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Research Report

Ketogenic diet does not impair spatial ability controlled by the hippocampus in male rats

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ABSTRACT

A ketogenic diet was recently shown to reduce glutamate accumulation in synaptic vesicles, decreasing glutamate transmission. We questioned whether a ketogenic diet affects hippocampal function, as glutamate transmission is critically involved in visuospatial ability. In the present study, male Wistar rats were maintained on a ketogenic diet containing 10% protein and 90% fat with complements for 3 weeks to change their energy expenditure from glucose-dependent to fat-dependent. Control rats were fed a diet containing 10% protein, 10% fat, and 80% carbohydrates. The fat-dependent energy expenditure induced by the ketogenic diet led to decreased body weight and increased blood ketone production, though the rats in the two groups consumed the same number of calories. The ketogenic diet did not alter food preferences for the control or high-fat diet containing 10% protein, 45% fat, and 45% carbohydrates. Anxiety in the open field was not altered by ingestion the ketogenic diet. However, rats fed the ketogenic diet performed better in the Y-maze test than rats fed the control diet. No difference was observed between the two groups in the Morris water maze test. Finally, Western blot revealed that the hippocampal expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-type glutamate receptor subunit 1 (GluR1) was significantly increased in mice fed a ketogenic diet. These results suggest that hippocampal function is not impaired by a ketogenic diet and we speculate that the fat-dependent energy expenditure does not impair visuospatial ability.

1. Introduction

A ketogenic diet is an extremely high-fat diet in which carbohydrates are largely substituted with fat for energy production. This diet increases ketone body production and the utilization of energy from fat instead of carbohydrates (Wheless, 2008), similar to starvation (Benoit et al., 1965). Thus, the liver mainly produces ketones (Bielohuby et al., 2011; Wheless, 2008). Ketone bodies become the main energy source for the central nervous system (Dietrich and Horvath,

Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GluR1, glutamate receptor subunit 1; GluR2, glutamate receptor subunit 2

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2011; Hallbook et al., 2011; Lutas and Yellen, 2012), though the reduction in carbohydrate metabolism is generally thought to lead to worse brain function. Clinical studies have reported that a ketogenic diet improves the convulsion states induced by central nervous system abnormalities, particularly those resistant to anticonvulsant drugs (Hori et al., 1997; Stafstrom, 1999). Although a large number of drugs have been developed to treat seizures, some patients continue to rely on fasting or a high-fat, low-carbohydrate ketogenic diet (Lutas and Yellen, 2012).

Juge et al. (2010) reported for the first time that a ketogenic diet competes with Cl^- for vesicular glutamate transport protein activation, inhibiting glutamate transmission *in vivo*. If this is the case, then the visuospatial ability of the hippocampus may be impaired by the ingestion of a ketogenic diet because glutamate and its receptors are essentially involved in such hippocampal functions (Maren and Quirk, 2004; Plant et al., 2006). Furthermore, if GABA action is enhanced, anxious behavior may be altered because GABA is a tranquilizing agent that treats anxiety and depression (Bough et al., 2007; Mohler, 2012; Vithlani et al., 2011).

On the other hand, a ketogenic diet may effectively reduce glucose metabolism (Mobbs et al., 2007) and improve diabetic symptoms and/or complications (Mobbs et al., 2013). A ketogenic diet was recently reported to reverse diabetic nephropathy, which is a profound diabetic complication (Poplawski et al., 2011). Thus, following a ketogenic diet may be beneficial, though there may be risks and users should be cautious (Schugar and Crawford, 2012). The present study aimed to clarify whether the fat-dependent energy expenditure caused by a ketogenic diet is harmful to the central nervous system. The focus of the study was the hippocampal visuospatial ability determined by behavior tests and biochemical analysis.

2. Results

Rats eating the ketogenic chow for 3 weeks had significantly decreased body weight compared to rats eating the control chow ($P<0.05$; Table 1). Serum β-butyric acid was significantly elevated in the ketogenic group compared to the control group ($P<0.05$) but no changes in blood glucose levels were observed ($P>0.9$). These findings indicate that the ketogenic diet reduced body weight and increased ketone production as expected.

