copying forms, and other sedentary activities. In

addition, your child will do some coordination activities (i.e., jumping jacks and tapping feet and fingers), balance activities (i.e., standing on a beam and walking forward heel-to-toe on a balance beam), running speed and agility activities (i.e., hopping on one foot or both feet), upper limb coordination activities (i.e., ball dribbling, catching, throwing), and lastly strength activities (i.e., jumping forward from a standing position).

Body composition: your child will complete a body composition test (DXA scan). For this scan, your child will lay still for a few minutes while a machine takes a picture of his or her body using a very weak x-ray. If your child is a girl and she had her first menses, the research team will conduct a urine pregnancy test before measuring her body composition. The pregnancy test is required by law and it is done to ensure the safety of your daughter because the x-rays might be harmful to the fetuses. If your daughter is pregnant, she will be excluded from participation in this project.

Physical activity: your child will be asked to wear a small device, smaller than a cell phone, called an accelerometer for eight days at four times: at baseline, after weeks 12 and 24, and after six months of participating in the program. Your child will wear the accelerometer strapped to a belt during all hours he or she is awake. Your child will remove the accelerometer to shower or bathe, to go to bed, and to swim. In addition, your child will complete a log everyday indicating all the physical activity he or she has done (e.g., physical education, playing outside, sports, biking, or even walking the dog). You or somebody you designate may help your child complete this log. You will return the accelerometer after 8 days of wearing it via a pre-stamped box provided to you at no cost.

During the second visit we will train you and your child on the activities and the use of the equipment we will be providing you for the physical activity program. During the training we will give you a manual to accompany the use of the media equipment as well as for the physical activity toolkit containing exercises and games. During the program, we will call you once a week to check on your child's involvement in the physical activity, troubleshooting, etc. during the initial six weeks and every other week afterwards. You will complete physical activity checklists for each day of the program and return them to CSUF as indicated in an envelope provided to you.

Ancillary Study Option

Your child is also invited to participate in an ancillary study investigating changes in blood markers associated with diabetes and cardiovascular disease such as cholesterol, glucose, and insulin, in response to the Active Play @ Home program. Participation in this ancillary study option will require one blood draw (30 mL or ~6 teaspoons) at baseline and another at the end of the PA program (Week 24). An experienced pediatric phlebotomist (a person trained in drawing blood from children) will draw blood from your child's arm using standard venipuncture technique such as when your child gets blood work done during a visit to the doctor. Blood draws will be completed in the morning following a 9- to 12-hour overnight fast (water allowed) the day of your visit.

As part of the research project "Family-based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome," Dr. Rubin and her collaborators would like to store some of your child's blood that is not needed for the measurements specified above that would otherwise be thrown away. If you agree, Dr. Rubin will keep the extra blood so that it may be used in future research to learn more about obesity, physical activity, and the risk of diabetes or cardiovascular disease. Even if the research that is done on your child's blood cannot be used to help your child, it might help other people who have obesity and risk for cardiovascular disease or diabetes.

Dr. Rubin will be responsible for making sure that your child's samples are protected and that the information provided about your child is kept confidential. Your child's samples will not be stored with any identifying information but instead will be given a code number to protect his/her identity. There are laws that require that research records that have your child's name on them may be shown to people who make sure that the research is being done correctly. As mentioned in this consent form, the US Army Medical Research Program and the Institutional Review Board have the legal right to review and copy your child's information related to this research. There will be no cost to you for any specimens collected and stored. Your child's blood will be used only for research and will not be sold. The choice to let Dr. Rubin keep your child's blood for doing research is entirely up to you. If you decide that your child's blood can be kept for research but you later change your mind, tell Dr. Rubin who will remove and destroy any of your child's blood that she still has. Otherwise, the samples may be kept until they are used up, or until Dr. Rubin decides to destroy them.

If you are interested in having your child participate in this ancillary study please check this box:

□ **Yes** □ **No** **Initials:** _____

If you agree that we can store some of your child's blood for future analyses please check this box:

□ **Yes** □ **No** **Initials:** _____

Potential Risks and Inconveniences

During or after exercising, your child might experience fatigue, shortness of breath, and muscle soreness. These risks are no different than those that children normally experience when they exercise or play. Your child may experience some discomfort from completing some of the tests as they may challenge your child physically and mentally. Because we understand that children with PWS may become more tired than children without the syndrome, we will provide continuous breaks during the testing to ensure the children are as rested as possible in between tests and activities. In addition, if your child is currently on lipid-lowering medication, diabetes medications, and/or blood pressure medication, he or she will be excluded from participation in this study.

During the sensory testing, all participants will be required to wear an overhead safety harness to ensure their safety during practice and test trials. During the motor proficiency test the person administering the test will be situated within close proximity to your child to be able to catch him/her if they were to fall. Moreover, test administrators will be trained in fall prevention to ensure your child's safety.

Also, if some of the questions feel too personal to answer, both you and your child can skip any question(s) you don't feel comfortable answering.

Blood draws may cause pain or a bruise at the puncture site. There is a slight risk of infection from these procedures. To avoid infection, the phlebotomist will take all possible precautions. These precautions include using sterile disposable equipment, and practicing sterile techniques during blood sampling. Only trained phlebotomists, adhering to standard operating procedures, will draw blood.

