NOTE

The Safety of Very-long-term Intake of a Ketogenic Diet Containing Medium-chain Triacylglycerols

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Abstract: We previously reported that consuming a ketogenic diet containing medium-chain triacylglycerols (MCTs) might be a valuable dietary strategy for endurance athletes. However, the long-term safety of the diet has not been established, and there is a concern that a higher intake of MCTs increases the liver triacylglycerol content. In this study, we found that consuming an MCT-containing ketogenic diet for 24 weeks decreased, rather than increased, the liver triacylglycerol concentration and did not aggravate safety-related blood biomarkers in male Wistar rats. Our results may therefore suggest that the long-term intake of a ketogenic diet containing MCTs may have no deleterious effects on physiological functions.

Key words: ketogenic diet, medium chain triacylglycerols, β -hydroxybutyrate, liver triacylglycerol, rat

1 Introduction

A very high-fat and extremely low-carbohydrate diet, known as the ketogenic diet, enhances the production of ketone bodies¹⁾, which have been suggested to be energy-efficient substrates²⁾. Consuming the ketogenic diet might therefore be an effective dietary strategy to improve athletic performance in endurance events³⁾, and this diet has recently begun to attract the attention of athletes⁴⁾. However, previous studies demonstrated low adherence among individuals who chose to consume the ketogenic diet, because it consists almost exclusively of fat, with an extremely limited carbohydrate content⁵⁾.

Medium-chain fatty acids (MCFAs), which consist of 8-10 carbon atoms, are transported via the portal vein to the liver and potentially promote the production of ketone bodies⁶⁾. We previously reported that incorporating medium-chain triacylglycerols (MCTs), which are composed exclusively of MCFAs, into ketogenic diets could allow for the consumption of a higher carbohydrate content and a lower fat content while preserving ketosis, and that consuming an MCTs-containing ketogenic diet (MKD) for 8 weeks substantially enhanced the ketone body utilization capacity in rat skeletal muscle⁷⁾. Although our results suggested that

the MKD might be a more feasible and effective diet for athletes, the safety of MKD intake over the long-term is not sufficiently supported by data. In particular, there has been a concern that long-term intake of the MKD impairs liver function, because a previous study reported that the intake of MCTs induced hepatic triacylglycerol accumulation⁸⁾. Thus, the purpose of the present investigation was to evaluate the effects of very long-term (24 weeks) intake of MKD on liver triacylglycerol levels and safety-related biomarkers in rats.

2 Experimental Procedures

2.1 Animals and diets

The treatment of the animals was conducted according to the methods of Fukazawa *et al.*⁷⁾. The animal room was maintained at $23 \pm 1^\circ\text{C}$ with $50 \pm 5\%$ humidity and illumination from 09:00 to 21:00. Nine-week-old male Wistar rats (Japan SLC, Inc. Shizuoka, Japan) were individually housed in cages and were treated in accordance with the national guidelines for the care and use of laboratory animals (Notification of the Prime Minister's Office of Japan). The

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Animal Experimental Committee of The University of Tokyo approved all experimental protocols (approval no. 29–10).

After a 9-day acclimation period, the rats were separated into three groups according to body weight. One group was fed a diet based on the AIN-93 M formula (CON group; $n = 11$), and the second and third groups were fed ketogenic diets containing long-chain triacylglycerols (LCTs) (LKD group; $n = 12$) or MCTs (MKD group; $n = 12$), respectively. The overall compositions and fatty acid compositions of each diet were described previously⁷⁾. The rats were allowed free access to water and to the diets, and their body weights and food intake were measured every second day.

The dietary treatment was continued for 24 weeks. During the dietary treatment, approximately 50- μ L blood samples were collected from the tail vein using heparinized microhematocrit capillary tubes (Thermo Fisher Scientific, Waltham, MA) to measure the plasma β -hydroxybutyrate (β HB) level every 4 weeks. The collected blood samples were immediately centrifuged to collect plasma, which was stored at -80°C until analysis.

