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Lauric Acid Stimulates Ketone Body Production in the KT-5 Astrocyte Cell Line

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Abstract: Coconut oil has recently attracted considerable attention as a potential Alzheimer's disease therapy because it contains large amounts of medium-chain fatty acids (MCFAs) and its consumption is thought to stimulate hepatic ketogenesis, supplying an alternative energy source for brains with impaired glucose metabolism. In this study, we first reevaluated the responses of plasma ketone bodies to oral administration of coconut oil to rats. We found that the coconut oil-induced increase in plasma ketone body concentration was negligible and did not significantly differ from that observed after high-oleic sunflower oil administration. In contrast, the administration of coconut oil substantially increased the plasma free fatty acid concentration and lauric acid content, which is the major MCFA in coconut oil. Next, to elucidate whether lauric acid can activate ketogenesis in astrocytes with the capacity to generate ketone bodies from fatty acids, we treated the KT-5 astrocyte cell line with 50 and 100 μM lauric acid for 4 h. The lauric acid treatments increased the total ketone body concentration in the cell culture supernatant to a greater extent than oleic acid, suggesting that lauric acid can directly and potently activate ketogenesis in KT-5 astrocytes. These results suggest that coconut oil intake may improve brain health by directly activating ketogenesis in astrocytes and thereby by providing fuel to neighboring neurons.

Key words: medium-chain fatty acid, lauric acid, ketone body, astrocyte

1 INTRODUCTION

In recent years there has been dramatic increase in dementia prevalence, which is predicted to further double every 20 years until 2040[1)]. Alzheimer's disease (AD), which is characterized by the progressive deterioration of cognitive function, is the leading cause of dementia. Although the mechanisms leading to AD pathology remain to be fully elucidated, it is now well documented that the uptake as well as metabolism of glucose, the predominant energy source the brain, are impaired in the brains of AD patients[2,3)].

Because many studies have linked the risks of AD to diet-modifiable conditions such as type 2 diabetes and hypertension[4–6)], dietary approaches may play an important role in preventing and managing AD. Such approaches have thus recently attracted a great deal of attention. Recent studies have suggested that dietary intake of coconut oil may be beneficial in the prevention and treatment of AD[7)]. Unlike most other dietary fats that are rich in long-chain fatty acids (LCFAs), coconut oil is composed of medium-chain fatty acids (MCFAs). MCFAs are transported via the portal vein to the liver[8,9)], where they can give rise to ketone bodies (KBs), which are thought to be able to compensate as an alternative energy source for the reduced brain glucose uptake in AD patients[10)]. However, the portal absorption of the MCFA lauric acid (C12:0), which is the major fatty acid constituent of coconut oil, might be less than one-fourth of the lymphatic absorption[11)]. Furthermore, in our hands, the coconut oil-induced increases in blood KB concentration are almost negligible, although there is a substantial elevation in the blood concentration of free fatty acid (FFA). This finding suggests that coconut oil may exert its favorable effects on cognitive function through mechanisms other than enhanced hepatic ketogenesis.

Astrocytes, which are the major class of glia cells in the brain of humans and other mammals, are crucial in regulating brain energy metabolism. As they are located around

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intraparenchymal blood capillaries, astrocytes are the first cellular barrier on the route to brain tissue encountered by nutrients and other substances. Accumulating evidence suggests that fatty acids can cross the blood-brain barrier and that astrocytes have a greater preference for fatty acids as their primary energy fuel and produce larger amounts of KBs, which are taken up by neighboring neurons and serve as oxidative fuels during hypoxia and hypoglycemia[12, 13]. Furthermore, the major regulatory and rate-limiting step of ketogenesis in astrocytes is carnitine palmitoyltransferase I (CPT-I)[14], which can be bypassed by MCFAs[8, 9]. These findings led us to hypothesize that (1) coconut oil intake would induce a large increase in blood lauric acid content and (2) lauric acid can serve as a preferential substrate for ketogenesis and/or stimulate ketogenesis in astrocytes but not hepatocytes, supplying neurons with KBs as an alternative energy source. To obtain preliminary evidence on this theory, we therefore reevaluated the blood MCFA and KB responses after oral administration of coconut oil to rats and examined the effects of treatment with lauric acid on KB production in KT-5 astrocyte cell cultures to determine whether lauric acid can increase astrocyte ketogenesis.