Compensation for Participation

If you decide to have your child participate, he or she will receive a gift card worth $30 after they complete each of the visits to CSUF and return the accelerometer. During the 24-week at-home program, if your child logs at least 3 days a week of physical activity, he or she will receive a gift card worth $60 every six weeks. Therefore, your child could receive up to $240 by the end of the study for

compliance with the physical activity schedule. After the program all the provided sports and media materials will be for your child to keep if he/she completes at least 70% of the scheduled days of physical activity during the six months of the study.

Youth participating in the blood markers ancillary study option will receive a $15 gift card for each blood draw.

You will receive a parking pass to park at a designated parking lot on campus and gas mileage at California State University, Fullerton rates ($0.50 per mile) up to $60 roundtrip. No other compensation is available for participating in this research study. You should be aware that there may be additional costs to you resulting from your child participating in the study.

Medical care for research related injury
If your child gets hurt or sick because of this research study, she or he can receive medical care at an Army hospital or clinic free of charge. He or she will only be treated for injuries that are directly caused by the research study. The Army will not pay for transportation to and from the hospital or clinic. If you have questions about this medical care, please contact Dr. Daniela Rubin (principal investigator for this study) at (657) 278-4704. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221. No other medical compensation for research related injury is available for participating in this study.

Voluntary Participation
Participation in this study is voluntary. You may refuse to allow your child to participate, or you may withdraw him or her from the study at any time without affecting any future relationship with California State University, Fullerton. Your child can also decide to stop participating at any time. There is no penalty for an early withdrawal. If you decide to withdraw your child from the study or he/she decides to do so, you should inform the researchers immediately and return all equipment to CSUF. Your child may also be removed from the study without your consent because of the following: a) based on the researcher's judgment to improve his/her health and welfare, and/or b) because your child has not followed the study procedures.

Confidentiality
Your child's records will be maintained confidential to the extent provided by law. Data will be stored in a locked file cabinet in a locked room or saved as an encrypted file on a password protected computer. Only the research team will have access to the data. In addition, representatives of the local Institutional Review Board or the US Army Medical Research and Materiel Command are eligible to review records as part of their responsibility to protect human subjects in research. Any information about this study will be presented (e.g., publications or presentations) in a group form and your child's name will never be used. Research records will be destroyed three years after the publication of the final papers.

If you have additional questions, you may contact Dr. Daniela Rubin at (657) 278-4704. For questions about your rights or your child's rights as a research participant, you may contact the California State University, Fullerton Institutional Review Board at (657) 278-7640.

By signing below you acknowledge that you have carefully read this form, and have had this research and the terms used in this Consent Form and their significance explained to you. You will receive a copy of this consent document for your files. If a person other than you is transporting your child to the location of the study (CSUF), please indicate the name of this person:

_____. Otherwise, please leave blank.

I agree to allow my child to participate in this research study ☐ **Yes** ☐ **No**

I consent that my child is healthy to participate in moderate to vigorous physical activity ☐ **Yes** ☐ **No**

_____ _____

Parent or Legal Guardian Name (**Printed**) Parent or Legal Guardian (**Signature**)

_____ _____

Child's Name Date

_____

Address of the subject

My signature as **witness** certifies that the subject signed this consent form in my presence as his/her voluntary act and deed.

_____

Name of Witness

_____ _____

Signature of Witness Date (same as above)

A witness has certified this consent form ☐ **Yes** ☐ **No**

CALIFORNIA STATE UNIVERSITY, FULLERTON

AUTORIZACION NOTIFICADA

"Programa Familiar de Ejercicio para Niños y Adolecentes con el Síndrome de Prader-Willi"

Invitación a Participar

Usted y su hijo han sido invitados a participar en un estudio llamado "Programa Familiar de Ejercicio para Niños y Adolecentes con el Síndrome de Prader-Willi." Esta investigación está auspiciada por el Departamento de Defensa de Estados Unidos. El Síndrome de Prader-Willi (SPW) es una condición genética que puede afectar la salud de un niño. El propósito de este proyecto es probar la efectividad de un programa de actividad física en el hogar (Juegos Activos en el Hogar) para incrementar los niveles de actividad física y mejorar la motricidad, la salud y la autoestima en su hijo. Este proyecto se llevara a cabo en la Universidad Estatal de California, Fullerton (CSUF, por sus siglas en Ingles). Se reclutara 25 jóvenes (8-16 años de edad) que tienen el Síndrome de Prader-Willi y 40 niños (8-11 años de edad) los cuales no tienen esta condición y están obesos (que tengan un Índice de Masa Corporal [IMC] arriba del 95 percentil por edad y sexo). Si su hijo se encuentra actualmente en medicamentos para bajar los niveles de los lípidos, para la diabetes, y/o la presión arterial, él o ella será excluido de la participación en este estudio. Este estudio será conducido en CSUF por Daniela Rubin, Ph.D., Jie Weiss, Ph.D., Debra Rose, Ph.D., Lenny Wiersma, D.P.E. y Kathleen S Wilson, Ph.D.