2.2 Tissue sampling

At the end of the dietary intervention, blood samples were taken via cardiac puncture under isoflurane anesthesia and centrifuged to collect serum. The liver was quickly dissected out, weighed, and then frozen in liquid N_2 and stored at -80°C until analysis. Intra-abdominal fat (the epididymal, mesenteric, and retroperitoneal fat pads combined) was removed and weighed.

2.3 Analytical procedure

2.3.1 Liver triacylglycerol

The liver triacylglycerol concentration was determined according to the method of Karasawa *et al.*⁹⁾.

2.3.2 Safety-related biomarkers in plasma and serum

The energy substrates and biomarkers in the plasma and serum were determined using kits obtained from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan) (Autokit 3-HB, NEFA C-Test Wako, Triglyceride E-Test Wako, LabAssay Cholesterol, Glucose C2 Test Wako, Transaminase C2 Test Wako, LabAssay Creatinine, A/G B-Test Wako).

2.4 Statistical analysis

Data are presented as the means $\pm$ standard errors of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA). Whenever the ANOVA indicated significant effects, a Tukey-Kramer test was used for post-hoc analysis. All statistical analyses were performed using BellCurve for Excel software (Social Survey Research Information, Tokyo). Statistical significance was defined as $p < 0.05$.

3 Results and Discussion

We previously reported that LKD and MKD intake both elevated the plasma β HB concentration in rats to a similar extent, even though the MKD contained more carbohydrates (18% of total energy vs. 1% in the LKD)⁷⁾. In this study, the LKD and MKD groups had significantly higher area-under-the-curve values for the plasma β HB concentration above the baseline during the 24-week intervention compared with the CON group (LKD, 304.2 ± 15.8 ; MKD, 347.2 ± 23.3 ; CON, 4.0 ± 0.3 mmol·day; $p < 0.001$, LCT or MKD vs. CON), whereas no significant difference was observed between the two ketogenic diet groups. These results confirmed our previous finding and provided additional evidence that a ketogenic diet containing MCTs is an effective and feasible dietary treatment for inducing ketosis, as the diet has relatively higher carbohydrate and lower lipid contents than standard ketogenic diets.

Consuming a ketogenic diet is widely used as an effective weight loss strategy¹⁰⁾. Our previous study also demonstrated that rats fed the LKD or MKD for 8 weeks had significantly lower body weights and intra-abdominal fat weights compared with CON-fed rats⁷⁾. Contrary to our previous finding, the LKD group had a similar final body weight and intra-abdominal fat weight relative to the CON group in the present study (Table 1), suggesting that the body-weight-lowering effects induced by the LKD had been lost during the longer-term (24-week) intervention. In contrast, the MKD-fed rats had significantly lower final body weights and intra-abdominal fat weights than the rats in the CON and LKD groups, despite their having a similar total energy intake relative to the CON group (Table 1). These results are consistent with previous findings showing that intake of MCTs decrease body weight and adiposity, possibly through an increase in energy expenditure¹¹⁾ and suggest that longer-term intake (>24 weeks) of the MKD still has body-weight- and body-fat-lowering effects. A previous study has demonstrated that dietary intakes of MCTs for 12 weeks increased uncoupling protein 1 (UCP1) content in brown adipose tissue, which mediates energy dissipation to generate heat, and boosted thermogenesis in mice¹²⁾. It is therefore plausible that the MKD-induced decreases in body weight and intra-abdominal fat weight might be mediated by the upregulation of UCP1 content, although we could not measure its expression in the present investigation.

