Carnitine and Coenzyme Q10 Levels in Individuals with Prader-Willi Syndrome

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Abstract

Background—Carnitine deficiency or coenzyme Q10 (CoQ10) deficiency may present with hypotonia, poor growth, easy fatigability, and apnea. This constellation of findings can also be seen in individuals with Prader-Willi syndrome (PWS). Animal studies indicate that increased fat mass due to obesity negatively correlates with both carnitine and CoQ10 levels in skeletal muscle. Increased body fat and obesity are characteristic of individuals with PWS. Currently there is no documentation of serum carnitine levels, and only one study investigating plasma CoQ10 levels, in individuals with PWS.

Methods—Fasting serum carnitine and plasma CoQ10 levels were measured in 40 individuals with molecularly confirmed PWS (ages 1–27 years; 19 F/21M), 11 individuals with early-onset morbid obesity of unknown etiology (ages 3–13 years; 5F/6M), and 35 control siblings from both groups (ages 1–24 years; 19F/16M).

Results—There were no significant differences among the 3 groups in either total carnitine, free carnitine, or CoQ10 levels. However, individuals with PWS had higher serum levels of carnitine esters (p=0.013) and higher ester-to-free carnitine ratios (p=0.0096) than controls suggesting a possible underlying impairment of peripheral carnitine utilization and mitochondrial energy metabolism in some individuals with PWS.

Conclusions—Serum sampling identified no significant differences in total and free carnitine or CoQ10 levels between individuals with PWS, obese individuals, and sibling control groups. Muscle biopsy or measurement in leukocytes or cultured skin fibroblasts could be a better method to identify abnormalities in carnitine and CoQ10 metabolism in individuals with PWS than peripheral blood sampling.

Introduction

Prader-Willi syndrome (PWS) is characterized by hypotonia and failure to thrive in infancy followed by weight gain and an increased appetite, obesity, multiple endocrinopathies, and developmental delay with cognitive impairment [Goldstone, 2004]. PWS is an imprinted condition with approximately 70% due to a *de novo* deletion in the paternally inherited chromosome 15 q11–q13 region, 25% from a maternal uniparental disomy of chromosome 15, and the remaining 5% from either microdeletions or epimutations of the imprinting center in the 15q11–q13 region (i.e. imprinting defects) [Goldstone, 2004; Cassidy and

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Driscoll, 2009; Bittel and Butler, 2005]. Although none of the genes involved in PWS are known to be associated with mitochondrial function or cellular metabolism, the similarities between individuals with PWS and those with mitochondrial myopathy (e.g. apnea, hypotonia and cognitive impairment) has raised suspicions that there may be a relationship between these conditions. Thus, the suggestion that there may be benefit from supplementing individuals with PWS with carnitine or coenzyme Q10 (CoQ10) has been raised [Butler et al, 2003; Eiholzer et al, 2008].

Carnitine is a natural antioxidant that improves cellular energy metabolism. Hypocarnitinemia may occur due to metabolic diseases or can be due to inadequate nutritional intake of carnitine-containing foods [Stephnes et al, 2007]. Carnitine supplementation has not been studied in individuals with PWS, but in other conditions has been shown to improve hypotonia, ataxia, activity levels, and alertness[6]. Infants and young children with Down syndrome, who have muscle hypotonia and delayed growth similar to that seen in individuals with PWS, have significantly lower carnitine levels than unaffected children of the same age, and supplementation with L-carnitine results in significant increases in visual memory and attention in this population [Seven et al, 2001]. Data from studies of rats with obesity show that lifelong obesity, such as is seen in individuals with PWS, corresponds with increased skeletal muscle accumulation of acylcarnitine esters and diminished expression of carnitine biosynthetic genes, resulting in diminished carnitine reserves in musculoskeletal tissue and perturbations in fatty acid oxidation, beta-oxidation, and impaired substrate switching from fatty acids to pyruvate [Noland et al, 2009]. The combination of lifelong obesity associated with this syndrome and the restricted calorie diet that individuals with PWS are typically prescribed to either prevent or reverse the obesity suggests that these individuals may be at higher risk for carnitine deficiency than the general population.