Descripción de Procedimientos

Usted y su hijo están invitados a participar en un programa de actividad física en el hogar que durará 24 semanas. A usted y a su hijo se les proporcionarán juegos de video (Nintendo Wii™ Fit Plus, Just Dance 2, y Just Dance 3), equipo deportivo y un manual de actividades y juegos para que usted y su hijo jueguen en su hogar durante un periodo de 24 semanas. Si usted permite que su hijo participe, le pediremos que se involucre en actividad física en el hogar usando los materiales que se les proporcionó por un mínimo de 25-45 minutos cada sesión, 3-4 días a la semana durante las 24 semanas. Además, su hijo visitara CSUF cinco veces en total: 2 visitas antes del comienzo del programa (una semana entre cada visita), otra a la doceava semana después del comienzo del programa, al fin del programa (semana 24), y seis meses después de haber completado el programa. Cada visita durara 3.5 horas. Si su hijo es puesto en el grupo de control, él o ella completara 6 visitas en vez de 5 y recibirá el programa 6 meses después de haber acordado participar.

Durante las cuatro visitas, el equipo de investigación examinará: 1) la habilidad sensorial y motriz de su hijo (por ejemplo, su habilidad de procesar diferentes tipos de información sensorial, coordinación motriz, fuerza muscular, equilibrio y agilidad), 2) la talla, el peso y la composición corporal de su hijo, 3) la actividad física de su hijo y 4) los sentimientos de su hijo sobre la actividad física y calidad de vida (usando encuestas). Usted llenará un formulario sobre la historia médica de su hijo. Su hijo también puede que complete un cuestionario que describa su desarrollo físico. Además, como padre/encargado legal, completará un cuestionario que incluye preguntas sobre: 1) información demográfica sobre usted y su familia, su nivel de estrés relacionado a la paternidad, su percepción de la función familiar, el sentido común de su eficacia, y su percepción de apoyo social; 2) su motivación a unirse al programa de actividad física en el hogar, su eficacia específicamente hacia ayudar a su hijo a ser activo, su intención en ayudar a su hijo a ser más activo físicamente y las influencias que usted usa para provocar que su hijo haga actividad física; y 3) un grupo de preguntas relacionadas a la calidad de vida de su hijo.

Exámenes de habilidad sensorial y motriz: el examen de organización sensorial requiere que su hijo este parado quietamente durante 6 diferentes condiciones (con los ojos abiertos, con los ojos cerrados, con el piso inmóvil, con el piso móvil y una combinación). Durante el examen su hijo estará en un arnés por si

él/ella pierde el equilibrio. Nosotros le explicaremos a su hijo que es lo que va a pasar en cada situación y completara las 6 condiciones dos veces, ayudando a familiarizarse con las tareas. Durante el examen de motricidad su hijo completará tales actividades como: dibujar, cortar un círculo de papel, copiar figuras y otras actividades sedentarias. Además, su hijo completará actividades de coordinación (por ejemplo, salto con palma y un ritmo de pies y manos), actividades de equilibrio (por ejemplo, pararse en una tabla de equilibrio y caminar en una línea recta de punta a punta), actividades de velocidad y agilidad (por ejemplo, brincando sobre un pie o dos pies), actividades de coordinación con los brazos (por ejemplo, botar un balón, atrapar, y aventar) y por último, actividades de fuerza (por ejemplo, salto de distancia).

Composición de grasa: su hijo completará una evaluación de la composición del cuerpo (DXA scan). Para esta evaluación su hijo se recostará sobre una maquina y se mantendrá quieto por unos minutos mientras la máquina toma una fotografía de su cuerpo usando un rayo x muy débil que no hace daño. Si su hija ha comenzado su menstruación, los investigadores tendrán que administrar una prueba de embarazo antes de medir su composición corporal. La prueba de embarazo es requerida por ley y lo hacemos para asegurar la salud de su hija ya que los rayos x pueden ser dañinos para el feto. Si su hija estuviese embarazada, será excluida de la participación en este proyecto.

Actividad física: Se le pedirá a su hijo que use una maquinita, de un tamaño más chico que de un celular, que se llama acelerómetro, por ocho días en cuatro diferentes puntos del estudio: entre las primeras visitas (visita 1 y visita 2), después de las semanas 12 y 24, y después de 6 meses de haber participado en el programa. Su hijo tendrá el acelerómetro puesto con un cinto a todas horas que él o ella este despierto(a). Su hijo se quitara el acelerómetro al bañarse, al irse a dormir y al nadar. Además, su hijo completará un registro diario por escrito para indicar toda la actividad física que él o ella hizo (por ejemplo, clase de educación física, jugar afuera, deportes, andar en bici, o caminar al perro). Usted o alguien que usted escoja puede ayudar a su hijo a llenar este registro. Usted regresara el acelerómetro después de haberlo usado por 8 días en un paquete estampado que se les proporcionará en sus visitas a CSUF sin costo adicional.

Durante la segunda visita los entrenaremos a usted y a su hijo sobre las actividades y el uso del equipo/material que se le proporcionará para el programa de actividad física. Durante el entrenamiento le daremos el manual que acompaña el uso de los equipos de video juegos y también para la bolsa de equipo de actividad física que contiene ejercicios y juegos. Durante el programa, le llamaremos cada semana para revisar como se está involucrando su hijo en la actividad física, para cualquier solución de problemas, etc. durante las primeras seis semanas y luego cada otra semana. Usted completará las listas de calificación de actividad física por cada día del programa y las regresará a CSUF como es indicado en un sobre que se les proporcionará.

