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A high-fat diet is deleterious to mice under glycolysis restriction

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Abstract

It is debated whether carbohydrate restriction has metabolic advantage for its variable weight loss. Five-week-old male mice fed a high-fat diet receiving a glycolytic inhibitor, 2-deoxyglucose, led to an absolute mortality within 9 days. They exhibited greater decreases in rectal temperature, appetite and decline in body weight accompanied by increasing total cholesterol level than the other groups. This study suggests that carbohydrate is necessary for adequate physical and metabolic performance when lipid-rich diet is loaded.

Key words: low-carbohydrate; ketoacidosis; dehydration

Introduction

Carbohydrate and lipid have a reciprocal relationship to provide a constant supply of energy through glycolysis and β -oxidation (Randle 1998). Plasma fatty acid downregulates glycolytic pathway (Schmid et al. 2004), whereas glycogen shortage in the liver generates ketone bodies by accelerating lipolysis (Izumida et al. 2013). Dysregulation of glucose tolerance and lipid metabolism underscores the high prevalence of atherosclerosis, ultimately increasing the morbidity and mortality of cardiovascular diseases (Poirier et al. 2006; Kanter et al. 2007). Several dietary regimens have recommended for preventing obesity or progression of metabolic syndrome (Kris-Etherton et al. 2003; Estruch et al. 2006). Specifically, low-carbohydrate diet with unlimited calories from fat and/or protein showed improvement in glycemic control in patients with type 2 diabetes mellitus (Westman et al. 2008) and decreasing body weight for obese subjects (Zinn et al. 2017). However, long-term intake of these regimens has declined the benefits and increased cardiovascular risk and all-cause mortality (Trichopoulou et al. 2007; Fung et al. 2010; Lagiou et al. 2012).

It is an unresolved question whether carbohydrate restriction has “metabolic advantage” for its variable weight loss (Westman et al. 2007). Fuel switch from glucose to fatty acid (ketone bodies) exerts protective roles in neuronal excitation (Lutas and Yellen 2013), but change in metabolic profiles may also affect whole-body functions (Ahima and Osei. 2001; Roohafza et al. 2012; Hung et al. 2013). To address this concern, we conducted experiments to administer a non-metabolite glucose analogue, 2-deoxyglucose (2-DG), to inhibit the glycolytic pathway in mice fed either standard chow or high-fat diet on physiological parameters.

Materials and Methods

All experimental procedures were in accordance with the guidelines of the Animal Ethical Committee at the University of Miyazaki (2013-505-4). This investigation also conformed to the Guide for the Care and Use of Laboratory Animals (8th edition, 2011).

Animals

C57BL/6J male mice were obtained from Charles River Inc., Yokohama, Japan. They were kept on a 12-hour light/12-hour dark cycle at 23 °C.

Chow diet composition

Standard rodent diet [CLEA Rodent Diet CE-2, CLEA Japan, Inc.] contains 339 kcal/100 g and is composed of 50 (gram %) carbohydrate, 25 (gram %) protein, 4 (gram %) fat and 21 (gram %) others [9 (gram %) moisture and 12 (gram %) fiber and ash]. The high-fat diet (D12108C, New Brunswick, NJ, United States) contains 452 kcal/100 g and is composed of 45 (gram %) carbohydrate, 23 (gram %) protein, and 20 (gram %) fat.

Experimental protocol

Five-week-old male mice weighing 17-23 g were housed individually in plastic cages with woodchip bedding for 1 week before use and were fed *ad libitum* with either the standard diet or high-fat diet. We gave 2-deoxyglucose (2-DG) dissolved in distilled water or control water to the respective group, with free access to the water bottle. We calculated the 2-DG dose so that it was administered at a constant dose normalized to

body weight (200 mg/kg/day). In the preliminary experiment, we increased the dose up to 1000 mg/kg/day of 2-DG, as a previous reference showed that the oral administration of 2-DG (8000 mg/kg) did not affect survival in mice (Vijayaraghavan et al. 2006). In the first experimental protocol, we examined the survival rate in respective groups. In another setting of experiment, we recorded body weight and quantities of food and liquid intake at 9:00 AM once a day. Core temperature was assessed using an electronic thermometer (BDT-100, Bio Research Center Co. Ltd., Nagoya, Japan) equipped with a rectal probe (RET-3, Bio Research Center Co. Ltd., Nagoya, Japan). On day 8, all mice in a fed state were euthanized by anesthesia with sodium pentobarbital (65 mg/kg, i.p.). Then, blood samples were collected by cardiac puncture and then mixed with 10 μ L heparin sodium. Detailed experimental protocols were described in Supplementary figure S1.

