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Energy Balance in Congenital Generalized Lipodystrophy Type I

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Abstract

INTRODUCTION—Congenital generalized lipodystrophy type one (CGL-1) is characterized by an absence of adipose tissue and decreased serum leptin levels. Low leptin levels in CGL-1 support the claim that these subjects are hypermetabolic and hyperphagic. The present study examines this claim.

DESIGN—We determined 24-hour energy expenditure (24-hr EE) (kcal) (n = 2) and RMR per kg of lean body mass (LBM) (n = 3) in CGL-1 and in 18 healthy control subjects. The 24-hr EE of control and subjects with CGL were compared with respect to kcal required/day relative to kg of LBM and with respect to RMR relative to kg of LBM. Fasting leptin, adiponectin, and 24-hour ghrelin levels were also measured in subjects with CGL-1.

RESULTS—The 24-hr EE per kg of LBM for the subjects with CGL-1 falls on the same regression line observed for this relationship in the controls. The RMR per kg of LBM in subjects with CGL-1 also was similar to controls. Both 24-hr EE and RMR were quite increased when reported per kg of total body weight. Subjects with CGL-1 also have decreased fasting leptin and adiponectin hormone levels and no pre-meal ghrelin rise.

CONCLUSION—People with CGL-1 have similar RMR and daily caloric requirements as healthy controls when these parameters are expressed as a function of LBM. Appetite regulating hormone levels in CGL-1 suggest multiple factors act to control appetite in these individuals.

Keywords

energy expenditure; energy intake; resting metabolic rate; basal metabolic rate; caloric intake; leptin; ghrelin; Bernardinelli-Seip Syndrome; Brunzell Syndrome (39)

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INTRODUCTION

Congenital generalized lipodystrophy is an autosomal recessive disorder characterized by absence of adipose tissue and leptin deficiency. Berardinelli first described CGL in 1954 in two children with hepatomegaly, marked muscular development, and milky serum [1]. In 1968, we reported a variant form of CGL in an African-American family with a similar phenotype that, in addition, featured cystic bone lesions [2]. More than thirty years later, the CGL variant associated with bone disease was classified as CGL-1, resulting from mutations in the gene for AGPAT2 (1-acylglycerol-3-phosphate O- acyltransferase 2), located at 9q34 [3–5]. AGPAT2, also known as LPAAT β gene (lysophosphatidic acid acyltransferase β), catalyzes the formation of phosphatidic acid, a critical component of phospholipids and triglyceride synthesis. The CGL variant initially described by Berardinelli [1] and later by Seip [6] is consistent with characteristics seen in CGL type two (CGL-2). CGL-2 results from mutations in the gene for seipin (at 11q13) which encodes an integral membrane protein of the endoplasmic reticulum [7, 8].

Both CGL variants have been reported to be associated with increased energy intake and increased RMR with respect to total body weight (TBW) [9]. The metabolic demands of individuals with congenital leptin deficiency [10] are consistent with the continuous pattern of food intake [1, 11–16] and low serum leptin levels reported in people with CGL [17]. Energy requirements are predominantly determined by lean body mass (LBM) since triglyceride stores in adipose tissue are metabolically inactive. Because adipose tissue is effectively absent in CGL, LBM accounts for a higher proportion of the TBW in these individuals. Therefore, energy expenditure in CGL reported as a function of TBW [9] may have been previously overestimated. To determine energy requirements in CGL-1, we compared the daily caloric intake and RMR (as a function of LBM) in CGL-1 and healthy control subjects.

RESEARCH DESIGN AND METHODS

Subjects

Eighteen weight stable control subjects (10 males and 8 females) and 3 subjects with CGL-1 were examined. The study was approved by the Human Subjects Institutional Review Committee of the University of Washington, and informed consent was obtained from all subjects after the nature of the procedures were explained to them. Subject #1-4, a 55 year-old man has a muscular appearance, bone cysts, acromegaloid features, type 2 diabetes mellitus, hypertension, coagulopathy, and hyperlipidemia (subject III-1 in reference 2). His 60 year-old brother (#1-1) has a history of type 2 diabetes, hypertension, a muscular appearance, and acromegaloid features (subject III-4 in reference 2). An additional CGL-1 subject, a 33 year-old African-American female (#3-1) not related to the other subjects, has type 2 diabetes mellitus, hypertension, a muscular appearance, acromegaloid features, bone cysts, history of acute pancreatitis (due to hypertriglyceridemia), and bipolar disorder. Subjects with CGL-1 were either homozygotes or compound heterozygotes for mutations in the AGPAT2 gene (Table 1).