Opción de Estudio Auxiliar

Su hijo también está invitado a participar en un estudio auxiliar que investiga los cambios en los marcadores sanguíneos asociados con la diabetes y las enfermedades cardiovasculares como el colesterol, la glucosa y la insulina, en respuesta al programa de Juegos Activos en el Hogar. La participación en esta opción de estudio auxiliar requiere una extracción de sangre (30 ml o ~6 cucharaditas) al inicio y otro al final del programa (Semana 24). Un flebotomista con experiencia pediátrica (una persona especializada en la extracción de sangre de los niños) sacará una muestra de sangre del brazo de su hijo utilizando la técnica de venipuntura estándar, como cuando a su hijo le hacen pruebas de sangre durante una visita al médico. La extracción de sangre se completará en la mañana después de una noche de ayuno de 9 horas (se permite agua) el día de su visita.

Como parte del proyecto de investigación "Programa Familiar de Ejercicio para Niños y Adolecentes con el Síndrome de Prader-Willi," la Dra. Rubin y sus colaboradores quisieran guardar lo que sobre de la muestra de sangre de su hijo que no se usaran para este estudio que de otra manera seria desechado. Si Ud. está de acuerdo, la Dra. Rubin guardar las muestras en un refrigerador bajo llave para que se puedan usar en un proyecto de investigación futuro y aprender más sobre la obesidad, actividad física, y el riesgo de la diabetes o enfermedad cardiovascular. Incluso si la investigación que se realiza con la sangre de su hijo no se puede utilizar para ayudar a su hijo, aun podría ayudar a otras personas que tienen obesidad y el riesgo de enfermedad cardiovascular o diabetes.

La Dra. Rubin será responsable de asegurarse de que las muestras de su niño estén protegidas y que la información proporcionada acerca de su hijo se mantenga confidencialmente. Las muestras de su hijo no se almacenaran con ninguna información de identificación, sino que se les dará un número de código para proteger la identidad de su hijo. Hay leyes que requieren que los expedientes de investigación que tienen el nombre de su hijo en ellos se pueden mostrar a las personas que se aseguran de que la investigación se está realizando correctamente. Como se menciona en este formulario de consentimiento, el Ejército de los EE.UU. Programa de Investigación Médica y el Comité de Ética (Institutional Review Board) tienen el derecho legal de revisar y copiar la información de su hijo en relación con esta investigación. No habrá ningún costo para usted por coleccionar y almacenar todos los especímenes. La sangre de su hijo será utilizada únicamente para investigación y no será vendida. La decisión de dejar que la Dra. Rubin se quede con la sangre de su hijo para hacer investigaciones es totalmente de usted. Si decide inicialmente que la sangre de su hijo puede ser guardada para un estudio futuro y luego cambia de opinión, simplemente notifique a la Dra. Rubin para que retire y destruya las muestras. De lo contrario, las muestras pueden mantenerse hasta que se agoten, o hasta que la Dra. Rubin decida destruirlos.

Si usted está interesado en que su hijo participe en este estudio auxiliar por favor, marque esta casilla:

□ **Si** □ **No** **Iniciales:** _____

Si usted esta de acuerdo en dejarnos almacenar las muestras de sangre de su hijo para usar en análisis futuros, por favor marque esta casilla:

□ **Si** □ **No** **Iniciales:** _____

Riesgos Potenciales o Inconvenientes

Durante o después del ejercicio, su hijo puede experimentar fatiga, falta de aliento, y dolor muscular. Estos riesgos no son diferentes de los que los niños normalmente experimentan cuando hacen ejercicio o juegan. Su hijo también puede sentir incomodidad al completar algunas de las pruebas ya que van a ejercer o retar a su hijo físicamente y mentalmente. Entendemos que niños con el Síndrome de Prader-Willi pueden sentirse más cansados que lo niños sin el síndrome y es por eso que ofreceremos descansos continuos durante las pruebas para asegurar que los niños estén lo más descansados posibles entre las pruebas y las actividades.

Durante las pruebas sensoriales, se les requiere a todos los participantes usar un arnés de seguridad para estar protegidos durante las prácticas y las primeras pruebas. Durante las pruebas de motricidad la persona administrando la prueba estará muy cerca de su hijo por si se cae lo podrán atrapar. Los administradores de pruebas estarán entrenados en prevención de caídas para la seguridad de su niño.

También tome en cuenta que si algunas de las preguntas se le parecen muy personales usted y su hijo pueden decidir no contestar cualquier pregunta que no se sientan cómodos contestar.

La extracción de sangre puede causar dolor o un morete en el sitio de la punción. Hay un riesgo ligero de infección de estos procedimientos. Para evitar la infección, el flebotomista tomará todas las precauciones posibles. Estas precauciones incluyen el uso de material desechable estéril, y la práctica de técnicas estériles durante el manejo de las muestras de sangre. Sólo un flebotomista capacitado, cumpliendo con los procedimientos de operación estándar, le extraerá sangre.