Blood analysis

We measured β -hydroxybutyric acid and glucose in whole blood using a disposable strip test (Abbott Japan, Co., Ltd.), and lactate, base excess, pH, hematocrit, and potassium by a blood gas analyzer (RAPIDLAB[®]1265, SIEMENS, Deerfield, IL, USA), immediately. The remainder of the blood was centrifuged at 3,000 rpm for 15 minutes at 4 °C, and measured the total cholesterol (Fuji DRI-CHEM 3500, FUJI FILM, Tokyo, Japan).

Statistical analysis

Data are shown as mean $\pm$ standard deviation. Statistical analyses were performed using GraphPad Prism 5 (La Jolla, CA, USA). The survival rate in the four groups was

analyzed by the log-rank test. Multiple comparisons between the groups were assessed by 2-way repeated-measures ANOVA or 2-way ANOVA, followed by Bonferroni post hoc test.

Results

High-fat diet-fed mice administered 2-DG exhibited an absolute mortality within 9 days, but the other 3 groups remained alive throughout the period (Figure 1). Rectal temperature was consistent in all groups between days 1 and 4, but the high-fat diet-fed mice administered 2-DG progressed to decrease it from day 5 to 8 (Figure 2A). Subsequently, high-fat diet mice administered 2-DG reduced the body weight (Figure 2B), and water consumption from day 6 to 8 (Figure 2C). Along with the decline in food intake (Figure 2D), the ratio of 2-DG over carbohydrate intake increased in mice fed high-fat diet (Figure 2E). In blood analysis, high-fat diet-fed mice administered 2-DG exhibited increases of β -hydroxybutyric acid and lactate levels, and decreases of base and pH. In addition, they revealed elevations of hematocrit and potassium ion levels (Supplementary figure S2A-F). Glucose concentration remained unchanged in respective group, but total cholesterol level raised in high-fat diet-fed mice administered 2-DG, despite the decrease of food (cholesterol) intake (Supplementary figure S3A and B).

Discussion

It has been debated whether lipid combusts in a flame of carbohydrate (Manninen 2004). Our data suggest that glycolytic pathway plays an important role for the lipid-metabolism to maintain the physiological responses.

In this study, glycolytic inhibition with 2-DG did not affect any physiological parameters on standard diet. However, high-fat diet with 2-DG progressed to decrease rectal temperature, food and drinking intakes, followed by declining the body weight. All mice used in this study gained the body weight from 19.0 ± 1.27 to 21.5 ± 1.35 g during the run-in for one week fed a standard diet. However, control mice fed a standard diet did not gain the body weight during the experimental periods. Repeated rectal probe insertion may have affected the growth of mice (Bae et al. 2007). Physiological parameters have varied since 5 days of 2-DG administration, and it corresponds that metabolic profile shifts from carbohydrate to lipid following the substantially reduced carbohydrate consumption (Soeters et al. 2012; Paoli et al. 2013). Carbohydrates produces 3-folds more thermogenesis than fat per calorie; on the contrary, 2-DG reduces temperature through hypothalamic neurons (Mobbs et al. 2007). High-fat diet has reduced the amounts of food and drinking intakes regardless of 2-DG administration, and 2-DG further decreased them in mice fed a high-fat diet. Ketone bodies (acetoacetate, β -hydroxybutyric acid and acetone) are an alternative energy source when carbohydrate is short, and the magnitude of ketosis is associated with the incomplete oxidation of fatty acid (Kwiterovich et al. 2003). They are increased in the plasma and urine under starvation, or high-fat/low-carbohydrate diets (Westman et al. 2007; Paoli et al. 2013). Our data may simulate a case (Shah and Isley 2006) that a woman who intakes low-carbohydrate diet (<20 g/day) with fat-rich meals developed ketoacidosis.

