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A case of congenital generalized lipodystrophy: metabolic effects of four dietary regimens. Lack of association of CGL with polymorphism in the lamin A/C Gene

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Sirs, Congenital generalized lipodystrophy (CGL) is an autosomal recessive disorder, characterized by severe metabolic derangement associated with the absence of subcutaneous adipose tissue. The exact genetic defect is unknown, and systematic studies of optimal dietary and pharmacological therapy for CGL have not been performed. We report a case of CGL, in which the patient's dietary/pharmacological management was optimized with the help of metabolic testing. In addition, DNA from this patient was screened for mutations in several candidate genes.

A 36-year-old female presented for treatment of diabetes mellitus uncontrolled with 0.8 units per kg of insulin per day. The diabetes was diagnosed 10 years earlier and was complicated by macroalbuminuria (533 µg/minutes), retinopathy, neuropathy, and peripheral vascular disease. Her parents were first cousins. She had generalized fat loss, prominent musculature, hepatomegaly, clitoromegaly, mild hirsutism and no acanthosis nigricans. Her height was 151 cm and weight was 49.2 kg (body mass index 21 kg/m²). Tetrapolar bioelectric impedance revealed a fat mass of 3% (1.5 kg). CT demonstrated no subcutaneous fat in the abdominal area, and visceral abdominal fat was approximately one third of that in age, height and body weight matched females. Her blood chemistry profile, pituitary, gonadal, adrenal and thyroid tests were normal. Serum leptin was 0.8 µg/l (10.8–16.6 µg/l is normal for females with BMI 21 ± 5 kg/m²). All research procedures were approved by the Institutional Review Board of the Hospital das Clinicas at the University, São Paulo, Brazil.

After the patient was hospitalized, baseline studies were performed, and four dietary regimens (Reg. 1–4, see Table 1) varying in energy and fat content, and with the addition of dL-fenfluramine with intensive insulin therapy were Instituted under carefully controlled conditions with a goal to maintain body weight and optimize glycaemic control. After each intervention, energy substrate metabolism was tested with a euglycaemic hyperinsulinaemic clamp (insulin infusion rate 10 mU × m⁻² × minutes⁻¹) and indirect calorimetry using a flow-through canopy gas analyser system in the resting state and after a glucose load (Felber *et al.*, 1987). The respiratory exchange ratios (RER) indicate (Table 1) that in all but the hypercaloric moderate-fat diet (Reg. 2), glucose was the primary fuel source after an overnight fast. This finding was supported by the low fasting free fatty acid availability in all regimens except for the hypercaloric moderate-fat regimen. The thermic effect of glucose did not change with any of the interventions, including dL-fenfluramine (Table 1). As measured by euglycaemic hyperinsulinaemic clamp exogenous glucose disposal did not

correlate with energy content in the diet. According to the clamp data (Table 1), treatment with a hypercaloric very low-fat diet (Reg. 3) and hypercaloric low-fat diet (Reg. 4) resulted in markedly greater exogenous glucose disposal than treatment with a hypercaloric moderate-fat diet (Reg. 2). However, the total glucose disposal determined by indirect calorimetry was not considerably different in the four regimens, suggesting that the disposal of glucose produced endogenously accounts for the difference between glucose disposal measured by the clamp *vs.* indirect calorimetry. Indeed, basal plasma glucose was higher after treatment with a moderate-fat diet (Reg. 2 *vs.* 3). Interestingly, with slightly higher basal plasma glucose levels, basal plasma insulin and C-peptide levels after an overnight fast (Table 1) were almost twice as low in Reg. 2 compared to Reg. 3. The lower endogenous insulin levels in Reg. 2 may be due to suppression by free fatty acids, which were at higher levels in Reg. 2.

Energy restriction in the diet has limitations in patients with CGL because of undesirable weight loss and persistent hunger (Reg. 1). However, as in our patient's case, modestly hypercaloric diets (Reg. 2 and 3, see Table 1) add a risk of hyperglycaemia that responds poorly to insulin. The exogenous glucose disposal rate was the highest and basal plasma glucose after an overnight fast normalized after the addition of dL-fenfluramine to a hypercaloric low-fat diet (Reg. 4). In addition, this drug in sharp contrast to dietary treatments alone, seems to be responsible for increased lipid oxidation after an oral glucose load, apparently at the expense of suprabasal glucose oxidation. However, even this decrease in suprabasal glucose oxidation may be a positive phenomenon in a patient with CGL if it reflects an overall decrease in glucose recycling and hepatic glucose production. Our findings are supported by data from Andersen *et al.* (1993), who have shown that lipid oxidation is increased and glucose oxidation is decreased during dL-fenfluramine treatment in obese nondiabetic patients. We suggest that the improvement in insulin sensitivity, despite no change in total energy intake and virtually the same nutrient composition after transition from dietary Reg. 3–4, is related to a dL-fenfluramine induced augmentation in fat oxidation and diminished cycling of the nutritional substrates. Clinically this positive effect was reflected by achievement of euglycaemia with just 1.1 U/kg of insulin per day.

