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Glucose-free/high-protein diet improves hepatomegaly and exercise intolerance in glycogen storage disease type III mice

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ABSTRACT

Glycogen disease type III (GSDIII), a rare incurable autosomal recessive disorder due to glycogen debranching enzyme deficiency, presents with liver, heart and skeletal muscle impairment, hepatomegaly and ketotic hypoglycemia. Muscle weakness usually worsens to fixed myopathy and cardiac involvement may present in about half of the patients during disease. Management relies on careful follow-up of symptoms and diet. No common agreement was reached on sugar restriction and treatment in adulthood.

We administered two dietary regimens differing in their protein and carbohydrate content, high-protein (HPD) and high-protein/glucose-free (GFD), to our mouse model of GSDIII, starting at one month of age. Mice were monitored, either by histological, biochemical and molecular analysis and motor functional tests, until 10 months of age.

GFD ameliorated muscle performance up to 10 months of age, while HPD showed little improvement only in young mice. In GFD mice, a decreased muscle glycogen content and fiber vacuolization was observed, even in aged animals indicating a protective role of proteins against skeletal muscle degeneration, at least in some districts. Hepatomegaly was reduced by about 20%. Moreover, the long-term administration of GFD did not worsen serum parameters even after eight months of high-protein diet. A decreased phosphofructokinase and pyruvate kinase activities and an increased expression of Krebs cycle and gluconeogenesis genes were seen in the liver of GFD fed mice.

Our data show that the concurrent use of proteins and a strictly controlled glucose supply could reduce muscle wasting, and indicate a better metabolic control in mice with a glucose-free/high-protein diet.

1. Introduction

Glycogen storage disease type III (GSDIII; OMIM #232400) is a rare autosomal recessive disease [1,2] caused by deficiency of glycogen debranching enzyme, one of the two enzymes responsible for glycogenolysis. Hepatomegaly, ketotic hypoglycemia, hyperlipidemia, elevated transaminases and failure to thrive are the usual presenting symptoms in the first year of life in GSDIIIa (the most common subtype) [1–3]. Hepatic symptoms usually improve and tend to resolve in adolescence although liver fibrosis and cirrhosis may develop [4,5]. Cardiac involvement, such as left ventricular wall thickness and mass increase, occurs in about half of the patients, but generally is stationary [3,6]. In young adults, myopathy initially presents mostly as exercise intolerance, with involvement of both proximal and distal muscle and elevated CK levels. A fixed myopathy with proximo-distal involvement of variable severity occurs in the following decades, eventually leading to loss of independent walking. Muscle weakness is often described also in young patients revealing that myopathy may occur earlier than usually reported [2].

To date, there is no cure for GSDIII and the current

Abbreviations: GSDIII, glycogen disease type III; GDE, glycogen debranching enzyme; HPD, high-protein diet; GFD, glucose-free diet; SD, standard diet; CKs, creatine kinases; BUN, blood urea nitrogen; M, male mice; F, female mice

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recommendations for management rely on follow-up of symptoms and dietary treatment to prevent hypoglycemia and maintain a constant glycaemia. Particularly, in infancy and childhood fasting hypoglycemia is less tolerated due to the age-dependent physiology. Euglycemia is crucial in GSDIII patients but it is not enough to prevent long-term complications such as myopathy and cardiomyopathy [7]. There is not a common agreement about sugar restriction: some authors do not suggest any sugar restriction [1], some others suggest avoiding simple sugars like fructose and lactose in favor of complex carbohydrates [2]. Moreover, carbohydrate overload should be avoided as it may increase glycogen storage, induce obesity and also insulin resistance [2]. Another emerging challenge is dietary treatment in adulthood.

A number of case reports focused on the improvement of myopathy and/or cardiomyopathy in adults and children that switched their diet in favor of high-protein diet [8–11] or high-fat/ketogenic diet [12–14]. Several benefits come from by using high-protein diet in the treatment of GSDIII: i) the metabolic pathway of gluconeogenesis is not damaged and proteins can be used as source to produce glucose; ii) the increase of dietary protein and the reduction of dietary carbohydrate may decrease glycogen storage in both liver and skeletal muscle; iii) increased protein intake may reduce muscle proteolysis both by increasing the availability of exogenous proteins for energy cell requirements and by enhancing muscle protein synthesis for the maintenance of muscle plasticity [2]. The administration of a high-protein diet in the management of GSDIII has its roots in the Sixties when Fernandes and van de Kamer observed that proteins induce a gradual and prolonged increase in blood glucose [15].