It remains to be elucidated the underlying mechanism by which high-fat diet-fed mice with 2-DG exhibited a high mortality. Low-carbohydrate on high-fat diet suppresses the appetite (Erlanson-Albertsson and Mei 2005), whereas lactate stimulates ketosis during starvation (Shah and Isley 2006). Specifically, increase of free fatty acid further

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decreases appetite in the setting of ketosis (Paoli et al. 2015). The increased ratio of 2-DG intake over dietary carbohydrate consumption may have exaggerated the metabolic profile to ketogenesis. Water consumption is strongly linked to food intake (Ellacott et al. 2010), and we speculate that the increased renal excretion of ketone bodies with sodium and water (Denke 2001), along with the lack of feeding behavior is attributable to the peripheral circulatory insufficiency (dehydration), leading to a high mortality. We did not assess the availability of glucose for whole body when 2-DG is administered. Glucose utilization after starvation depends on tissues in which glucose concentration keeps at levels sufficient to ensure an appropriate supply to the brain (Cherel et al. 1988; Janigro 1999). 2-DG does not only inhibit the glycolytic pathway but also it interfere the various metabolic processes, including the pentose phosphate pathway (Urakami et al. 2013). Inhibition of syntheses of nucleic acid and NADPH by 2-DG may also affect the growth and outcome of these mice. It is noted that energy balance in mice is substantially different from humans. Mice expend a greater amounts of daily energy budget than humans to maintain core temperature (Ellacott et al. 2010), explaining the detrimental consequence within a short period in this study. In consistent with a report (Ingram et al. 2004), our data shows that 2-DG did not affect the plasma concentration of glucose in each group of mice. However, it should be considered that glucose strip test can measure 2-DG as well as glucose (data not shown). Increase of cholesterol level in low-carbohydrate diet with unlimited calorie/high fat diet is explained by the increase of cholesterol intake; however, our study shows that cholesterol levels raised in high-fat diet-fed mice administered 2-DG, despite the malnutrition. 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) is a common intermediate in the mevalonate and ketone bodies, which is formed from acetoacetyl

CoA. Carbon unit of acetoacetate is incorporated directly into HMG-CoA and used for synthesizing cholesterol (Geelen et al. 1983). Our experiment suggests that benefit of low-carbohydrate diet may depend on the combination of other ingredients. Strict restriction of carbohydrate intake with high-fat regimen is effective to reduce the body weight, but it may have potential to increase the adverse effects.

Conclusion

This study suggests an important role for carbohydrate on lipid metabolism. In addition, our data may imply that strict glycolysis restriction might be harmful on high-fat diet.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Figure legends

Figure 1. Effect of 2-DG on survival rate in the 4 groups of mice

Parentheses indicate the number of mice used in the experiment. HFD: high-fat diet; 2-DG: 2-deoxyglucose. Statistical analysis was performed by Log-rank test.

Figure 2. Effects of 2-DG on physiological parameters such as rectal temperature (A), body weight (B), drinking (C), food intake (D), and the ratio of 2-DG to carbohydrate (E) in the 4 groups of mice. HFD: high-fat diet; 2-DG: 2-deoxyglucose.

Statistical analysis was performed by two-way repeated-measures ANOVA followed by Bonferroni's post hoc test. It was not possible to calculate the statistical analysis (E), because of lack of value due to zero g for food consumption in mice fed a high-fat diet administered 2-DG at day 8 [[†], standard diet/water vs. HFD/water; [#], standard diet/water vs. HFD/2-DG; ^{*}, standard diet/2-DG vs. HFD/2-DG; [‡], HFD/water vs. HFD/2-DG].

^{†, #, *} p<0.05; ^{††, ##, **, ‡‡} p<0.01; ^{†††, ###, ***, ‡‡‡} p<0.001. A-C (n=9); D-E (n=7)

Figure 1