The controls were matched to the subjects with CGL-1 by LBM. In control subjects, the measurements of LBM and daily caloric intake were performed in 10 healthy males and 8 healthy females. Studied previously, these individuals had a mean age of 44 (± 14) years, a mean LBM of 66 kg (± 17), a mean weight of 104 kg (± 20), and a mean height of 175 cm (± 10) [18].

Genotype Analysis

For exons 2–6 at 9q34, the following amplification primer pairs were designed based on the sequence of the human AGPAT2 gene in GenBank:

1. EXONS 2–4. Fragment size = 1.1kb.
 AGPAT2 I1F: 5' CAGCCTCGGCTGCGGGATCTG 3'
 AGPAT2 I4R: 5' CACCTGCTGCCTTAAGCCAGCCTC 3'
2. EXON 5. Fragment size = ~255 bp.
 AGPAT2 I4F: 5' CCCATGGCAGCAGCGAAGGC 3'
 AGPAT2 I5R: 5' CTCCGTGACTGTAGGGTCAGGC 3'
3. EXON 6. Fragment size = ~ 300 bp
 AGPAT2 I5F: 5' CGAGGCCAGGGGCAGCAGGCTG 3'
 AGPAT2 I6R: 5' CCAGCCATCGGCTTCCACCTG 3'

Fragments of exons 2–4 were initially denatured at 94°C followed by 35 cycles of denaturation at 94°C for 15 sec, annealing at 66°C for 2 min, and polymerization at 72°C for 4 min. Exons 5 and 6 fragments were initially denatured at 94°C followed by 35 cycles of denaturation at 94°C for 15 sec, annealing at 64°C for 30 sec, and polymerization at 72°C for 30 sec, followed by a final step of 4 min at 72°C.

The PCR products were gel-purified and the DNA was extracted using the Qiagen MinElute Gel Extraction kit (Cat. #28604), and both strands of DNA were directly sequenced using the Applied Biosystem Bigdye Terminator kit and protocol. The amplification primers were used in sequencing reactions.

AGPAT2 Activity

Blood was obtained after a 12 hour fast in EDTA tubes and peripheral blood mononuclear cells (PBMCs) were isolated and plated on culture dishes. AGPAT activity from PBMC cell lysates was assayed as previously described [19]. Total activity of the different AGPAT isoforms is predominantly based on AGPAT1 and AGPAT2 activity. Total AGPAT enzyme activity was obtained by incubation in the presence of DMSO (8μM); AGPAT2 activity was obtained after incubation with a specific AGPAT1 inhibitor, CT 11493 (10μM). For each patient sample, one control sample (of healthy, non-CGL middle-aged men) was processed on the same day in the same assay. All samples were assayed in duplicate.

Diets

Each control and CGL-1 subject was admitted to the General Clinical Research Center at the University of Washington Medical Center. The controls were admitted in 1988, while individuals with CGL-1 were admitted in 2004. All subjects were limited to weight maintaining liquid formula diets divided into three or five equal servings between 8AM and 8PM. No other meals were provided. In addition, subjects' physical activity levels were limited to activities of daily living and short walks. In the studies, 45–55% of the total calories was derived from carbohydrates (maltodextrose), 30–40% from fats (corn oil and butterfat), and 15% from proteins (nonfat milk). Liquid formula containers of all subjects were rinsed with water and drank to ensure an accurate measurement of daily caloric intake. Complete vitamin and iron supplements were provided.

Body weight was measured each morning on a metabolic scale immediately upon arising with the same limited clothes on each occasion.