CoQ10 is an essential component of the mitochondrial respiratory chain and a scavenger of reactive oxygen species, which is deficient in some individuals with reduced energy expenditure and cardiac or skeletal muscle dysfunction [Butler et al, 2003; Littarru and Tiano, 2005]. Individuals with idiopathic CoQ10 deficiency have reduced skeletal and cardiac muscle function, as is seen in individuals with PWS, suggesting that patients with PWS may have an underlying deficiency of CoQ10. It is frequently used by parents of infants with PWS, with anecdotal data that it improves the energy level in some of these infants. One study measured plasma CoQ10 levels in individuals with PWS and showed that reduced levels were associated with obesity, regardless of etiology, and that there were no significant differences between individuals with PWS and obese controls [Butler et al, 2003]. A study investigating the effects of supplementation with CoQ10 in young children with PWS found that growth hormone therapy and CoQ10 supplementation had equal effects on psychomotor development of the infants [Eiholzer et al, 2008].

To date, there has been only one study documenting CoQ10 levels in individuals with PWS, but no studies that have assessed carnitine levels in this condition [Butler et al, 2003]. Given the similarities in clinical phenotype between individuals with PWS and those with mitochondrial myopathies, our hypothesis was that individuals with PWS would have lower total and free serum carnitine levels than either obese controls or their normal weight sibling controls, and that they would have lower plasma CoQ10 levels than sibling controls. Based on the previous study investigating CoQ10 levels in individuals with PWS, we hypothesized that there would be no significant difference in CoQ10 levels as compared to the obese control group. We prospectively measured fasting serum carnitine profiles (total, free, and esterified carnitine) and plasma CoQ10 levels in a group of patients who were enrolling in a family-based natural history study of PWS and early-onset obesity.

Methods

Individuals participating in this study were a subset from a larger study investigating the natural history of PWS and other non-PWS individuals with early-onset morbid obesity (EMO) who were in good health at the time of testing (Table I). We measured fasting serum carnitine levels (total, free, and esters) in 40 individuals with molecularly confirmed PWS (ages 1–27 years; 19 females and 21males), 11 individuals with EMO of unknown etiology (ages 3–13 years; 5 females and 6 males), and 35 normal weight control siblings from both groups (ages 1–24 years; 19 females and 16 males). Twenty-three of the individuals had PWS due to paternal deletion of 15q11–q13 (PWS-D) and 17 had PWS due to maternal uniparental disomy (PWS-UPD) of chromosome 15. Fasting plasma CoQ10 levels were measured in 21 individuals with PWS, 21 normal weight sibling controls, and 8 individuals with EMO. This study was approved by the University of Florida IRB and all of the parents signed informed consent.

Sample Analysis

All samples were sent to ARUP laboratories (Salt Lake City, UT) according to their specifications. Carnitine profiles were analyzed using tandem mass spectrometry and CoQ10 levels were measured using high performance liquid chromatography. Carnitine and CoQ10 deficiency were defined as values below the reference ranges for age.

Statistical Methods

Carnitine and CoQ10 levels were compared amongst the three groups using a one-way analysis of variance. Two-sample, two-sided t-tests are only reported if the three-way comparisons were significant. Significance was defined as $p < 0.05$ for all measures.

Results

There were no significant differences in either total or free serum carnitine or plasma CoQ10 levels among the groups (Table II). Individuals with PWS had higher serum levels of esterified carnitine as compared to the control sibling group ($p=0.013$) but there were no differences between those with PWS and the EMO group ($p=0.25$) or between the control group and the individuals with EMO ($p=0.52$; Table II). The individuals with PWS also had significantly higher esterified-to-free carnitine ratios compared to the control sibling group ($p=0.0096$). Due to the small sample size of children with EMO, the difference in esterified-to-free carnitine ratios was not statistically significant between this group and those with PWS ($p=0.084$; Table II). There was no difference in the ratio of esterified-to-free carnitine between subjects with EMO and controls ($p=0.97$; Table II). No significant differences were found between individuals with PWS-D or PWS-UPD ($p=0.7$) for either serum carnitine levels or plasma CoQ10 levels.

