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Original Research

Long-term ketogenic diet contributes to glycemic control but promotes lipid accumulation and hepatic steatosis in type 2 diabetic mice

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ABSTRACT

The ketogenic diet (KD) has been widely used in weight and glycemic control, although potential side effects of long-term KD treatment have caused persistent concern. In this study, we hypothesized that the KD would ameliorate the progression of diabetes but lead to disruptions in lipid metabolism and hepatic steatosis in a mouse model of diabetes. In type 2 diabetic mouse model, mice were fed a high-fat diet and administered streptozotocin treatment before given the test diets for 8 weeks. Subsequently, ameliorated glucose and insulin tolerance in KD-fed diabetic mice was found, although the body weight of high-fat diet– and KD-fed mice was similar. Interestingly, the weight of adipose tissue in KD mice was greater than in the other groups. The KD diet resulted in higher serum triacylglycerol and cholesterol levels in diabetic mice. Moreover, the KD-fed mice showed greater hepatic lipid accumulation. Mice fed the KD showed significant changes in several key genes such as sterol regulatory element-binding protein, fibroblast growth factor 21, and peroxisome proliferator–activated receptor α, which are all important in metabolism. In summary, KD ameliorates glucose and insulin tolerance in a mouse model of diabetes, but severe hepatic lipid accumulation and hepatic steatosis were observed, which should be considered carefully in the long-term application of KD.

1. Introduction

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin resistance, or both [1]. As the most common form of diabetes, type 2 diabetes mellitus (T2DM) or non–insulin-dependent diabetes mellitus is strongly associated with obesity, cardiovascular disease, uremia, and even

Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; FGF21, fibroblast growth factor 21; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; KD, ketogenic diet; mRNA, messenger RNA; NAFLD, nonalcoholic fatty liver disease; NM, nondiabetic mice; PCR, polymerase chain reaction; SREBP2, sterol regulatory element-binding protein; STZ, streptozotocin; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglyceride; TNF-α, tumor necrosis factor α.

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Alzheimer disease [2-4]. With an increase in sedentary lifestyles and the consumption of high-fat and high-sugar foods, obesity and T2DM have become a serious health problem. A healthy diet and physical activity to achieve weight loss are an efficient way to prevent or postpone the development of type 2 diabetes in high-risk individuals [5].

Although antidiabetic drugs have been widely used in treating T2DM, a growing number of nondrug therapies have become popular since considerable attention to drug safety. Among these strategies, nutritional therapy has been recommended by the American Diabetes Association for all people with type 2 diabetes [6]. In particular, a low-carbohydrate diet is one of the most popular dietary therapies for patients with diabetes or obesity in recent clinical studies [7,8]. Interestingly, the consumption of a high-fat diet and high intake of saturated fat used to be associated with an increased risk of type 2 diabetes, but this association disappears when combined with a low-carbohydrate or noncarbohydrate diet known as the ketogenic diet (KD). Ketogenic diet was first reported as a kind of high-fat, adequate-protein, low-carbohydrate diet for treatment for epilepsy in the 1920s [9]. The classical KD provides 90% of calories from long-chain fats, a minimum of 1 g/kg of protein, and minimal carbohydrates, resulting in the generation of acetoacetate and β-hydroxybutryate (ketone bodies) [10,11]. Furthermore, several researchers have shown that a KD plays an important role in weight loss and glycemic control, and it may be a treatment for obese or diabetic patients [12-14]. Furthermore, a low-carbohydrate diet has greater beneficial effects on glycemic control compared with a low glycemic index diet [8,15]. The KD is known to prevent the development of diabetes and reverse the damage to the pancreas in streptozotocin (STZ)–induced diabetic rat models [16]. A low-carbohydrate diet may result in malnutrition and lack a variety of vitamins [17]; however, few clinical or animal studies have considered the adverse effect of long-term KD treatment for diabetes.

In the present study, we hypothesized that KD would have effects on lipid metabolism and hepatic steatosis in the treatment of diabetes. Thus, we attempted to mimic long-term KD treatment in a mouse model of diabetes to investigate the potential side effects. We demonstrated that KD led to improved glycemic control in diabetic mice, although it also contributed to lipid accumulation and hepatic steatosis in type 2 diabetic mice. These results extend the novel effect of KD in long-term treatment and possibility for controlling diabetes.

2. Methods and materials

2.1. Animals

Six-week-old male C57BL/6 mice (Shanghai Laboratory Animal Company, Shanghai, China) were maintained in the Laboratory Animal Center of East China Normal University. They were housed in a temperature-regulated (22°C ± 2°C) facility with a 12-hour light/12-hour dark cycle and free access to chow and pure water. All animal experiments conformed to the regulations drafted by the Association for Assessment and Accreditation of Laboratory Animal Care in Shanghai and were approved by the East China Normal University Center for Animal Research.

2.2. Type 2 diabetic mouse model and diets

Mice fed a high-fat diet (Shanghai Laboratory Animal Company) for 4 weeks then, STZ (Sigma-Aldrich, St. Louis, MO, USA) was administered to mice by intraperitoneal injection daily for 3 consecutive days at a dose of 85 mg/kg body weight in freshly prepared sodium citrate buffer (pH 4.5). An injection of sodium citrate buffer alone served as a buffer control. The glucose levels of mice fasted for 8 hours were measured once a week using the blood from the tail vein and a glucose monitor (ACCU-CHEK Active; Roche, Basel, Switzerland). One week after STZ injection, mice with a fasting blood glucose level higher than 11.6 mmol/L on 3 consecutive days were regarded as type 2 diabetic mice and divided into 2 subgroups, one receiving high-fat diet and the other receiving KD. A standard chow was fed to the control group. The ingredient composition of the all diet fed to mice was listed in Table 1.

2.3. Mice and procedures

Blood glucose, body weight, food intake, and water intake of each group were measured once a week to monitor the general changes during the experiment. In the end, the mice were fasted overnight and euthanized by cervical dislocation to obtain tissues and blood samples. Tissues were weighed and stored at –80°C for subsequent analysis.

2.4. Intraperitoneal glucose/insulin tolerance test

Three days before euthanasia, an intraperitoneal glucose/insulin tolerance test was performed on the fasted mice.

Table 1 – Ingredient composition of the diets fed to mice

	g/100 g total diet		
	SD	HFD	KD
Fish guano	4	2.18	–
Soybean meal	22	12.01	–
Corn starch	25	13.65	–
Wheat	34	18.56	–
Soybean	2	1.09	–
Bran	4	2.18	–
Yeast	1.5	0.82	–
Soybean oil	2	1.09	–
Lard	0	16.9	70
Casein	0	10.2	20
Sucrose	0	14	–
Maltodextrin	0.5	2.47	–
Choline bitartrate	0.2	0.11	–
Dietary fiber	–	–	5.5
Salt	0.4	0.4	0.38
Lysine	0.75	0.41	0.7
L-Cystine	0.4	0.55	0.38
Mineral mix	0.15	0.26	0.14
Vitamin mix	0.2	0.2	0.19
Calcium hydrophosphate	1.6	1.57	1.5
Calcium carbonate	1.3	1.28	1.22

Nutrient composition: standard diet: protein (22.1%), carbohydrate (52%), fat (5.28%), fiber (4.12%); high-fat diet: protein (22.3%), carbohydrate (44.6%), fat (19.8%), fiber (2.1%); KD: protein (20%), carbohydrate (0%), fat (70%), fiber (5.5%).
Abbreviations: SD, standard diet; HFD, high-fat diet; KD, ketogenic diet.