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Short communications

A very low carbohydrate ketogenic diet increases hepatic glycosphingolipids related to regulation of insulin signalling

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ABSTRACT

Low carbohydrate ketogenic diet (KD) produces several ameliorating effects against metabolic abnormalities found in obese patients. However, mechanisms underlying the effect of KD are poorly understood. We report here that KD increases gangliosides, which were recently identified as a novel modulator involved in the regulation of cellular insulin signalling. We found that the *ob/ob* mice had low liver ganglioside content, whereas feeding KD increased this to approximately equivalent levels observed in wild-type mice. This result indicates that KD improves the low level expression of gangliosides in *ob/ob* mouse liver. On the other hand, feeding KD specifically increased only monosialoganglioside GM3 in wild-type mice. This work revealed the potential utility of low carbohydrate diet including KD as a functional food targeting insulin-related metabolic abnormalities.

1. Introduction

Low carbohydrate ketogenic diet (KD) has historically been known as a method for managing seizures. Recently, this diet has been utilized for the treatment of metabolic abnormalities in obese patients. KD can induce weight loss, decrease intrahepatic triglyceride content, and improve metabolic parameters in obese patients (Schugar & Crawford, 2012). From these remarkable effects, utilization of KD as functional food for controlling obesity has been proposed. However, several adverse effects have also been found in KD-fed rodents. For example, long-term maintenance on KD evokes hepatic steatosis, inflammation, and ER stress in C57BL/6 wild-type (WT) mice (Garbow et al., 2011). Systemic glucose intolerance and insulin resistance have also been found in KD-fed WT mice (Garbow et al., 2011; Jornayvaz et al., 2010). Thus, characterization of the effect of KD at the molecular level *in vivo* is necessary to provide more effective use of KD as functional food.

Recent *in vivo* and *in vitro* studies revealed that gangliosides, a class of acidic glycosphingolipid, participate in the regulation of insulin signalling in cellular membranes (Herzer, Meldner, Gröne, & Nordström, 2015; Kabayama et al., 2007; Tagami et al., 2002; Yamashita et al., 2003). For example, genetically engineered mice lacking gangliosides showed abnormally enhanced insulin sensitivity in skeletal muscle (Yamashita et al., 2003) and the basal hypothalamus (Herzer et al., 2015). Kabayama and colleagues also found that ganglioside plays an important role in the insulin receptor signal

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transduction (Kabayama et al., 2007). These findings indicate that ganglioside is a novel modulator for insulin signalling, and prompted us to investigate the influence of KD on ganglioside content *in vivo*.

We report here that KD increases ganglioside content in liver of WT and leptin deficient mutant (*ob/ob*) mice in a different manner. This finding represents a novel effect of KD on insulin signalling and provides important information about the utilization of low carbohydrate diet including KD as a functional food for controlling obesity in both healthy individuals and obese patients.

2. Materials and methods

2.1. Animals, dietary studies, and biochemical analyses

WT and *ob/ob* mice of the inbred strain C57BL/6J (Charles River Laboratories Japan, Yokohama, Japan) were maintained as reported previously (Okuda & Morita, 2012). CE-2 (CLEA Japan, Tokyo, Japan) was used as regular chow and is composed of 58.2% carbohydrate, 12.6% fat, and 29.2% protein by calorie. F3666 (Bio-Serv, Frenchtown, NJ, USA) was used as the KD and is composed of 1.7% carbohydrate, 93.9% fat, and 4.4% protein by calorie. Five-week-old mice were raised on either a chow or KD diet for a period of seven weeks, after which, samples were collected. Biochemical analyses were performed as reported previously (Okuda & Morita, 2012). The Committee for Experiments involving Animals of the National Institute of Advanced Industrial Science and Technology (AIST) approved all animal experiments.