We performed single-stranded conformational polymorphism (SSCP) and DNA analysis to search for mutations in the coding regions, exon-intron junctions and proximal promoter regions of the β_3 -adrenergic receptor (β_3 AR) and the peroxisome proliferator-activated receptor-gamma (PPAR γ) genes. We did not detect mutations in the β_3 -AR gene in this patient, similar to studies by Silver *et al.* (1997) and Vigouroux *et al.* (1997) in other patients with CGL. The only polymorphism in the PPAR γ gene in this patient was a silent nucleotide substitution at the position 1431 (CAC^{His}→CAT^{His}). This variant was also present in several nonlipodystrophic controls. Similarly, we (unpublished) and colleagues (Vigouroux *et al.*, 1998) did not find unique mutations in the PPAR γ gene in other patients with CGL. Heterozygosity for several missense mutations in the lamin A/C gene on chromosome 1 has been associated with familial partial lipodystrophy (Cao & Hegele 2000). DNA sequence analysis of the lamin A/C gene revealed that our patient was heterozygous for the previously reported polymorphism at codon 583 [GCC^{Ala}→GAC^{Ala}] (Speckman *et al.* 2000). The patient was also heterozygous for a C2737T polymorphism in intron 8, which introduces a potential new splice donor site (GC→GT). However, this novel intron variant was also present in three of nine normal controls.

In conclusion, we show that a low-fat diet with supplemental dL-fenfluramine and insulin therapy may be an effective method to manage hyperglycaemia in a patient with CGL. Studies of the genes for lamin A/C, PPAR γ and β_3 -AR in our patient failed to detect pathogenic alterations. Uncovering the genetic defect of CGL will allow a better understanding of the pathogenesis of this disease and provide insights into adipocyte

development and function, as well as potential treatments for CGL and perhaps other metabolic disorders such as obesity and type 2 diabetes.

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Table 1
Effects of dietary modifications, insulin therapy and addition of dL-fenfluramine in a patient with CGL

Regimen	Baseline	1	2	3	4*	Reference values
Dietary energy intake (MJ/24 h)	–	7.5	9.2	9.2	9.2	
% Fat in diet	–	31	31	8	15	
% Carbohydrate in diet	–	52	52	78	70	
Duration of regimen (days)	–	21	42	27	52	
Body weight (kg)	49.2	47.8	51.3	51.9	50.9	N/A
Insulin requirements (U/kg per 24 h)	0.8	0.8	2.0	2.4	1.1	N/A
Glycohemoglobin (%)	10.6	9.7	8.3	7.8	6.2	6–8
Plasma triglycerides (mmol/l)	7.1	2.3	3.8	5.1	3.0	<1.8
Plasma total cholesterol (mmol/l)	5.33	4.55	4.55	3.54	3.44	<5.20
HDL cholesterol (mmol/l)	0.85	0.70	0.57	0.75	0.57	35–65
Free fatty acids (mmol/l)	–	110	270	138	162	200–490
Baseline plasma glucose (mmol/l)	8.0	7.7	14.1	12.3	6.1	4.2–6.4
Baseline plasma insulin (pmol/l)	–	152	227	434	138	34–138
Baseline C-peptide (nmol/l)	–	153	87	303	80	17–66
Glucose disposal** ($\text{mg} \times \text{m}^{-2} \times \text{minutes}^{-1}$)	–	64.7	5.6	88.0	104.5	N/A
Steady state plasma glucose (mmol/l)	–	5.2	5.2	5.4	5.3	N/A
Steady state plasma insulin (pmol/l)	–	812	799	771	785	N/A
Respiratory exchange ratio***	–	0.89	0.82	0.90	0.91	0.79–0.85
Resting energy expenditure*** (MJ/24 h)	–	5.3	7.6	6.5	6.1	5.3–6.9
Non-oxidative glucose disposal ($\text{mg} \times \text{m}^{-2} \times \text{minutes}^{-1}$)	–	232.6	199.3	251.1	251.4	170–217
Basal glucose oxidation ($\text{mg} \times \text{m}^{-2} \times \text{minutes}^{-1}$)	–	97.4	88.9	123.6	115.5	42–82
Suprabasal glucose oxidation ($\text{mg} \times \text{m}^{-2} \times \text{minutes}^{-1}$)	–	43.3	40.2	27.7	12.7	53–68
Change in basal fat oxidation after a 75-g glucose load ($\text{mg} \times \text{m}^{-2} \times \text{minutes}^{-1}$)	–	–8.3	–15.5	–4.7	+4.7	–10 to –35

* With dL-fenfluramine.

** Euglycaemic hyperinsulinaemic clamp ($10 \text{ mU} \times \text{m}^{-2} \times \text{minutes}^{-1}$).

*** Indirect calorimetry.