Compensación por Participar

Si usted decide que su niño participe, él/ella recibirá una tarjeta de regalo con un valor de $30 después de que complete cada de las cuatro visitas y regrese el acelerómetro. Durante el programa de 24 semanas de actividad física en el hogar, si su hijo mantiene su registro de actividad física por lo menos 3 días a la semana, él/ella recibirá una tarjeta de regalo con un valor de $60 cada seis semanas. Por lo tanto, su hijo puede recibir hasta $240 al final del estudio por cumplir con el programa de actividad física. Después del programa todos los materiales y equipos deportivos e interactivos que se le surtió a su niño serán de él o ella solamente si completa no menos de 70% de los días programados de actividad física durante los seis meses del estudio.

Los niños que participen en el estudio de sangre auxiliar recibirán una tarjeta de regalo de $15 por cada muestra de sangre.

Usted recibirá un pase para estacionarse dentro de la universidad si es necesario y se le reembolsara sus millas recorridas ($0.50) hasta un máximo de $60 por viaje redondo en base a precios provistos por la Universidad del Estado de California, Fullerton. No hay otra compensación disponible por participar en este estudio. Usted debe saber que puede haber otros costos adicionales que resulten de la participación en este estudio.

Cuidado médico por lesiones relacionadas con esta investigación

Si su niño se lastima o se enferma por este estudio, él/ella puede recibir cuidado médico en un hospital o clínica del Ejército sin cargo. Él/ella solo será tratado por lesiones que han sido directamente causadas por este estudio de investigación. El Ejército no pagará por transporte hasta o desde el hospital o clínica. Si usted tiene preguntas sobre el cuidado médico, hable con la investigadora principal para este estudio (Dra. Daniela Rubin, teléfono 657-278-4704). Si usted paga de su bolsillo por gastos médicos por lesiones causadas en este estudio en otro lugar, contacte a la investigadora principal. Si este problema no se resuelve, contacte al personal legal de el Ejercito de los EE.UU. Programa de Investigación Médica (U.S. Army Medical Research and Materiel Command, USAMRMC Office of the Staff Judge Advocate) oficina legal, al número (301) 619-7663/2221. Ninguna otra compensación para las lesiones relacionadas con la investigación es disponible por participar en este estudio.

Participación Voluntaria

La participación en este estudio es voluntaria. Usted puede negarse a que su hijo participe o puede retirarlo del estudio en cualquier momento sin afectar cualquier futura relación con la Universidad Estatal de California, Fullerton. Su niño puede también interrumpir su participación en cualquier momento. No hay ninguna penalidad por retirarlo antes de finalizar el estudio. Si usted decide retirar a su hijo del estudio, o si él/ella decide hacerlo, debe informar a los investigadores inmediatamente y regresar todo el material a la Universidad. Su hijo puede ser retirado del estudio sin su consentimiento de acuerdo a lo siguiente: A) basado en el juicio del investigador por mejorar la salud y bienestar, B) porque su hijo o Ud. no ha seguido los procedimientos del estudio.

Confidencialidad

Los registros de su hijo se mantendrán confidenciales en la medida en que lo requiere la ley. Los datos serán guardados dentro de un archivero bajo llave en un cuarto bajo llave o serán guardados en un archivo codificado en una computadora con acceso bajo un código específico. Solo el equipo de investigación tendrá acceso a los datos. También los representantes de la Comité de Etica (IRB, pos sus siglas en Ingles) o el Ejercito de los EE.UU. Programa de Investigación Médica (US Army Medical Research and Materiel Command) pueden revisar los datos como parte de su responsabilidad de proteger a las personas que participan en estudios de investigación. Toda información acerca de este estudio se presentará (por ejemplo, publicación o presentaciones) en forma global y los nombres de su hijo y de los otros niños/as no se usaran nunca. Una vez que se complete el estudio, todos los registros de investigación se guardarán por tres años después de la publicación y luego se destruirán.

Si tiene preguntas adicionales, puede comunicarse con la Dra. Daniela Rubin al (657) 278-4704. Para contestar las preguntas acerca de sus derechos y de los derechos de su hijo como participante de un estudio de investigación, puede comunicarse con la Comité de Revisión Institucional de la Universidad del Estado de California, Fullerton al (657) 278-7640.

Al firmar este documento, Ud. indica que ha leído cuidadosamente este formulario y que se le ha explicado este estudio de investigación, los términos empleados en esta Autorización Notificada y su significado. Ud. recibirá una copia de este documento de autorización para guardar en sus archivos. Si alguien ajeno a usted se encargo de proveer transportación a su hijo al estudio (CSUF), por favor indique el nombre de esa persona: _____. De otra manera, deje en blanco el espacio.

Autorizo que mi hijo participe en este estudio de investigación ☐ **Si** ☐ **No**

Accedo que mi hijo está en buena condición para participar en actividad física moderada o rigoroso. ☐ **Si** ☐ **No**

_____ _____

Nombre del Padre, Madre o Encargado legal (**En letra**) Firma del Padre, Madre o Encargado legal

_____ _____

Nombre del Niño/a Fecha

_____

Dirección del Participante

Mi firma como testigo certifica que el participante firmo esta autorización notificada en mi presencia en un acto voluntario.