Within the group of individuals with PWS, total carnitine deficiency was seen in 15% (5/40) and free carnitine deficiency was found in 17.5% (7/40). Total and free carnitine deficiency were only found in one individual with EMO (9%; 1/11). However, interestingly, we found low levels of total carnitine in 23% of the sibling controls (8/35), with low free carnitine levels in 17% (6/35). Amongst the sibling controls who had evidence of carnitine deficiency or defects in carnitine metabolism, 87.5% (7/27) were siblings of individuals with PWS rather than EMO (1/8). Two individuals from the PWS group had both a low free carnitine level (18 and 12 umol/L, respectively) and a high carnitine ester-to-free ratio (1.4 and 2.8, respectively). Both of these children had an abnormal acylcarnitine profile, which demonstrated an increased concentration of long-chain acylcarnitines – both saturated and unsaturated (C14, C14:1, C16, C18, C18:1, and C18:2) (Table III).

Although there were no significant differences in serum carnitine levels between individuals with PWS-D and PWS-UPD, there were differences in the prevalence of carnitine deficiency between the groups. Nine percent of individuals with PWS-D had total and free carnitine deficiency, whereas 18% in the PWS-UPD group had total carnitine deficiency and 35% had free carnitine deficiency.

Surprisingly, none of the individuals in this study had low plasma CoQ10 levels. Mean CoQ10 levels were nearly identical between PWS and controls (0.77 ± 0.2 and 0.78 ± 0.3, respectively) and not significantly different than EMO (mean 0.67 ± 0.2; p=0.6) (Table IV).

Discussion

Individuals with deficiencies of either carnitine or CoQ10 have hypotonia and reduced energy expenditure, in addition to other metabolic abnormalities [Stephens et al, 2007; Littarru and Tiano, 2005]. Individuals with PWS have long been known to have significant hypotonia and reduced energy expenditure compared to age-, gender-, and weight-matched controls, which suggests impaired energy metabolism is a part of the syndrome. Measurement and replacement of carnitine and CoQ10 may be of benefit to these patients. In this study, we found no significant differences in serum total and free carnitine or plasma CoQ10 levels between individuals with PWS and either obese controls or normal-weight sibling controls. We found that, as a group, those with PWS had significantly higher esterified-to-free carnitine ratios than either individuals with EMO or normal-weight controls. This finding suggests that PWS may be associated with a defect in fatty acid metabolism in some individuals.

An elevated esterified-to-free carnitine ratio occurs when mitochondrial energy metabolism is impaired, resulting in an increased load of short chain organic acids esterified to CoA, which are transesterified to carnitine for export from muscle tissue into the circulation [Calvani et al, 2000]. While the overall carnitine ester-to-free ratio was higher in PWS as compared to the EMO and sibling control groups, only 2 individuals with PWS had an abnormal ratio that was above the normal reference range for their age in addition to having a low free carnitine level. In addition, these same 2 individuals had very similar elevations on their acylcarnitine profiles. These findings suggest that there may be a small subset of individuals with PWS (approximately 5% based on our results from this cohort) who have a true secondary carnitine deficiency, while other individuals with this syndrome may have a functional carnitine insufficiency due to either impaired carnitine metabolism or insufficient intake of foods containing carnitine. Thus, a subset of individuals with PWS may benefit from carnitine supplementation. We found a surprising number of siblings of individuals with PWS (7/27) had low serum carnitine levels, but only 1 sibling of an individual with EMO had similarly low levels. We hypothesize that caloric intake (and thus total nutrient intake) may be reduced for the entire family, in order for the reduced-calorie diet typically prescribed for individuals with PWS to be accepted by the affected child.

Anecdotal reports suggest benefit of CoQ10 supplementation in individuals with disorders of energy metabolism, such as is seen in PWS. The only study measuring CoQ10 levels in individuals with PWS done prior to this study found differences in CoQ10 levels between those with PWS and normal-weight controls. We were not able to reproduce this result, as there were no individuals in this study with low CoQ10 levels, and we found no significant difference in CoQ10 levels amongst the 3 comparison groups. The different results between these 2 studies may be indicative of the unreliability of plasma measurements of CoQ10 [Battino et al, 2001]. Additionally, plasma measurements of CoQ10 may underestimate the prevalence of low CoQ10 levels in the muscle, thus indicating that blood sampling for CoQ10 levels is not the best method for identifying deficiencies in this syndrome [Duncan et

al, 2005]. We have seen individuals with PWS in the clinic setting with documented low serum levels of CoQ10 who had clinical improvement with treatment, as well as others who have not benefitted from this therapy, as assessed by parental questionnaires assessing the effectiveness of supplements [PWSA, unpublished data].