2.2. Western blot analysis

Protein extraction and western blotting were performed as reported previously (Okuda, Fukui, & Morita, 2013). Anti-SCD1 (C12H5), anti-GRP78/Bip (C50B12), anti-IR-β (4B8), and anti-GAPDH (D16H11) were purchased from Cell Signaling Technology (Danvers, MA, USA). An image analyser (ImageJ, http://imagej.nih.gov/ij/) was used to measure the intensities of the detected bands.

2.3. Glycosphingolipid extraction and thin layer chromatography (TLC)

Glycosphingolipid extraction and TLC were performed as reported previously (Okuda, Furukawa, & Nakayama, 2009). Briefly, total lipids from 100 mg of liver were sequentially extracted with chloroform/methanol/water 2:1:0 and 1:2:0.8 (*v/v/v*). Gangliosides and neutral glycosphingolipids were separated by Folch partitioning, and purified using a Sep-Pac C18 cartridge (Waters, Milford, MA, USA) or Iatrobeads 6RS-8060 (Mitsubishi Kagaku Iatron, Tokyo, Japan), respectively. Purified gangliosides corresponding to 10 mg of liver were analysed on HPTLC plates (Merck, Darmstadt, Germany) with a solvent system consisting of chloroform/methanol/water (60:35:8, *v/v/v*), and were visualized using orcinol–H_2SO_4. ImageJ was used to measure the ganglioside intensities on the TLC plates.

2.4. Statistical analysis

After determination of variance by F-test, statistical significance was determined using the two-tailed Student's t-test, with statistical significance defined as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Effects of KD on metabolic parameters and protein expression in WT and ob/ob mice

We have recently developed a KD-fed animal model using *ob/ob* mice and reported the resultant liver phenotypes (Okuda & Morita, 2012). In this study, we implemented the KD-fed animal model using *ob/ob* as well as WT mice. The metabolic parameters of the WT and *ob/ob* mice are summarized in Table 1. In *ob/ob* mice, the KD significantly reduced high blood

Table 1 – Effects of KD feeding on metabolic parameters in mice.

	c57BL/6		*ob/ob*	
	Chow	KD	Chow	KD
Serum				
Blood glucose (mg/dl)	290.93 ± 75.70	262.83 ± 65.07	374.71 ± 92.69	171.17 ± 47.55***
β-Hydroxybutyrate (mmol/l)	0.17 ± 0.08	3.98 ± 0.98***	0.34 ± 0.17	1.29 ± 0.80*
Insulin (ng/ml)	ND	0.62 ± 1.52	19.34 ± 10.50	1.61 ± 0.79*
Triglycerides (mg/dl)	194.64 ± 90.74	108.33 ± 32.94	139.33 ± 34.12	182.67 ± 65.56
Cholesterol (mg/dl)	58.81 ± 11.12	141.36 ± 41.73**	153.57 ± 36.87	215.86 ± 23.75*
Liver				
Weight (g)	0.98 ± 0.06	0.98 ± 0.07	3.74 ± 0.34	1.24 ± 0.24***
Total triglycerides (mg)	18.72 ± 4.97	62.17 ± 16.76***	246.20 ± 93.71	57.14 ± 21.56**
Triglycerides/liver (mg/g)	19.17 ± 5.68	63.65 ± 16.44***	65.75 ± 24.70	48.79 ± 16.93
Total cholesterol (mg)	4.30 ± 2.56	15.14 ± 3.16***	53.92 ± 18.65	12.34 ± 4.54**
Cholesterol/liver (mg/g)	4.49 ± 2.90	14.78 ± 6.01**	15.58 ± 3.42	10.62 ± 3.57*
Diet intake (kcal/day)	10.48 ± 0.46	10.41 ± 1.50	18.56 ± 0.40	17.27 ± 2.43

Abbreviations: Chow, regular chow diet; KD, ketogenic diet; ND, not detected; *ob*, obese.
Data are presented as means ± s.d.; n = 5–7. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Chow vs KD. Significant differences were assessed using the Student's t-test. The values for *ob/ob* mice include previously reported data (Okuda & Morita, 2012).