Three groups of rats were fed a control, ketogenic, or high-fat diet for 3 weeks and then allowed to freely choose either ordinary chow (control diet) or a high-fat diet. The control group preferred to obtain more of their total calories from ordinary chow. The average proportion of daily caloric intake obtained from a high-fat diet during a 7-day interval was 34.6±6.3% in the control group ($n=5$), 73.7±2.9% in the high-fat diet group ($n=4$), and 73.5±9.3% in the ketogenic diet group ($n=4$) (ANOVA, $P<0.05$; Fig. 1a). Thus, the high-fat and ketogenic groups preferred to obtain a greater proportion of calories from the high-fat diet than from ordinary chow (post-hoc analysis, $P<0.05$). However, the total caloric intake did not differ among the groups (ANOVA, $P>0.4$; Fig. 1b).

We used the open field test to examine anxiety in control diet-fed ($n=11$) and ketogenic diet-fed ($n=8$) rats. The total distance moved was significantly greater in the ketogenic diet group (2628±117 mm vs. 2226±122 mm, $P<0.05$; Fig. 2b). This result suggests an increase in locomotor activity. Importantly, the time spent in the center area did not differ significantly between the two groups (control group: 14.6±2.9 s, $n=8$; ketogenic group: 9.3±1.9 s, $n=11$; $P>0.2$; Fig. 2c); the rats in both groups spent almost all their time in the walled, peripheral area

Table 1 – Effects of a ketogenic diet on body weight, blood glucose, and serum ketones.

	Control group	Ketogenic group
Body weight (g)	361±4.3 ($n=22$)	330±4.1 ($n=24$)*
Blood glucose (mM)	5.4±0.1 ($n=6$)	5.6±0.1 ($n=5$)
β-butyric acid (mM)	0.3±0.07 ($n=6$)	1.8±0.09 ($n=5$)*

* $P<0.05$.

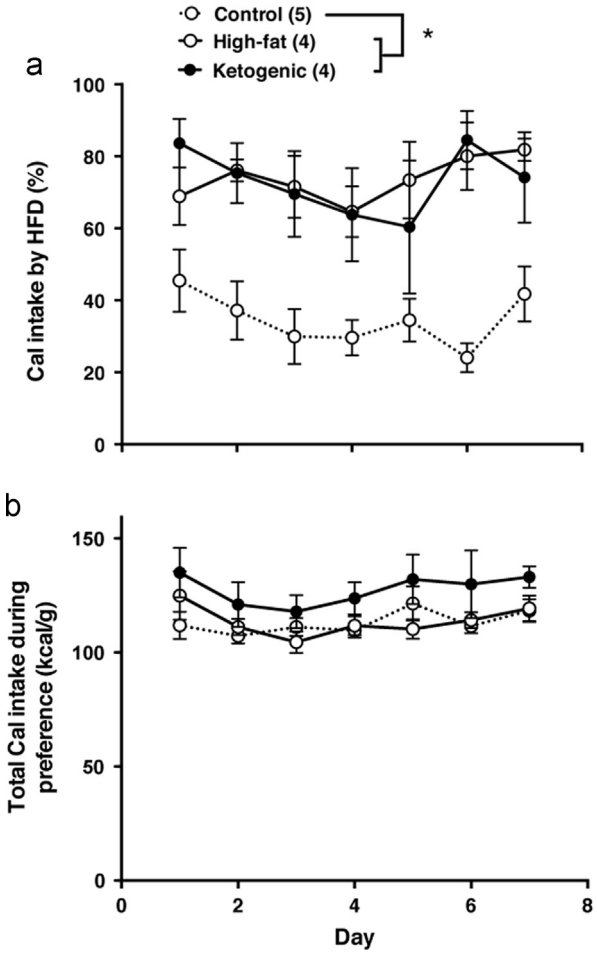

Fig. 1 – The effect of prior dietary experience on food preference. Rats were fed a control diet, a high-fat diet, or a ketogenic diet for 3 weeks and then given the choice of a control or high-fat diet for 1 week. (a) Food preference expressed as the proportion of calories obtained from a high-fat diet (HFD). (b) The total caloric intake was calculated each day for 1 week. Data are presented as mean±SEM. Numbers in parentheses refer to the number of rats. *$P<0.05$ vs. rats fed control chow.