Lean Body Mass

LBM was measured by underwater weighing with pulmonary N₂ washout or by dual-energy X-ray absorptiometry (DEXA) scan (GE Lunar Prodigy). Measurement of LBM by underwater weighing and DEXA scan are highly correlated [20, 21].

Resting Metabolic Rate

RMR was measured in the same way for all controls and CGL-1 subjects. The calculation of RMR was performed by hood indirect calorimetry in the morning as subjects awakened and before they arose from bed as previously described [18]. All hood calorimetry instruments used were calibrated for both gas sensor and total airflow.

Daily Energy Expenditure and Resting Metabolic Rate as Functions of Total Body Weight and Lean Body Mass

LBM is highly correlated with energy metabolism [22–24], and 24-hour energy expenditure is reported per unit of LBM when comparing individuals of different body compositions. As previously reported, we measured the daily caloric intake of 18 healthy individuals as a function of LBM and total body weight (Figs. 1a and 1b) [18]. Similarly, in the same cohort, the relationship between RMR as a function of LBM and total body weight was measured (Figs. 2a and 2b).

For study subjects, their LBM determined their daily energy expenditure by using the linear regression equation correlating LBM and 24-hr energy expenditure in controls (Fig. 1a). For #1-1 and #1-4, their weight was maintained for five and eight days, respectively, with a weight stability of +40 g/day mean change for #1-1 and +50 g/day mean change for #1-4. In addition, all individuals with CGL were limited only to activities of daily living. Slight adjustments of 5–10% initially were made to 24-hr caloric intake to achieve weight stability. Therefore, discounting significant caloric losses, at a steady weight the 24-hour energy expenditure is approximated by the 24-hour caloric intake over the period of weight stability for each individual with CGL (24-hr caloric intake = 24-hr energy expenditure).

Statistics

For controls, we calculated the mean and standard deviation of their age, total body weight, LBM, height, and plasma ghrelin levels. We used linear regression to determine the correlation between 24-hr energy expenditure with LBM and total body weight (Figs. 1a and 1b). We utilized the same method to determine the correlation between RMR with LBM and total body weight (Figs. 2a and 2b).

Serum Markers for Appetite and Insulin Resistance

Serum leptin and adiponectin levels were measured by Northwest Lipid Research Laboratory in Seattle. The leptin assay utilizes ¹²⁵I-labeled human leptin and a human leptin antiserum to determine the level of serum leptin levels. The lowest level of leptin that can be detected by this assay is 0.5 ng/ml when using a 100 µl sample size. Each sample is analyzed in duplicate. The adiponectin assay is based on a commercial radioimmunoassay kit and uses ¹²⁵I-labeled murine adiponectin and a multispecies anti-adiponectin serum. This is a double antibody assay using PEG aided precipitation. The assay has a sensitivity of 1 ng/ml when using a 100 µl sample size. Each sample is analyzed in duplicate. 24-hour ghrelin assays were measured as previously described [25]. Twenty-four hour ghrelin levels were determined in the CGL subjects after three weeks of meals served at the same time of the day to each subject (0800, 1200, and 1730). The mean 24-hour ghrelin levels for previously described ten weight stable control subjects (9 women and 1 man) with the same meal times (0800, 1200, and 1730) was used for comparison [25]. Glycosylated hemoglobin

levels were measured by the University of Washington Medical Center Clinical Laboratories.

RESULTS

AGPAT2 Activity and Genotypes

The AGPAT2 activity in the three CGL patients was reduced by 70–76% compared to that in normal controls (Fig. 3) [26]. There was no compensatory increase in AGPAT1 (data not shown). Subjects #1-1 and #1-4, who were compound heterozygotes for the IVS4 -2A to G and R68X mutations, have similar AGPAT2 activity. In addition, AGPAT2 activity was reduced by 70% in #3-1 who is a compound heterozygote for the IVS4 -2A to G and the novel mutation P112L.