_____

Nombre del Testigo

_____ _____

Firma del Testigo Fecha (la misma de arriba)

Un testigo certifico esta autorización notificada ☐ **Si** ☐ **No**

CSUF IRB
APPROVED FOR USE
HSR# 12-0211
Approved: 5-11-12
Expires: 5-10-13

Fecha de preparación: Abril 4, 2012

CALIFORNIA STATE UNIVERSITY, FULLERTON

INFORMED CONSENT TO PARTICIPATE IN RESEARCH

"Family-based Exercise Intervention for Children and Adolescents with Prader-Willi Syndrome: Parents' Decisions to have their Children Involved in Physical Activity"

Invitation to Participate
I have been asked to take part in a research study investigating parents' decisions to have their child with Prader-Willi Syndrome (PWS) (ages 8 to 16 years old) get involved in physical activity (PA). Parental involvement has been shown to be important in both prevention and treatment of childhood obesity in children without PWS. For children and adolescents, parents are the primary mediators of change in adopting a healthy lifestyle and reducing sedentary behaviors. A total of 20 parents of youth with PWS (13 at the California State University, Fullerton (CSUF) site and 7 at the University of Florida, Gainesville site) will be randomly invited to participate in the decision reporting intervention in addition to participating in the Active Play @ Home physical activity program. Parents of youth participating in the PA program that are not randomly invited to be part of this study will form part of the control group. This research study will be conducted at CSUF by Dr.'s Jie Weiss, Daniela Rubin, Debra Rose and Lenny Wiersma.

Description of Procedures
If I agree to be in this research study, I will participate in a 24-week decision reporting intervention on how to help my child with PWS be more physically active. I agree to visit CSUF four times during this research study: 2 visits before the intervention (1 week apart), at the 12th week during the intervention, and at the end of the intervention (week 24). Each visit will be about 2 hours long. The first two visits will be orientation sessions, one week apart.

In my first visit, the research team will lend me a handheld computer/personal digital assistant (PDA) and teach me how to use it. In addition, the research team will give me two memory cards to record my responses in the PDA (one already inserted into the PDA). I will be asked to answer questions every other day/week on the PDA for a 24-week period of time. The research team will set up beeping signals in the PDA every other day to remind me to answer the questions and remind me of having my child(ren) be involved in PA. During my second visit to CSUF (one week after the first meeting), I will bring the PDA for any troubleshooting along with any questions.

In order to secure the data being collected from the PDA and to minimize the amount of visits to campus for downloading, one additional memory card will be provided to me during the first and third visits. At the completion of week six and week 18, I will swap memory cards and send in the provided pre-stamped envelope to CSUF the used memory card for weeks 1-6 and 13-18, respectively. During the 3rd and 4th meeting at CSUF (12th and 24th week of intervention), I will bring the PDA to have the data downloaded by the research team and answer some assessment questions. On the last meeting (week 24) I will return the PDA (and PDA accessories) to the research team and will receive the final incentive.

Parents participating in the PDA control study group will not receive a PDA.

Potential Risks and Inconveniences
Participation in this study will take about 5-10 minutes of my time each day to answer the questions on a PDA three days a week for 24 weeks. There are no known risks associated with my participation in this research beyond those of everyday life. Also, if some of the questions feel too personal to answer, I can skip any question(s) I don't feel comfortable answering.

Voluntary Participation
Participation in this study is voluntary. I may refuse to participate, or withdraw any time without penalty.

Compensation for Participation
For my participation in this 24-week study, I will receive in the mail every six weeks a gift card worth $60 for a total of $240. For each six-week period, I will need to complete at least 80% of the responses in the PDA reports (every other day/week) on my decision making process in supporting my child to do PA, and return in the mail (in the pre-stamped envelopes provided by CSUF) the memory card for weeks 1-6 and 13-18 immediately after completing each six-week period. For weeks 7-12 and 19-24, I will have the data downloaded by the research team during visit three and visit four, respectively. Approximately two weeks following the last site visit to CSUF, I will receive an additional $50 bonus gift card via mail if I provide 80% of responses in the PDA during the entire intervention (24 weeks) and return the PDA without any damage. I will take good care of the PDA. No other compensation is available for participating in this research study. Parents in the control group will not receive any additional incentives other than those provided to their child for participation in the physical activity program.

Medical care for research related injury
If you should get hurt or sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. Treatment will only cover injuries that are directly caused by the research study. The Army will not pay for transportation to and from the hospital or clinic. If you have questions about this medical care, please contact Dr. Daniela Rubin (principal investigator for this study) at (657) 278-4704. If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U.S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221. No other medical compensation for research related injury is available for participating in this study.

Confidentiality
Confidentiality of my research records will be strictly maintained to the extent allowed by law. All of the information collected from me will be kept in an electronic database that is set up in a designated, password-protected computer in a locked research office (CSUF, Kinesiology and Health Sciences Building Office #236). Data collected via the PDA will be downloaded to the designated computer that is also accessible solely to the research team in the research office. In the database, respondents will be identified only by an ID code number, not by their names or addresses. The list linking respondents' names with their ID codes will be stored in a separate file with a separate password.

Only the research team will have access to the data. In addition, representatives of the local Institutional Review Board or the US Army Medical Research and Materiel Command are eligible to review records as part of their responsibility to protect human subjects in research. Any information about this study will be presented (e.g., publications or presentations) in a group form and your name will never be used. Research records will be destroyed three years after the publication of the final papers. I understand that any information resulting from this research project that personally identifies my information will not be voluntarily released or disclosed by these entities without my separate consent, except as specifically required by law.

