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Short-term effects of ketogenic diet on anthropometric parameters, body fat distribution, and inflammatory cytokine production in GLUT1 deficiency syndrome

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ABSTRACT

Objective: The aim of this study was to evaluate the effects of a 12-wk ketogenic diet (KD) on inflammatory status, adipose tissue activity biomarkers, and abdominal visceral (VAT) and subcutaneous fat (SAT) in children affected by glucose transporter 1 deficiency syndrome GLUT1 DS.
Methods: We carried out a short-term longitudinal study on 10 children (mean age: 8.4 y, range 3.3–12 y, 5 girls, 5 boys) to determine fasting serum proinflammatory cytokines (high sensitivity C-reactive protein, tumor necrosis factor-α interleukin-6), adipocyte-derived chemokines (leptin and adiponectin), lipid profile, homeostatic model assessment-insulin resistance (HOMA-IR), quantitative insulin sensitivity index (QUICKI), anthropometric measurements, and VAT and SAT (by ultrasonography).
Results: Children showed no significant changes in inflammatory and adipose tissue activity biomarkers, blood glucose, lipid profile, anthropometric measurements, VAT, and SAT. Fasting insulin decreased (6 ± 3.2 μU/mL versus 3 ± 2 μU/mL; $P = 0.001$), and both HOMA-IR and QUICKI indexes were significantly modified (1.2 ± 0.6 versus 0.6 ± 0.4; $P = 0.002$; 0.38 ± 0.03 versus 0.44 ± 0.05; $P = 0.002$, respectively).
Conclusions: Only HOMA-IR and QUICKI indexes changed after 12 wk on a KD, suggesting that over a short period of time KD does not affect inflammatory cytokines production and abdominal fat distribution despite being a high-fat diet. Long-term studies are needed to provide answers concerning adaptive metabolic changes during KD.

Introduction

The ketogenic diet (KD) is an isocaloric, high-fat, low-carbohydrate diet that induces production of ketone bodies (i.e., β-hydroxybutyrate and acetoacetate), mimicking the biochemical changes of starvation [1]. For classic KD, nutrient composition is expressed as the weight ratio of fat to protein and carbohydrate. The most common ratios are 4:1 and 3:1, equal to 3 or 4 g fat to 1 g protein + carbohydrates, providing ≥0.8 g/kg of protein for body weight. This means that 85% to 90% of the dietary calories are derived from fat and <2% to 3% are derived from carbohydrates.

KD has been used since the 1930s as an adjuvant therapy in patients with drug-resistant epilepsy who were suffering different types of seizure and epilepsy syndromes [2,3], and it is the treatment of choice in glucose transporter 1 deficiency syndrome (GLUT1 DS) [4].

SB and AB designed the research. SB, IGN, CT, CF, RSDA, AB, PV, VDG, and AT conducted the research. SB performed the statistical analysis, wrote the manuscript, and had primary responsibility for final content. All the authors read and approved the final manuscript. None of the authors reported a conflict of interest.

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GLUT1-DS is a rare form of encephalopathy caused by dominant mutations in *SLC2 A1* (solute carrier family 2 member 1) encoding GLUT-1, a facilitated glucose transporter [5]. Because GLUT-1 is the main glucose transporter across the blood–brain barrier, its deficiency leads to hypoglycorrhachia and impairment in energy cerebral metabolism [4], resulting in serious developmental delays, seizures, and complex motor disorders [6]. In this neurometabolic condition, ketone bodies can be used as an alternative energy substrate, switching brain metabolism from glucose to ketones and leading to a powerful improvement in neurologic symptoms (seizures, complex motor disorders, and cognitive impairment) [4]. Obviously, a stable chronic ketosis status must be maintained throughout life by strict adherence to a KD, which can sometimes be applied with a lower ketogenic ratio (3:1 or 2.5:1) according to individual brain sensitivity to ketone bodies [5].

High dietary fatty acid consumption, particularly long-chain saturated fatty acids, has a well-known negative effect on endothelial function, which is mediated by the production of adipocyte-derived chemokines (leptin and adiponectin), proinflammatory cytokines (tumor necrosis factor [TNF]-α, interleukin [IL]-6, high-sensitivity C-reactive protein [hs-CRP]) [7–9] and macrophage accumulation in the vascular wall [10]. An underlying mechanism responsible for the production of proinflammatory cytokines and adipocyte-derived chemokines is the increase in abdominal visceral fat [11,12].

As a consequence, the continuous use of KD for several decades could increase cardiovascular risk. Studies conducted to date on cardiovascular risk factors are controversial. One study demonstrated a significant increase in atherogenic apolipoprotein B (ApoB)-containing lipoproteins and a decrease in the antiatherogenic high-density lipoprotein cholesterol (HDL-C) after 6 mo on a KD, in 141 children with difficult-to-treat seizures [13]. Another study found a proatherogenic lipoprotein profile, without elevation in total cholesterol (TC) or low-density lipoprotein cholesterol (LDL-C), in 10 prepubertal children after at least 6 mo on a classical 3:1 diet compared to 10 control children [14]. The study also evidenced lower leptin levels that were interpreted as a consequence of low body fat content. However, in a second study [4], a moderate elevation of TC was evidenced in only 2 of 15 children on the diet, for a time period of between 2 and 5.5 y. Moreover, the lipid profile alterations (increase in LDL-C, ApoB and the ratios of TC to LDL-C and LDL-C to HDL-C) and the worsened arterial function observed within the first year of KD treatment in another study seems to be reversible, and was not significant after 24 mo of treatment [15]. With regard to the metabolic activity of adipose tissue, only one case–control study has investigated the effects of KD on adipokines showing a stable adipokine pattern [16], whereas there have been no previous studies to investigate the KD effect on inflammatory status and abdominal fat distribution. More studies are needed to clarify changes in adipose tissue distribution and activity, and to establish the modification of early markers of inflammatory status during KD.