24-Hour Energy Expenditure and Lean Body Mass

There is a higher correlation in controls between daily energy expenditure and LBM ($r^2 = 0.88$, $P < 0.001$) than between daily energy expenditure and total body weight ($r^2 = 0.77$, $P < 0.001$) (Figs. 1a and 1b). When 24-hour energy expenditure is expressed as a function of LBM, the two CGL-1 subjects (#1-1, #1-4) fall near the regression line formed by the 18 control subjects (Fig. 1a, Table 2). When the daily energy expenditure is expressed as a function of total body weight, values for the subjects with CGL-1 fall above the regression line formed by control subjects (Fig. 1b, Table 2).

Resting Metabolic Rate and Lean Body Mass

The correlation is stronger in controls between RMR and LBM ($r^2 = 0.92$, $P < 0.001$; $\text{RMR} = 20.7 (\text{LBM}) + 217$) than RMR and total body weight ($r^2 = 0.83$, $P < 0.001$) (Figs. 2a and 2b). When the RMR of three CGL-1 subjects (#1-1, #1-4, #3-1) is compared to 18 controls as a function of LBM, the values for two of the three subjects fall near the regression line formed by the control subjects (Fig. 2a, Table 2). In contrast, when the RMR is expressed as a function of total body weight, the measured values for all subjects with CGL-1 are well above the regression line formed by control subjects (Fig. 2b, Table 2).

Leptin, Ghrelin, and Adiponectin

Fasting leptin and adiponectin levels in the CGL individuals were very low (Table 1). In addition, the 24-hour profile of ghrelin in #1-1 and #1-4 were more than two standard deviations below that of the mean for previously described controls (Fig. 4) [25]. The subjects with CGL-1 also did not have the expected pre-meal spike or post-meal decline in ghrelin levels observed in controls.

DISCUSSION

Lean body mass is the most significant determinant of daily energy balance in individuals restricted to activities of daily living. In this study, there is a high correlation between LBM and daily caloric intake in control subjects. Since CGL is associated with near absence of adipose tissue, which normally is comprised mainly of metabolically inactive triglyceride mass, the energy parameters of these individuals have to be determined in terms of their relatively increased proportion of fat free mass. Therefore, to control for the different amounts of adipose tissue in control and CGL subjects, their energy requirements are corrected for LBM to facilitate accurate comparisons.

Three main reasons appear to be responsible for the hypothesis that CGL subjects are hypermetabolic and hyperphagic (i.e., ingesting an excessive number of calories). First, in the past, energy balance in subjects with CGL has been reported in terms of total body

weight, instead of lean body mass [9]. Second, due to a lack of adipose tissue and therefore an inability to store sufficient long-term energy, subjects with CGL are observed eating frequent meals. Finally, congenital lipodystrophy is associated with markedly low serum leptin levels [17], which would be predicted to lead to increased food intake [10].

Since individuals with CGL do not gain weight with their apparent hyperphagia [9], subjects with both types of CGL have been described as hypermetabolic. However, the RMR may have been overestimated in CGL-2 when reported with respect to total weight and it appears to be normal in CGL-1, in whom individual metabolic rate data have not been reported [27]. When RMR values for CGL-1 are recorded with regards to total body weight (rather than LBM), as previously reported in subjects with CGL, they are up to 95% higher than the standard values for healthy controls (Table 2). The relation of RMR with lean body mass for the controls ($\text{RMR} = 20.7(\text{LBM}) + 217$) in this study is remarkably similar to that reported by Mifflin et al. ($n = 238$ men: $\text{RMR} = 22.5(\text{LBM}) + 209$; $n = 245$ women: $\text{RMR} = 20.8(\text{LBM}) + 360$) [24]. Their data add validity to our data in a much smaller cohort for RMR. In the present study, the RMR in two of three CGL-1 subjects fell within ten percent of predicted values for control subjects with similar LBM. Determination of RMR requires overnight fasting and the only CGL-1 subject who appears to have an increased metabolic rate, #3-1, has bipolar disorder and was noncompliant with the requirement for overnight fasting.