I have carefully read and am fully competent to sign this Consent Form. I understand that this form with my signature will be kept by the researchers in a locked cabinet for three (3) years; at the end of the three years, this consent form will be destroyed. All paper and pencil data collected will also be kept in a locked cabinet for three years after the study is completed, and afterwards it will be destroyed.

If I have any questions about the study or wish to report a research-related problem, I may contact Dr. Weiss at (657) 278-4388. For questions about my rights as a research participant, I may contact the California State University, Fullerton Regulatory Compliance Coordinator at (657) 278-7640.

By signing below I acknowledge that I have carefully read this form, and have had this research and the terms used in this Consent Form and their significance explained to me. I will receive a copy of this consent document for my files.

_____ _____

Name (**Printed**) Date

_____

Signature

_____

Address of the subject

My signature as **witness** certifies that the subject signed this consent form in my presence as his//her voluntary act and deed.

_____

Name of Witness (**Printed**)

_____ _____

Signature of Witness Date (same as above)

A witness has certified this consent form ☐ **Yes** ☐ **No**

CALIFORNIA STATE UNIVERSITY, FULLERTON

AUTORIZACION NOTIFICADA

"Programa Familiar de Ejercicio para Niños y Adolecentes con Síndrome de Prader-Willi: Decisiones de los Padres en tener a sus Hijos involucrados en Actividad Física"

Invitación a Participar
He sido invitado a participar en un estudio que investiga las decisiones de los padres en tener a sus hijos con el Síndrome de Prader-Willi (SPW) (de 8 a 16 años de edad) involucrados en actividad física (AF). Se ha mostrado que el envolvimiento de los padres es muy importante en la prevención y el tratamiento de la obesidad infantil en niños sin el SPW. En los niños y adolescentes, los padres son los mediadores primarios de cambio al adoptar un estilo de vida saludable y reducir el comportamiento sedentario. Un total de 20 padres de jóvenes con el SPW (13 en la Universidad Estatal de California, Fullerton [CSUF, por sus siglas en Ingles] y 7 en el campus de la Universidad de Florida, Gainesville) serán invitados al azar para participar en el programa de decisiones reportadas además de participar en Juegos Activos en el Hogar, el programa de actividad física. Padres de jóvenes participando en el programa de AF que no serán invitados al azar para este estudio formaran parte del grupo de control. Este estudio de investigación será conducido en CSUF por los Drs. Jie Wiess, Daniela Rubin, Debra Rose y Lenny Wiersma.

Descripción de Procedimientos
Si decido ser parte de este estudio de investigación, participare en el programa de decisiones reportadas por 24 semanas sobre cómo ayudar a mi hijo/a con SPW ser más activo/a físicamente. Estoy de acuerdo en visitar el campus de CSUF cuatro veces durante el estudio de investigación: 2 visitas antes del programa (1 semana entre cada visita), otra a la doceava semana después del comienzo del programa y otra al final del programa (semana 24). Cada visita durara 2 horas. Las primeras dos visitas serán orientaciones con una semana entre cada visita.

En mi primera visita, el equipo de investigación me prestara un Asistente Personal Digital/Electrónico, una computadora portátil (APD o en Ingles, Personal Digital Assistant [PDA]) y me enseñaran a usarlo. También, el equipo de investigación me dará dos tarjetas de memoria para grabar mis respuestas en el APD (una ya estará en el APD). Se me pedirá responder preguntas cada otro día/semana en el APD por un periodo de 24 semanas. El equipo de investigación pondrá alarmas para que suenen en el APD cada otro día para recordarme que necesito responder las preguntas y recordarme en involucrar a mi hijo/a(s) en AF. Durante mi segunda visita a CSUF (una semana después de la primera visita), traeré el APD para cualquier solución de problemas que necesite con cualquier pregunta que tenga.

Para obtener la seguridad de los datos coleccionados del APD y para minimizar las visitas al campus para bajar la información, se me proveerá una tarjeta de memoria adicional durante mi primera y tercera visita. Al final de la semana seis y la semana 18, yo intercambiare las tarjetas de memoria y mandare a CSUF en un sobre (proporcionado con estampilla por CSUF) las tarjetas de memoria usadas de las semanas 1-6 y 13-18, respectivamente. Durante la 3ra y 4ta visita a CSUF (la 12va y 24ta semana de la intervención) yo traeré el APD para que el equipo de investigación bajen mis datos y responderé unas preguntas de evaluación. En la última visita (semana 24) yo regresare el APD (y sus accesorios) al equipo de investigación y recibiré el último incentivo.
Los padres que participan en el grupo de control de dicho programa no recibirán un APD.

Riesgos Potenciales e Inconvenientes
La participación en este estudio tomara alrededor de 5-10 minutos de mi tiempo cada día para contestar las preguntas en el APD tres días a la semana por 24 semanas. No se conoce algún riesgo asociado a mi participación en esta investigación además de los que vienen día en día. También, si algunas de las preguntas se me parecen muy personales, puedo decidir no contestar cualquier pregunta que me siento incómodo respondiendo.