The most serious concern associated with the intake of MCTs is hepatic triacylglycerol accumulation. Shinohara *et al.* demonstrated that rats fed a diet containing MCTs (with 11% of the total energy from MCTs) for 1 month had significantly higher triacylglycerol levels in the liver compared with rats fed the AIN-93-based control diet⁸⁾. In the present study, contrary to our expectations, 24-week intake of the MKD, which had a higher MCT content (with 59% of the total energy from MCTs), decreased, rather than increased,

Table 1 Body weight, total energy intake, and intra-abdominal fat and liver weights in rats.

	CON	LKD	MKD
Initial body weight (g)	237 ± 2	247 ± 2	245 ± 2
Final body weight (g)	454 ± 7	440 ± 9	385 ± 10*** §§§
Total energy intake (kcal)	11154 ± 120	9859 ± 219***	10868 ± 274
Intra-abdominal fat weight (g)	40.8 ± 1.3	46.5 ± 1.6	30.4 ± 2.2*** §§§
Liver weight (g)	14.8 ± 0.4	11.0 ± 0.4***	11.6 ± 0.3***

Values are means ± SEM, n = 12, except in the CON group (n = 11). *** indicates significant differences from the values obtained in the CON group at $p < 0.001$. §§§ indicates significant differences from the values obtained in the LKD group at $p < 0.001$.

Fig. 1 Effects of long-term intake of the LKD or MKD on triacylglycerol concentration in the rat liver. Values are means ± SEM, n = 12, except in the CON (n = 11). *** and * indicate significant differences from the values obtained in the CON group at $p < 0.001$ and $p < 0.05$, respectively. §§§ indicates significant differences from the values obtained in the LKD group at $p < 0.001$.

the liver triacylglycerol concentration and liver weight in rats, whereas the LKD-fed group had a significantly higher liver triacylglycerol concentration compared with the CON group (Fig. 1). In addition, no significant differences in the serum glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) concentrations, which are well-known biochemical markers for liver disorder, were observed among the three groups (Table 2). Based on these results, it is likely that longer-term intake of the MKD has no deleterious effects on liver function. However, a

previous study reported that the intake of MCTs for 1 year significantly increased the liver triacylglycerol concentration and serum GOT and GPT concentrations in mice¹³. Future studies may therefore be required to evaluate the effects of longer-duration MKD intake (~1 year). In addition, the underlying mechanism of the MKD-induced lower hepatic triacylglycerol concentration needs to be determined.

Some previous studies indicated that a ketogenic diet induces significant increases in the blood lipid levels¹⁴, which is a risk factor for the development of atherosclerosis¹⁵. Our results were consistent with these findings, as they showed that the plasma free fatty acid concentration was significantly higher in the LKD group than in the CON group and that the plasma triacylglycerol level in the LKD group also tended to be higher than in the CON group (Table 3). In contrast, such increases in plasma lipids were not observed in the MKD group (Table 3). MCFAs are absorbed through the portal vein without re-esterification in enterocytes. Moreover, MCFAs are metabolized rapidly in liver mitochondria and are converted into ketone bodies, which are then released into blood³. Because of these unique characteristics, MCFAs are less likely to induce increase in blood triacylglycerols and free fatty acids levels. Actually, previous studies reported that intake of MCTs prevented increases in the blood lipids^{16–18}. The present findings are consistent with those findings and suggest that a ketogenic diet containing MCTs has a less atherogenic effect.

As shown in Table 3, the plasma glucose, serum total protein, and serum albumin concentrations in the MKD group were not significantly different from those in the CON group and were within the normal ranges¹⁹. In addition,

Table 2 Serum GPT and GOT concentrations in rats.

	CON	LKD	MKD
Serum GPT (IU/L)	38.7 ± 3.2	45.7 ± 3.0	44.0 ± 5.3
Serum GOT (IU/L)	138 ± 16	124 ± 11	111 ± 12

Values are means ± SEM, n = 12, except in the CON group (n = 11).

Table 3 Energy substrates and safety-related biomarkers in plasma and serum.