In the past, both abnormally high and normal measurements of resting metabolism have been reported in undifferentiated CGL. Schwartz et al first reported RMR data in CGL and found them to be normal [28]. More recently, Savage et al attributed the increased RMR in a group of subjects with various lipodystrophies to their relatively higher LBM [29]. Studies by Petersen and Oral, who measured basal metabolic rate before and after leptin therapy, reported no change and a mean decrease from 1920 to 1580 kcal/day, respectively [13, 30]. Klein reported an RMR of 30% above normal in his single subject [31]. Seip reported elevated levels in four CGL subjects, with values as high as 73% above normal [9]. However, the data on hypermetabolism in these studies are either an average for a group of subjects that includes different types of lipodystrophy or are based on subjects who have characteristics associated with CGL-2. The current study reports that individuals with CGL-1 have normal metabolic rates.

Several studies describe energy use in lipodystrophy. One study found statistically insignificant increases in energy expenditure while others reported energy use in lipodystrophic subjects but did not include control values [15, 16, 31, 32]. Furthermore, these studies do not distinguish between different types of lipodystrophy [15, 16, 31, 32] and report the daily energy use as a mean value for the entire group [16, 31, 32]. The best example of this is Seip's examination of seven individuals with CGL whom he describes as hyperphagic [9]. However, in addition to not reporting energy balance in terms of LBM, daily caloric intake results are provided for only a pair of subjects. In fact, as the phenotypic heterogeneity between the two types of CGL has become more apparent [12, 33], Seip's subjects appear to have CGL-2. Still, because energy use is most accurate when reported as a function of LBM, these energy balance values are exaggerated. Three other individuals with CGL in the same study are described as having a "voracious appetite," but no caloric measurements are presented so the degree of overeating is unclear. In fact, many assertions of hyperphagia in CGL are not based on discernible measurements of energy intake by investigators, but rather by observation of continuous feeding behavior or the subjects' own perception of eating habits [1, 11–14]. Therefore the premise that CGL-1 is associated with increased daily caloric intake is based primarily on studies of different types of lipodystrophy, overestimated results, and investigator observation.

Decreased serum leptin levels are characteristic of CGL and consistent with the lack of adipose tissue. The adult subjects with CGL-1 in this study had low leptin levels, similar to those in published reports (17). However, if daily energy intake in CGL-1 is not increased in the presence of very low leptin levels, then leptin alone may not determine food-seeking behavior, food intake, and energy utilization in this setting. The role of leptin as an appetite regulator in CGL-1 may be explained by either increased leptin sensitivity or by interaction of leptin with other regulatory factors (e.g., insulin and/or ghrelin). Low serum leptin levels result in leptin hypersensitivity in congenital leptin deficiency [34] or following surgical removal of adipose tissue [35, 36]. Studies of the latter intervention showed an unchanged or decreased level appetite with liposuction. However, because of the very low levels of the serum leptin in CGL, even an exaggerated response to the hormone seems unlikely to prevent compensatory hyperphagia. In addition, if sensitivity to leptin action is increased in congenital lipodystrophy, the response would have to be tissue-specific since the gonadal response to leptin is reduced in CGL-1 [37]. Therefore, whereas the sensitivity to leptin signaling in the ovaries may exhibit no change, the hypothalamic response would have to be exaggerated. A more likely explanation for the lack of overeating in CGL-1 is that appetite relies on the interaction among multiple factors beyond just leptin. Insulin resistance in CGL-1, associated with decreased adiponectin levels in subjects of this study, may lead to hyperinsulinemia, which could counteract the appetite stimulating affect of low serum leptin levels and result in appetite suppression in the central nervous system. Like leptin, central insulin infusion decreases food intake by modulating appetite-regulatory neuropeptides in the hypothalamus and other sites in the brain. Insulin may also affect another appetite stimulant, ghrelin. Persistent hyperinsulinemia in CGL-1 may explain the lack of preprandial increases in ghrelin levels, because of insulin's known inhibitory action on ghrelin secretion (Fig. 4) [38]. The 24-hour ghrelin levels in #1-1 and #1-4 are persistently low and do not manifest the normal meal-time spikes. It is possible that without this stimulus for initiating meals, subjects with CGL-1 are inclined to graze for food, less driven to eat bolus meals at specific, habitual intervals. Following meals, the subjects do not have the expected decrease in ghrelin levels. Interestingly, the 24-hour ghrelin levels for #1-1 resemble those in gastric-bypass patients [25].