Supplementation with carnitine or CoQ10 in individuals with PWS has potential to benefit. Following the measurements of serum levels of carnitine and CoQ10 20 families elected to try carnitine supplementation (50 mg/kg/day divided twice a day) regardless of the serum carnitine profile results. Thirteen of these twenty families (65%) reported subjective improvement of exercise tolerance and daytime alertness with carnitine supplementation. Three of these families provided independent documentation from physical therapists, who were unaware of treatment, reporting improvements in exercise tolerance after the children began carnitine supplementation. However, 7 of the 20 families who tried carnitine supplementation discontinued it due to lack of benefit and/or side effects of therapy. Parental reports on both of the children with an abnormal acylcarnitine profile who were treated with carnitine therapy indicated an improvement in exercise tolerance. As this data was subjective, no attempts to objectify this data were made. However, serum carnitine levels and acylcarnitine profiles were repeated while the children were receiving supplementation, and they had normalized. Ten families started CoQ10 supplementation (50 mg/day) and 5 (50%) of these families reported benefits in daytime alertness with this therapy. All of the individuals who were reported to benefit from CoQ10 were under 3 years of age, so it is difficult to know whether the improvements in alertness were due to the CoQ10 or would have occurred as part of the natural history of PWS.

Most individuals get adequate amounts of carnitine and CoQ10 from their diets, but because individuals with PWS are often on extremely restricted diets to avoid obesity, they may have dietary deficiencies of both carnitine and CoQ10. Carnitine is most prevalent in animal products, such as meat, fish, and dairy products, but it is rare in fruits and vegetables. Due to concerns about obesity the diets of individuals with PWS are typically rich in fruit and vegetables while often being low in dairy products and red meats. It is interesting to note that 87.5% of the control siblings who had low total and free carnitine were siblings of the individuals with PWS rather than EMO. This suggests that perhaps the entire family is on a restricted diet for the child with PWS and, thus, may all have a need for additional carnitine. Although individuals with EMO should also be on a restricted calorie diet to counteract obesity, because these children did not have a diagnosis, none of the families of the children with EMO were utilizing dietary restriction prior to participating in our study.

Measurements of carnitine and CoQ10 from muscle biopsy, leukocytes, or cultured fibroblasts are considered a more reliable assessment of stores than measurement of serum or plasma levels, as the pools of carnitine and CoQ10 are different in the erythrocyte than in the muscle [Duncan et al, 2005; Kohlstadt, 2009]. To date, no studies have reported results of muscle biopsies for carnitine or CoQ10 deficiency in individuals with PWS. Ours is the first study to evaluate serum carnitine profiles in individuals with PWS. This study is limited by its cross-sectional nature, but the results are intriguing, and suggest that a small percentage of individuals with PWS may have an underlying disorder of carnitine metabolism. Many parents have independently started their young children with PWS on these supplements. Questionnaires sent to parents by the Prader-Willi Association of the United States indicate that some children experience a subjective improvement in exercise tolerance and daytime alertness with these supplements [PWSA, unpublished data]. Clearly further research is warranted with respect to rigorous clinical trials of these supplements in PWS, as well as elucidation of the etiology in the subset that have a true secondary carnitine deficiency due to dietary management.