	CON	LKD	MKD
Plasma glucose (mg/dL)	133 ± 2	146 ± 9	116 ± 3 ^{§§}
Plasma triacylglycerol (mg/dL)	219 ± 23	271 ± 49	180 ± 13
Plasma free fatty acid (mEq/L)	0.813 ± 0.056	1.51 ± 0.16***	0.866 ± 0.046 ^{§§§}
Plasma total cholesterol (mg/dL)	88.4 ± 9.1	76.7 ± 14.6	38.8 ± 3.9** [§]
Serum total protein (g/dL)	7.72 ± 0.15	8.82 ± 0.93	6.61 ± 0.13 [§]
Serum albumin (g/dL)	4.68 ± 0.05	4.26 ± 0.07	4.74 ± 0.58
Serum creatinine (mg/dL)	0.653 ± 0.067	0.698 ± 0.045	0.402 ± 0.036** ^{§§}

Values are means ± SEM, n = 12, except in the CON group (n = 11). *** and ** indicate significant differences from the values obtained in the CON group at $p < 0.001$ and $p < 0.01$, respectively. §§§, §§ and § indicate significant differences from the values obtained in the LKD group at $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively.

tion, serum creatinine was significantly lower in the MKD group than in the CON and LKD groups. It is therefore less likely that longer-term intake of a ketogenic diet containing MCTs would have unfavorable effects on physiological functions such as the glucose and protein metabolisms and kidney function.

4 Conclusion

Long-term intake of a ketogenic diet containing MCTs may have no deleterious effects on physiological functions and may be a safer dietary treatment than a conventional ketogenic diet.

Conflicts of Interest

Shin Terada received grants from the Nisshin Oillio Group, Ltd., Tokyo, Japan, while this study was being conducted. The other authors declare that they have no conflicts of interest.

References

- 1) Robinson, A.M.; Williamson, D.H. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev* **60**, 143-187 (1980).
- 2) Sato, K.; Kashiwaya, Y.; Keon, C.A.; Tsuchiya, N.; King, M.T.; Radda, G.K.; Chance, B.; Clarke, K.; Veech, R.L. Insulin, ketone bodies, and mitochondrial energy transduction. *Faseb J* **9**, 651-658 (1995).
- 3) Chang, C.K.; Borer, K.; Lin, P.J. Low-carbohydrate-high-fat diet: Can it help exercise performance? *J. Hum. Kinet* **56**, 81-92 (2017).
- 4) Volek, J.S.; Freidenreich, D.J.; Saenz, C.; Kunces, L.J.; Creighton, B.C.; Bartley, J.M.; Davitt, P.M.; Munoz, C.X.; Anderson, J.M.; Maresh, C.M.; Lee, E.C.; Schuenke, M.D.; Aerni, G.; Kraemer, W.J.; Phinney, S.D. Metabolic characteristics of keto-adapted ultra-endurance runners. *Metabolism* **65**, 100-110 (2016).
- 5) Shai, I.; Schwarzfuchs, D.; Henkin, Y.; Shahar, D.R.; Witkow, S.; Greenberg, I.; Golan, R.; Fraser, D.; Bolutin, A.; Vardi, H.; Tangi-Rozental, O.; Zuk-Ramot, R.; Sarusi, B.; Brickner, D.; Schwartz, Z.; Sheiner, E.; Marko, R.; Katorza, E.; Thiery, J.; Fiedler, G.M.; Bluhner, M.; Stumvoll, M.; Stampfer, M.J. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N. Engl. J. Med.* **359**, 229-241 (2008).
- 6) Bach, A.C.; Babayan, V.K. Medium-chain triglycerides: An update. *Am. J. Clin. Nutr.* **36**, 950-962 (1982).
- 7) Fukazawa, A.; Koike, A.; Karasawa, T.; Tsutsui, M.; Kondo, S.; Terada, S. Effects of a ketogenic diet containing medium-chain triglycerides and endurance training on metabolic enzyme adaptations in rat skeletal muscle. *Nutrients* **12**, 1269 (2020).
- 8) Shinohara, H.; Ogawa, A.; Kasai, M.; Aoyama, T. Effect of randomly interesterified triacylglycerols containing medium- and long-chain fatty acids on energy expenditure and hepatic fatty acid metabolism in rats. *Biosci. Biotechnol. Biochem.* **69**, 1811-1818 (2005).
- 9) Karasawa, T.; Kondo, S.; Fukazawa, A.; Koike, A.; Tsutsui, M.; Terada, S. Effects of dietary fat restriction on endurance training-induced metabolic adaptations in rat skeletal muscle. *J. Oleo Sci.* **70**, 235-262 (2021).
- 10) Jornayvaz, F.R.; Jurczak, M.J.; Lee, H.Y.; Birkenfeld, A.L.; Frederick, D.W.; Zhang, D.; Zhang, X.M.; Samuel, V.T.; Shulman, G.I. A high-fat, ketogenic diet causes hepatic insulin resistance in mice, despite increasing energy expenditure and preventing weight gain. *Am. J. Physiol. Endocrinol. Metab.* **299**, E808-815 (2010).
- 11) St-Onge, M.P.; Ross, R.; Parsons, W.D.; Jones, P.J. Medium-chain triglycerides increase energy expenditure and decrease adiposity in overweight men. *Obes. Res.*

