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Effect of Short-Term Ketogenic Diet on Redox Status of Human Blood

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ABSTRACT

The present study investigated the effect of a ketogenic diet on the blood redox status of healthy female subjects. Twenty healthy females with mean body mass index of 21.45 ± 2.05 kg/m^2 were provided a low-carbohydrate (55 ± 6 g; 13% total energy), high-fat (138 ± 16 g; 74% total energy), calorie-restricted (−465 ± 115 kcal/d) diet. The followings were tested prior to and after 14 days consumption of the diet: Whole body, body weight and total body fat; blood, complete blood count, red blood cells, white blood cells, hemoglobin, and hematocrit; plasma, 3-β-hydroxybutyrate, total antioxidative status, and uric acid; red blood cells, total sulfhydryl content, malondialdehyde, superoxide dismutase activity, and catalase activity. After 14 days, weight loss was significant whereas no changes were detected in body fat. No alterations were observed in blood count or morphology. 3-β-hydroxybutyrate, total antioxidative status, uric acid, and sulfhydryl content were significantly increased. There were no alterations in malondialdehyde, or superoxide dismutase or catalase activity. The present study demonstrates that 14 days of a ketogenic diet elevates blood antioxidative capacity and does not induce oxidative stress in healthy subjects.

INTRODUCTION

THE EFFECTS OF KETOGENIC DIETS (KD) and ketosis on oxidative stress and antioxidative capacity varies in different diseases and systems. In neurologic disorders including Alzheimer's and epilepsy, ketosis induced by administration of ketone bodies or calorie and carbohydrate restricted KDs elevates antioxidative capacity in the central nervous system of animal models, and improves the conditions of patients.[1–6] In contrast, high fat and ketosis may induce oxidative stress in the blood and elevate the risk of vascular diseases.[7,8] In patients with diabetes, elevated ketone bodies decreases the antioxidative capacity, induces oxidative modification of low-density lipoproteins, and causes development of vascular diseases.[9–11]

Although numerous studies have addressed the effects of hypocaloric nonketogenic diets on oxidative stress and redox status,[12] the effect of hypochaloric ketogenic diets on the redox status of healthy subjects has not been studied.

As the popularity of short-term KDs remains high among healthy subjects for lowering body

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mass,[13,14] there emerges need for understanding the effect of those diets on oxidative stress and antioxidative capacity of healthy subjects. The present study investigated the effect of 14 days of KD on oxidative stress and antioxidative capacity in the blood of healthy female subjects.

METHODS

Subjects

Twenty recreationally active and healthy nonsmoking women took part in the present study. Subjects were between 21–23 years with average body mass index (BMI) of 21.45 ± 2.05 kg/m^2.

The subjects did not have neurologic disorders, hypertension, or diabetes mellitus, and were not engaged in tobacco use. Subjects abstained from any medications, alcohol, or antioxidant supplements during the study. Participants gave consent on the study and experimental procedures.

Characteristics of the diet

Subjects were provided a low-carbohydrate (55 ± 6 g), high-fat (138 ± 16 g), and calorie-restricted (−465 ± 115 kcal/d) diet. Those values correspond to 13% and 74% of total energy for carbohydrate and fat, respectively. The diet was based on animal products enriched in fat including pork, beef, butter, lard, cheese, eggs, and up to 100 g/d of vegetables and fruits. The subjects received a list of products they could consume, information about the protein, carbohydrate, fat, and energy content of the food products, and recipes to prepare meals accordingly.

Body mass and body composition

Body mass and body composition were measured in the morning of the day before the beginning of the study, and in the morning after the fourteenth day of the study. Body mass was measured to the nearest 0.1 kg, and body fat expressed as percentage of body mass to the nearest 0.1%. Body fat was assessed by measurements of bioelectrical impedance analysis (BIA).

Blood collection and analysis

Blood was drawn from the antecubital vein after 16 hours fasting, in the morning of the day before the beginning of the study, and in the morning after the fourteenth day of the study. The red blood cells (RBC) and plasma were collected and the following tests were performed.

Blood. Complete blood count (CBC) and morphology was tested using Sysmex SF-3000 Automated Hematology Analyzer (GMI, Inc., Ramsey, MN).

Plasma. 3-β-hydroxybutyrate (3HB) was measured using kinetic assay at 340 nm based on oxidation of 3HB to acetoacetate by 3HB dehydrogenase, and reduction by 3HB dehydrogenase of NAD^+ to NADH. This test was performed using the commercially available assay reagents (Randox Laboratories, Ltd., County Antrim, UK).

Total antioxidant status (TAS) of fresh plasma was determined by testing the ability of the plasma to inhibit oxidation by H_2O_2 of 2,2-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS®; Randox Laboratories Ltd.) in the presence of metmyoglobin. Plasma was mixed with Chromogen (ABTS® and metmyoglobin) and optical density at 600 nm was measured before and 3 minutes after addition of H_2O_2.

Plasma uric acid (UA) was measured by standard colorimetric method (Randox Laboratories, Ltd.). This assay is based on conversion of UA by uricase to allantoin and hydrogen peroxide that oxidizes 3,5-dichloro-2-hydroxybenzenesulfonic acid and 4-aminophenazone to form a red-violet quinoneimine compound measured at 520 nm.

Red blood cells. Catalase (CAT) activity was determined by measuring at 240 nm the decrease in H_2O_2 concentration.[15] CAT activity is expressed as units per gram (U/g) hemoglobin.

Superoxide dismutase (SOD) activity was measured as described.[16] This method utilizes xanthine and xanthine oxidase to generate superoxide that reacts with 3-(4-iodophenyl)-2-(4-nitrophenyl)-5-phenyltetrazolium (INT) to form a red formazan dye that was measured at 505 nm. SOD activity is expressed as U/g hemoglobin.