Acknowledgments

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Table I

Characteristics of Subjects

Group	Mean Age	P value (3 way)	Gender	BMI SDS	P value (3 way)
PWS	8.19 (± 7.57)	0.47	19 F/21 M	1.45 (± 1.31)	0.19
EMO	7.91 (± 4.44)		5 F/6 M	2.92 (± 0.84)	
Normal weight controls	9.31 (± 6.22)		19 F/16 M	0.48(± 0.91)	

Table II

Carnitine Profiles in Individuals with PWS, EMO, and sibling controls

	N	Total Carnitine (NL range: 35–90 umol/L)		Free Carnitine (NL range: 25–55 umol/L)		Carnitine Esters (NL range: 4–36 umol/L)		Ester/free Ratio (NL range: 0.1–1.0)	
p-value (3-way)		0.44		0.084		0.043*		0.02*	
		Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
PWS	40	43.4	9.8	29.7	9.3	13.7	6.7	0.52	0.45
Normal weight Controls	35	42.6	8.1	32.8	6.9	9.7	5.6	0.31	0.20
EMO	11	46.9	12.1	35.6	7.9	11.2	6.5	0.31	0.15

Pairwise P-values for carnitine esters: PWS vs. controls: $p = 0.013$ PWS vs. EMO: $p = 0.25$ Controls vs. EMO: $p = 0.52$

Pairwise P-values for esterified to free carnitine ratio: PWS vs. controls: $p = 0.0096$ PWS vs. EMO: $p = 0.081$ Controls vs. EMO: $p = 0.97$

Table III

Abnormal Acylcarnitine Profiles for Two Children with PWS

Acylcarnitine Profile	Child #1	Child #2	Reference Range
C2, Acetyl	11.23	14.32	3.69–24.71 mol/L
C3, Propionyl	0.84	0.31	0.00–0.97 mol/L
C4, Iso-/Butyryl	0.18	0.13	0.00–0.50 mol/L
C5, Isovaleryl/2Mebuty	0.31*H	0.05	0.00–0.28 mol/L
C6, Hexanoyl	0.06	0.05	0.00–0.12 mol/L
C8:1, Octenoyl	0.13	0.16	0.00–0.63 mol/L
C8, Octanoyl	0.05	0.10	0.00–0.23 mol/L
C10:1, Decenoyl	0.14	0.27	0.00–0.41 mol/L
C10, Decanoyl	0.15	0.30	0.00–0.35 mol/L
C5-DC, Glutaryl	0.04	0.04	0.00–0.07 mol/L
C12:1, Dodecenoyl	0.03	0.04	0.00–0.16 mol/L
C12, Dodecanoyl	0.09	0.09	0.00–0.12 mol/L
C12-OH, 3-OH-Dodecanoy	0.00	0.01	0.00–0.02 mol/L
C14:2, Tetradecadienoy	**0.29*H**	**0.27*H**	**0.00–0.12 mol/L**
C14:1, Tetradecenoyl	**0.45*H**	**0.50*H**	**0.00–0.23 mol/L**
C14, Tetradecanoyl	**0.15*H**	**0.15*H**	**0.00–0.07 mol/L**
C14:1-OH, 3-OH-Tetrade	0.02	0.02	0.00–0.03 mol/L
C14-OH, 3-OH-Tetradeca	0.02	0.02	0.00–0.02 mol/L
C16:1,Palmitolevl	0.05	0.05	0.00–0.05 mol/L
C16, Palmitoyl	**0.22*H**	**0.18*H**	**0.00–0.10 mol/L**
C16:1-OH, 3-OH-Palmito	0.02*H	0.01	0.00–0.01 mol/L
C16-OH, 3-OH-Palmitoyl	0.02*H	0.01	0.00–0.01 mol/L
C18:2, Linoleyl	**0.24*H**	**0.20*H**	**0.00–0.08 mol/L**
C18:1,Oleyl	**0.33*H**	**0.36*H**	**0.00–0.16 mol/L**
C18, Stearoyl	**0.07*H**	**0.08*H**	**0.00–0.05 mol/L**
C18:2-OH, 3-OH-Linoley	0.01	0.01	0.00–0.01 mol/L

Acylcarnitine Profile	Child #1	Child #2	Reference Range
C18:1-OH, 3-OH-Oleyl	0.01	0.01	0.00–0.01 mol/L
C18-OH, 3-OH-Stearoyl	0.00	0.00	0.00–0.01 mol/L

Table IV

Coenzyme Q10 in Individuals with PWS, EMO, and sibling controls

	N	Coenzyme Q10 levels (NL range 0.4–1.6 mg/L)
p-value (3-way)	50	0.61
PWS	21	0.77 ± 0.2
Normal weight Controls	21	0.78 ± 0.3
EMO	8	0.67 ± 0.2