- 11, 395-402 (2003).
- 12) Zhang, Y.; Xu, Q.; Liu, Y.H.; Zhang, X.S.; Wang, J.; Yu, X.M.; Zhang, R.X.; Xue, C.; Yang, X.Y.; Xue, C.Y. Medium-chain triglyceride activated brown adipose tissue and induced reduction of fat mass in C57BL/6J mice fed high-fat diet. *Biomed. Environ. Sci.* **28**, 97-104 (2015).
- 13) Tucci, S.; Flögel, U.; Sturm, M.; Borsch, E.; Spiekerkoetter, U. Disrupted fat distribution and composition due to medium-chain triglycerides in mice with a β -oxidation defect. *Am. J. Clin. Nutr.* **94**, 439-449 (2011).
- 14) Ellenbroek, J.H.; van Dijck, L.; Töns, H.A.; Rabelink, T.J.; Carlotti, F.; Ballieux, B.E.; de Koning, E.J. Long-term ketogenic diet causes glucose intolerance and reduced β - and α -cell mass but no weight loss in mice. *Am. J. Physiol. Endocrinol. Metab.* **306**, E552-558 (2014).
- 15) Ziemann, S.J.; Melenovsky, V.; Kass, D.A. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb. Vasc. Biol.* **25**, 932-943 (2005).
- 16) Sung, M.H.; Liao, F.H.; Chien, Y.W. Medium-chain triglycerides lower blood lipids and body weight in streptozotocin-induced type 2 diabetes rats. *Nutrients* **10** (2018).
- 17) Calabrese, C.; Myer, S.; Munson, S.; Turek, P.; Birdsall, T.C. A cross-over study of the effect of a single oral feeding of medium chain triglyceride oil vs. canola oil on post-ingestion plasma triglyceride levels in healthy men. *Altern. Med. Rev.* **4**, 23-28 (1999).
- 18) Nonaka, Y.; Takagi, T.; Inai, M.; Nishimura, S.; Urashima, S.; Honda, K.; Aoyama, T.; Terada, S. Lauric acid stimulates ketone body production in the KT-5 Astrocyte cell Line. *J. Oleo Sci.* **65**, 693-699 (2016).
- 19) Clinical Laboratory Parameters for Crl:WI (Han) [Internet]. Wilmington, MA: Charles River Laboratories, Inc. https://www.criver.com/sites/default/files/resources/rm_rm_r_Wistar_Han_clin_lab_parameters_08.pdf. Accessed 17 March 2020.

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