Recently, we developed a knock-out mouse model (GSDIII mouse model) that reproduces the major features of GSDIII like hepatomegaly, progressive glycogen storage in skeletal muscle and liver, and muscle impairment [16]. We administered to GSDIII mice two types of high-protein diet differing in protein and carbohydrate content. Mice fed with glucose-free/low-carbohydrate diet (GFD) showed significant improvement of muscle performance, decreased glycogen accumulation and reduction of hepatomegaly.

2. Material and methods

2.1. Animals and experimental protocols

All studies were approved by the Experimentation Committee (OPBA) of the University of Milan and the Italian Ministry of Health (Authorization number: 1169/2016-PR) and were performed in accordance with Italian guidelines for the use of laboratory animals. Mice were maintained on a 12:12 h light-dark cycle in a temperature- and humidity-controlled environment, and were allowed to free access to standard (SD) or special diets (chow) and water.

The GSDIII mouse model was previously described [16]. Both GSDIII and WT mice were fed with special diets starting from weaning at 1 month of age. High-protein (HPD; 49 kJ% protein, 31 kJ% carbohydrates, 20 kJ% fat) and glucose-free/low-carbohydrate (GFD; 66 kJ% protein, 2 kJ% carbohydrates, 32 kJ% fat) diets were from ssniff ® (ssniff Spezialdiäten GmbH, Soest, Germany). For detailed diet compositions see Table 1.

A motorized treadmill (Panlab LE8708; Panlab Harvard Apparatus, Spain) was used to assess muscle performance. Before testing, mice were trained for two consecutive days on a 5° incline for 15 min starting at a speed of 5 cm/s and increasing the velocity of 5 cm/s every 5 min. The treadmill is supplied with an electrified grid that administer a mild shock (0.2 mA) to provide motivation. The exercise test was administered after a 5 min of warm up at a speed of 10 cm/s. The mice were tested at 30 cm/s for 5 min on a 5° incline. The test was interrupted after 5 min or when the mouse could no longer keep pace with the belt.

Table 1
Composition of the diets administered in this study.

Nutrients	Standard diet (SD)	High protein diet (HPD)	Glucose free diet (GFD)
	%		
Crude protein	19.1	45.9	53.4
Crude fat	4.8	8.3	11.4
Crude fiber	3.8	5.0	20.3
Starch	35.4	9.6	0.1
Dextrin	n.a.	6.9	–
Sugar (sucrose, glucose)	4.6	11.0	< 0.1
Others	32.3	13.3	14.7
Total	100	100	100

n.a. not available.

2.2. Serum analysis and biochemistry

Blood glucose was measured using test strips in a FreeStyle Optium H system (Abbott Diabetes Care). Blood samples were collected from tail vein.

For serum testing and biochemistry analysis, mice were anesthetized and blood was sampled by puncture of vena cava in the sub-hepatic tract. The procedure was followed by euthanasia. Serum was sent to Charles River Laboratories for cholesterol, triglycerides, ALT, AST, ALP, creatine kinases (CKs), blood urea nitrogen (BUN) and creatinine determinations.

Glycogen content was determined as previously described [16,17].

The activities of glycolytic enzymes and of enzymes of each respiratory chain complex were measured in tissue homogenates as previously described [18,19]. The activity of each complex was normalized to that of citrate synthase.

2.3. Histological analysis

For light microscopy studies, fresh organs and tissues were processed according to standard methods [20]. Routine stains with and without prior diastase digestion were performed with hematoxylin and eosin and periodic acid–Schiff (PAS).

2.4. Glucose and glycogen metabolism expression profile

We evaluated expression profiling of 84 genes involved in both glucose and glycogen metabolism using the Mouse Glucose Metabolism RT^2 Profiler™ PCR Array (PAMM-006Z; SABiosciences, QIAGEN). Liver and skeletal muscle (vastus) tissues from 2 month-old mice were analyzed for WT, SD-KO and GFD-KO (n = 4). Equal amounts of total RNA (0.5 µg for liver and 0.8 µg for vastus) were reverse transcribed using the RT^2 First Strand Kit (QIAGEN). Real-Time qPCR was performed in a 7500 Real Time PCR System (Applied Biosystems). The analysis of expression profiling was performed using the $\Delta\Delta C_T$ method using the online free software available at the PCR Array Data Analysis Web portal (www.SABiosciences.com./pcrarraydataanalysis.php). Raw data were normalized to the housekeeping genes included in the array. Differentially expressed genes were identified using a 2-tailed *t*-test and changes in gene expression were presented as fold change increase or decrease (P value < 0.05).

2.5. Statistical analysis

The survival time was calculated using the Kaplan–Meier log rank test. Experimental groups were compared using Student's *t*-test and a P value < 0.05 was considered statistically significant. Error bars represent standard deviation.