Taken together, the data suggest that all subjects with CGL-1 are not hyperphagic or hypermetabolic and also that, at the very least, past energy expenditure in CGL-2 may have been overestimated. In all, energy balance data for individuals with CGL-1 is scant. Based on our findings, even though persons with CGL-1 eat more frequently than healthy individuals do, their energy intake is spread throughout the day and they consume the same number of total calories as do individuals with a more typical, meal-dominated eating pattern.

There are limitations to this study with regards to the small sample size and the timeline within which subjects and controls were studied. Control and study subject comparisons of measured LBM and RMR were completed at different times. But the two techniques used in this study to determine LBM have a high correlation. In determining RMR, the hood calorimetric methods were very similar and the machine was calibrated in the same way. In order to more accurately determine 24-hr energy expenditure, the study could have accounted for gastrointestinal (fat) and urinary (glucose) caloric losses. However, the energy loss from these sources is minimal (unpublished data) and does not account for the discrepancy between the measurement of 24-hr energy expenditure with regards to total body weight versus LBM. In addition, potential sources of energy loss do not account for the wide discrepancy in RMR when measured with regards to total body weight instead of LBM (Table 2).

More studies are needed in both types of CGL to clarify energy parameters and metabolism. Several larger studies have characterized larger groups of subjects with each type of CGL. However, this is one of few studies designed to evaluate metabolism specifically in one type of CGL. In the least, the results in this study suggest that data on energy usage in other CGL subjects should be reexamined in order to treat these individuals more effectively.

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FIG. 1.

FIG. 1a. Lean Body Mass vs. 24-Hour Energy Expenditure

FIG. 1b. Total Body Weight vs. 24-Hour Energy Expenditure

FIG. 2.

FIG. 2a. Lean Body Mass vs. Resting Metabolic Rate

FIG. 2b. Total Body Weight vs. Resting Metabolic Rate

FIG. 3.
AGPAT2 Activity in CGL-1 (solid bars) and Control (striped bars) Subjects.

FIG. 4.
24-Hour Plasma Ghrelin Levels in Subjects with CGL-1 and Mean 24-Hour Ghrelin Levels in 10 Control Subjects with Error Bars Representing Two Standard Deviations

TABLE 1

Metabolic Measurements and AGPT2 mutations in Subjects with CGL-1

Subject	Sex	Age	% Body Fat	Weight (kg)	Height (cm)	BMI (kg/m ²)	Leptin* (ng/ml)	Adiponectin [†] (mcg/ml)	HbA1c (%)	Mutations in AGPT2
#1-1	M	60	5.6	73.0 [§]	178	23.0	0.9	0.85	11.5	IVS4-2A→G R 68X
#1-4	M	55	6.9	94.0	177	30.0	1.3	0.45	7.8	IVS4-2A→G R 68X
#3-1	F	33	4.9	73.1	162	27.9	1.2	0.20	9.5	IVS4-2A→G P112L

* Normal Values 2-5.6 ng/ml for men and 3.7-11.1 ng/ml for women

[†] Normal Values 1.5-10.3 mcg/ml

[§] Patient has left total leg amputation

TABLE 2

Predicted and Measured 24-hour Energy Expenditure and RMR in Subjects with CGL-1

SUBJECT	#1-1	#1-4	#3-1
Predicted 24-hr EE/kg of LBM	2948	3387	-
Measured 24-hr EE	2466	3170	-
% Deviation from Predicted 24-hr EE/kg of LBM	-16.4	-6.4	-
Predicted 24-hr EE/kg of Total Body Weight	2295	2682	-
% Overestimation of Predicted 24- hr EE/kg of total weight	7.5	18.2	-
SUBJECT	#1-1	#1-4	#3-1
Predicted RMR/kg of LBM	1643	2053	1656
Measured RMR/kg of LBM	1725	2218	2095
% Deviation of Measured RMR from Predicted RMR	5.0	8.0	26.5
Predicted RMR/kg of Total Body Weight	1075	1417	1077
% Overestimation of Predicted RMR/kg of Total Weight	60.5	56.5	94.5