RBCs were tested for sulfhydryl group (SH) content using dithionitrobenzoic acid (DTNB) at 412 nm as described.[17] Reduced glutathione (GSH) was used to prepare standard curve, and SH content is expressed as micromole (μmol) GSH per gram (g) hemoglobin.

Malondialdehyde (MDA) content was measured by reacting RBCs with N-methyl-2-phenylindole dissolved in acetonitrile and methanol, followed by adding HCl. Samples were incubated at 45°C for 1 hour, centrifuged at 3000 rpm/min for 10 minutes, and the optical density of the supernatant was measured at 586 nm.

The hemoglobin content of RBCs was measured with Drabkin's Reagent containing potassium cyanide that forms cyanmethemoglobin absorbing at 540 nm. Standard hemoglobin solution (Randox Laboratories, Ltd.) was used to prepare standard curve. All other chemicals and reagents were obtained from Sigma (St. Louis, MO).

Ethics

The present study was approved by the Local Ethical Committee of Gdansk (KB-1207/05) and procedures followed in accordance with institutional guidelines.

Statistics

Data are presented as mean ± standard deviation. Paired *t* test was used to compare the data using Statistica 7.0 software (StatSoft Inc., Tulsa, OK). Differences with $p < 0.05$ were considered statistically significant.

RESULTS

The effects of KD are shown in Table 1. No changes were observed in CBC or blood cells morphology, as well as white blood cell count (WBC), RBC, hemoglobin, and hematocrit values are shown. Body mass was significantly decreased from 61.7 ± 9.9 kg to 60.0 ± 9.6 kg. Change in total body fat from 27.1% ± 5.9% to 25.1% ± 5.5% was not significant. 3HB, TAS, UA, and SH content were significantly increased. MDA content was not altered. Activities of CAT and SOD remained unchanged.

Table 1. Effect of the Ketogenic Diet on the Blood of Healthy Subjects

Parameter	*Before diet*	*After diet*
D3HB (mmol/L)	0.03 ± 0.06	0.73 ± 0.08[a]
WBC (g/L)	6.51 ± 1.31	5.91 ± 1.31
RBC (T/L)	4.64 ± 0.25	4.57 ± 0.22
Hemoglobin (g/dL)	13.56 ± 0.82	13.54 ± 0.86
Hematocrit (%)	41.26 ± 1.84	40.41 ± 1.78
TAS (mmol/L)	1.53 ± 0.02	1.74 ± 0.12[a]
UA (μmol/L)	233.91 ± 40.61	285.63 ± 50.29[a]
SH (μmol GSH/g Hg)	9.43 ± 2.38	14.59 ± 3.58[a]
MDA (nM/g Hg)	5.92 ± 0.64	6.00 ± 0.51
CAT (U/g Hg)	271.65 ± 31.50	286.46 ± 39.88
SOD (U/g Hg)	631.36 ± 108.72	611.28 ± 76.89

[a]Statistically significant ($p < 0.05$) difference.

Data are expressed as mean ± standard deviation (SD).

D3HB, 3-β-hydroxybutyrate dehydrogenase; WBC, white blood cell count; RBC, red blood cell count; TA's, total antioxidant status; UA, uric acid; SH, sulfhydryl group; MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase.

DISCUSSION

The present study administered to healthy subjects a 14-day calorie-restricted, low-carbohydrate, and high-fat KD. No changes were observed in CBC or blood cells morphology. Because the diet did not cause negative changes in global measurements of nutritional state including glucose, albumin, and lipid profile,[18] it seems that 14 days KD does not impair nutritional state.

3HB that is a marker of ketosis[19] significantly increased, indicating subjects underwent ketosis.

Ketosis caused by KDs induces oxidative stress in the plasma of patients with diabetes,[9] whereas KDs elevate antioxidative capacity in patients with neurological disorders.[5] The present study found a significant increase in UA and TAS in the plasma of healthy subjects after 14 days of KD. The diet used in the present study was made of animal products enriched in purines that increase the plasma UA.[20] UA scavenges oxidative free radicals and protects against oxidative stress.[21,22] This finding suggests that 14 days KD increases the antioxidative capacity in healthy subjects.

High level of ketone bodies causes lipid peroxidation and lowers glutathione in type 1 diabetes.[11]

However, the present study found that lipid peroxidation was not increased but SH content was significantly increased in the healthy sub-

jects. High blood glucose in diabetic patients facilitates lipid peroxidation and decreases SH content,[23] whereas KDs are carbohydrate restricted and do not increase blood glucose in healthy subjects.[18] Additionally, the KD used in the present study increases high density lipoprotein (HDL) in healthy female subjects.[18] HDL is a potent antioxidant preventing accumulation of oxidized lipids.[24]

The present study did not detect changes in CAT or SOD activity. These findings provide additional evidence for lack of elevated oxidative stress after 14 days of KD in healthy subjects.

Taken together, the present study shows that 14 days of KD increases antioxidative capacity in the blood of healthy subjects. Elevated antioxidative capacity was not an adaptive response to increased oxidative stress, because no evidence was obtained indicating increased oxidative stress. Increased antioxidative capacity was also not due to inactivation of antioxidative enzymes, because SOD and CAT activities were not decreased. Thus, it seems that antioxidative effects of 14 days carbohydrate and calorie restriction superseded the possible oxidative stress induced by high fat and ketosis. Such effect might derive from alteration in macronutrient content, as well as elevated UA, HDL, or amino acid turnover. Further studies are required to test the effect of long-term KD for the redox status of healthy subjects.

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