We planned a longitudinal study on the effects of a 12-wk KD on inflammatory cytokines production (biomarkers of inflammatory status and adipose tissue activity) and visceral fat in a sample of patients with GLUT1 DS.

Materials and methods

Participants

GLUT1 DS is a rare disease. To date, about 200 patients have been identified in the world mainly in the United States and Europe [17]. The Norway National Registry indicates a point prevalence of 2.6 per 1 million inhabitants [18]. In Italy, a national registry of GLUT1 DS is not yet available and the prevalence cannot be estimated.

We prospectively enrolled 10 children (5 girls and 5 boys, mean age 8.4 y, range 3.3–12 y) diagnosed with GLUT1 DS at the Department of Child Neurology and Psychiatry, Fondazione IRCCS Istituto Neurologico C.Mondino in Pavia, Italy, from October 2010 to November 2012. The general characteristics of the children are represented in Table 1.

All the patients met the clinical criteria for GLUT1 DS diagnosis: All underwent a lumbar puncture in the fasting state (after 5–6 h of fasting); a blood sample for glucose measurement was obtained immediately before the procedure to avoid stress-related hyperglycemia [5]. A cerebrospinal fluid-to-blood glucose rate <0.6 was considered suspicious for GLUT1 DS. Subsequently, for definite confirmation, all the patients were submitted to *SLC2 A1* mutation analysis.

Study design

This was a 3-mo, prospective, single-center, single-arm study of the metabolic effects of KD in patients with GLUT1 DS treated with the diet.

The main outcome measures were the changes from the baseline of inflammatory status, adipose tissue activity biomarkers, and abdominal fat distribution.

Pre- and postintervention assessment included nutritional status evaluation by anthropometry, resting metabolic rate, abdominal body fat distribution by ultrasonography, dosages of proinflammatory cytokines, and markers of inflammation (hs-CRP, TNF-α, and IL-6), adipose tissue activity biomarkers (leptin, adiponectin, and fatty free acid [FFA]), glucose metabolism (glucose, insulin, and C-peptide), lipid profiles (triacylglycerols [TGs], TC, LDL-C, and HDL-C), hepatic enzymes (glutamic pyruvic transaminase, glutamic oxaloacetic transaminase, and gamma glutamyl transpeptidase), uric acid, and creatinine.

All measurements were performed at the International Center for the Assessment of Nutritional Status (ICANS-Milan University). After pretreatment evaluation, a KD treatment was implemented at the Human Nutrition and Eating Disorders Research Centre (see later).

According to the 2011 Italian consensus on KD therapy [19], neurologic evaluations and electroencephalography (EEG) was performed after 1 and 3 mo at the Department of Child Neurology and Psychiatry, Fondazione IRCCS Istituto Neurologico C. Mondino in Pavia. The following neurologic symptoms were monitored: paroxysmal dyskinesias, dysarthria, ataxia, spasticity dystonia, and muscle strength. Moreover, caregivers completed a daily record on alertness and activity.

The study protocol received institution review board approval and complied with all tenets of the Helsinki declaration. Children's parents provided written informed consent before the beginning of the study.

Anthropometric measurements

Anthropometric measurements were taken by the same trained dietitian according to conventional criteria and measuring procedures [20].

Body weight (BW, kg) and body height (BH, cm) were measured to the nearest 100 g and 0.5 cm, respectively. Body mass index (BMI) was calculated using the formula: BW (kg)/BH^2 (m^2). Sex-specific BMI-for-age percentiles and *Z* scores were calculated from the 2000 Centers for Disease Control and Prevention (CDC) growth charts [21]. In accordance with CDC guidelines, a *Z* score below the fifth percentile was considered underweight, a score between the 5th and 85th percentile was considered normal weight, a score ≥85th percentile and <95th

Table 1
General characteristics of the sample and ketogenic diet ratio

Patients	Sex	Age (y)	SLC2 A1 testing	KD ratio
1	F	10.1	Positive	3.5:1
2	M	3.5	Positive	3:1
3	F	6.1	Positive	3:1
4	M	12	Positive	3:1
5	F	6.8	Positive	2:1
6	M	7.7	Positive	3:1
7	F	10.6	Positive	3:1
8	M	9.8	Positive	2.5:1
9	F	8.4	Positive	4:1
10	M	8.8	Positive	2.5:1

KD, ketogenic diet
SLC2 A1 gene mutation; KD ratio is ketogenic diet ratio, fat g/(protein g plus carbohydrates g)