Participación Voluntaria
Participación en este estudio es voluntaria. Yo puedo negar mi participación, o retirarme a cualquier momento sin penalización.

Compensación por Participar
Por mi participación en este estudio de 24 semanas, recibiré en el correo cada seis semanas una tarjeta de regalo con un valor de $60 lo cual acumulara a un total de $240. Por cada periodo de seis semanas, necesitare completar por lo menos 80% de las respuestas en los reportes del APD (cada otra día por semana) sobre mi proceso de decisión en apoyar a mi hijo/a en hacer AF, y regresar por correo (en el sobre con estampilla proporcionado por CSUF) las tarjetas de memoria por las semanas 1-6 y 13-18 inmediatamente después de completar cada periodo de seis semanas. Para las semanas 7-12 y 19-24, mis datos serán bajados por el equipo de investigación durante la tercera y cuarta visita, respectivamente. Aproximadamente dos semanas después de la última visita a CSUF, recibiré una tarjeta de regalo adicional con un valor de $50 por correo si completo el 80% de las respuestas en el APD durante todo el programa (las 24 semanas) además de regresar el APD sin algún daño. Tendré buen cuidado del APD. No hay otra compensación disponible por participar en este estudio. Los padres del grupo de control no recibirán ningún incentivo adicional a los que recibirán sus niños por su participación en el programa de actividad física.

Cuidado médico por lesiones relacionadas con esta investigación
Si usted se lastima o se enferma por estar en el estudio, puede recibir cuidado médico en un hospital o clínica del Ejército sin cargo. El tratamiento solo cubrirá lesiones directamente causadas por el estudio de investigación. El Ejército no pagara por transporte al o del el hospital o clínica. Si Ud. tiene preguntas sobre el cuidado médico, por favor contacte a la Dra. Daniela Rubin (investigadora principal para este estudio) al teléfono 657-278-4704. Si Ud. paga de su bolsillo por gastos médicos por lesiones causadas en este estudio en otro lugar, contacte a la investigadora principal. Si este problema no se resuelve, contacte al personal legal de U.S. Army Medical Research and Materiel Command (USAMRMC), al número (301) 619-7663/2221. No hay otra compensación médica para las lesiones relacionadas con esta investigación al participar en este estudio.

Confidencialidad
Se mantendrá estricta confidencialidad de mis records en la medida en que lo requiere la ley. Todos mis datos serán guardados en un archivo electrónico que estará codificado en una computadora designada con acceso bajo un código protegido dentro de una oficina de investigación cerrada con llave (en el edifico de Kinesiología y Ciencias de Salud en la Universidad del Estado de California, Fullerton, oficina #236). Los datos coleccionados del APD (Asistente Personal Digital/Electrónico, la computadora portátil) también serán guardados en una computadora con acceso bajo un código protegido en la oficina de investigación lo cual solo el equipo de investigación tendrá acceso. En la base de datos, los participantes no serán identificados por sus nombres o domicilios si no con un numero de código ID. La lista que conecta los participantes a los números de ID serán guardados en un archivo por separado con un código diferente.

Solo el equipo de investigación tendrá acceso a los datos. Tambien los representantes del comité de protección de los derechos de los participantes (IRB, por sus siglas en Ingles) o el Ejercito de los Estados Unidos (US Army Medical Research and Material Command) pueden revisar los datos como parte de su responsabilidad de proteger a las personas que participan en estudios de investigación. Toda la información acerca de este estudio se presentara (por ejemplo, publicaciones o presentaciones) en forma global y su nombre nunca será usado. Una vez que se complete el estudio, todos los registros de investigación se destruirán después de tres años de la publicación. Entiendo que cualquier información que resulte de este estudio que me identifique personalmente no será liberada voluntariamente ni se revelara sin mi autorización, excepto en la medida que lo requiera la ley.

He leído cuidadosamente este formulario y estoy completamente capacitado/a para firmar esta Autorización Notificada. Entiendo que esta forma con mi firma será guardado por los investigadores en un archivo con llave por tres (3) años; al final de los tres años, esta forma de notificación será destruida. También se guardara en un archivo con llave todo papel y datos coleccionados en lápiz por tres años después de la finalización del estudio, y al cumplir los tres años serán destruidos.

Si tengo cualquier pregunta sobre el estudio o deseo reportar algún problema relacionado al estudio, puedo comunicarme con la Dra. Weiss al (657) 278-4388. Para preguntas sobre mis derechos como participante del estudio, puedo comunicarme con el Coordinador de Acatamiento de las Regulaciones de la Universidad del Estado de California, Fullerton al (657) 278-7640.

Al firmar este documento, estoy indicando que he leído cuidadosamente este formulario y que se me ha explicado este estudio de investigación, los términos empleados en esta autorización notificada y su significado. También recibiré una copia de este documento de autorización para guardar en mis archivos.

_____ _____

Nombre (**En letra**) Fecha

_____

Firma

_____

Domicilio del Participante

Mi firma como **testigo** certifica que el participante firmo esta autorizacion notificada en mi presencia en un acto voluntario.

_____

Nombre del Testigo (**En letra**)

_____ _____

Firma del Testigo Fecha (la misma de arriba)

Un testigo certifico esta autorización notificada ☐ **Si** ☐ **No**