RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Nutritional Phases in Prader–Willi Syndrome

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Received 11 September 2010; Accepted 25 January 2011

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:
Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold J-A, Kimonis V, Dykens E, Butler MG, Shuster JJ, Driscoll DJ. 2011. Nutritional phases in Prader–Willi syndrome.
Am J Med Genet Part A 9999:1–10.

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.

Grant sponsor: NIH; Grant numbers: HD061222, RR019478, 1K24 HD01361, 1K23 DK081203; Grant sponsor: Department of Defense; Grant number: W81XWH-08-1-0025; Grant sponsor: National Center For Research Resources; Grant number: K30RR022258; Grant sponsor: National Institutes of Health Clinical and Translational Science Award, National Center for Research Resources; Grant number: 1UL1RR029890; Grant sponsor: Hayward Foundation.

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Published online 00 Month 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.33951

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 ≥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 ± 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 ± 3.5 (n = 16)	38.1 ± 3.3 (n = 28) All PWS 38.2 ± 3.0	38.1 ± 2.8 (n = 28)	39.2 ± 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 ± 0.56 (n = 16)	2.9 ± 0.62 (n = 28)	2.7 ± 0.51 (n = 28)	3.46± 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 ± 3.98 (n = 14)	50.2 ± 3.94 (n = 22)	48.7 ± 3.0 (n = 24)	51.6 ± 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 ± 1.65 (n = 14)	11.5 ± 1.53 (n = 22)	11.2 ± 1.8 (n = 24)	13.5 ± 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 ± 5.4		35.4 ± 5.0	31.2 ± 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.

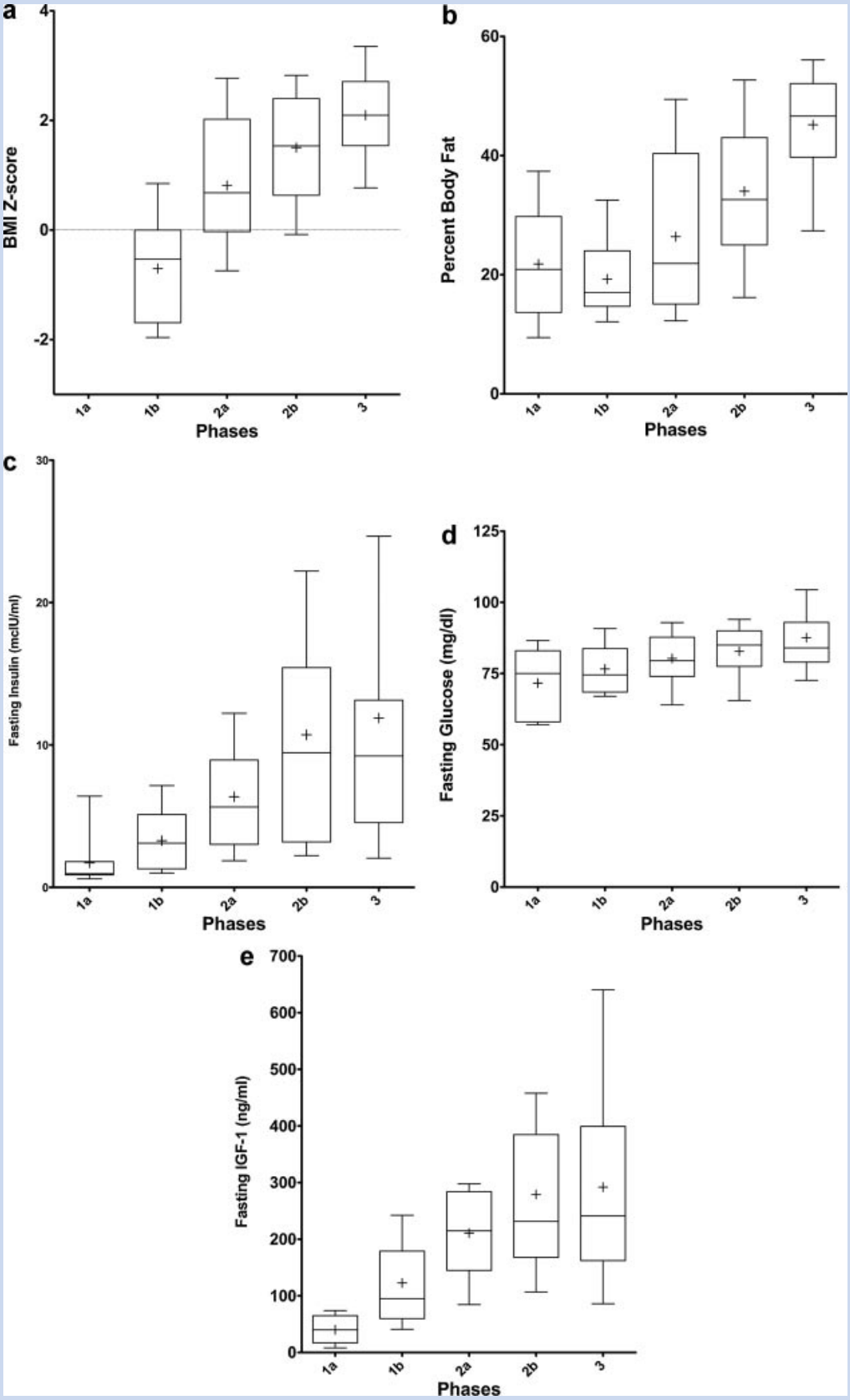

FIG. 1. All figures contain information presented as boxplots. The bottom of the box indicates the 25th centile, the line within the box indicates the median, the cross within the box indicates the mean, and the top of the box indicates the 75th centile. The whiskers above and below the box indicate 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.

ACKNOWLEDGMENTS

The authors would like to thank Soo Kim, M.D. for her assistance with the MS-MLPA assays; Fred Kweh for assistance with the figures, and Douglas Theriaque, M.S. for database support. The authors acknowledge the gracious participation and provision of information provided by the families in the Rare Disease Natural History Study for which Dr. Mary Lou Oster-Granite, Health Scientist Administrator at NICHD, provided invaluable guidance, support, and encouragement. Funding support provided by: NIH U54 grants HD061222 and RR019478 (DJD, JLM, JAG, VK, ED and MGB); Department of Defense W81XWH-08-1-0025 (DJD and JLM); National Institutes of Health 1K24 HD01361 (DJD); NIH 1K23 DK081203 (JLM); K30RR022258 from the National Center For Research Resources (JLM); National Institutes of Health Clinical and Translational Science Award, National Center for Research Resources 1UL1RR029890 (JJS); and the Hayward Foundation (DJD). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Department of Defense.

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