2 EXPERIMENTAL

2.1 Materials

The KT-5 astrocyte cell line was obtained from Japanese Collection of Research Bioresources (Osaka, Japan). The medium-chain triacylglycerol (MCT) oil, which is composed exclusively of caprylic acid (C8:0) and capric acid (C10:0), and coconut oil were obtained from the Nisshin OilliO Group Ltd. (Tokyo, Japan). High-oleic sunflower oil was purchased from Showa Sangyo Co., Ltd. (Tokyo, Japan). Cell culture reagents were obtained from Thermo Fisher Scientific Inc. (Waltham, MA). Fatty acids were purchased from Sigma-Aldrich (St. Louis, MO). Reagents for measurements of fatty acid composition were obtained from Wako Pure Chemicals (Osaka, Japan).

2.2 Experiment 1: Effects of oral lipid administrations on plasma KB concentrations and fatty acid compositions in rats

2.2.1 Treatment of animals

Animal treatment was conducted according to the methods of Terada *et al.*[15]. Briefly, 7-week-old male Sprague-Dawley rats were obtained from CLEA Japan Inc. (Tokyo, Japan) and housed individually in cages. The animal room was kept at 23 ± 1℃ with 50 ± 5% humidity and illumination from 09:00 to 21:00 h. All animals were treated in accordance with the guidelines for the care and use of laboratory animals (Notification of the Prime Minister's Office in Japan). The experimental protocols were approved by the Animal Experimental Committee of The University of Tokyo. During the acclimation period, the rats were given access to water and standard powdered food ad libitum (CE-2; CLEA Japan Inc.).

Table 1 Fatty acid compositions of test oils.

Fatty acid[$]	Sunflower	Coconut	MCT
	g/100 g fatty acids		
8:0	ND	8.3	73.9
10:0	ND	6.4	25.8
12:0	ND	46.7	0.3
14:0	ND	17.5	ND
16:0	ND	9.0	ND
18:0	3.4	3.1	ND
18:1	86.1	6.6	ND
18:2	7.7	1.9	ND
18:3	1.0	ND	ND
Others	1.8	0.5	0.0

Sunflower, high-oleic sunflower oil; MCT, medium-chain triacylglycerol oil; Coconut, coconut oil; ND, not detected.
[$] Numbers are carbon atoms: double bonds.

2.2.2 Lipid administrations

On the day before the experiment, rats were given food equal to 75% of the amount of food eaten daily during the acclimation period. Between 11:00 and 12:00 h on the next day, the rats were orally administered 10 mg/g body weight of either high-oleic sunflower oil (Sunflower group, n = 5), coconut oil (Coconut group, n = 5), or MCT oil (MCT group, n = 5) using a stainless-steel gavage needle with a ball diameter. The fatty acid compositions of the oils, as measured by gas chromatography[16], are listed in Table 1. Blood samples were collected from the tail vein into capillary tubes immediately before and 2 h and 4 h after the lipid administrations. The capillary tubes were then centrifuged and plasma samples were stored at −20℃ until analysis. The total KB, triacylglycerol (TG), and FFA concentrations and fatty acid composition in the plasma were determined as described below. The areas under the curves (AUCs) for total KB, TG, and FFA concentrations above the baselines (before oral administration) were determined using the trapezoidal rule.

2.3 Experiment 2: Effects of treatments with LCFAs and MCFAs on KB production in KT-5 astrocyte cells

2.3.1 Cell culture

KT-5 astrocyte cells, which stain positive for glial fibrillary acidic protein (GFAP, the astrocyte marker), were cultured in 100-mm polystyrene culture dishes in Ham's F12 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 4 mM HEPES, and 10% fetal bovine serum (FBS). KT-5 cells were maintained in a humidified incuba-

tor at 37℃ under an atmosphere of 5% CO_2. When KT-5 cells were approximately 80–90% confluent, the cells attached to the dishes were trypsinized with 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) and centrifuged. The pellet was resuspended in the culture media and the cells were plated in 12-well plates.

2.3.2 Treatment with fatty acids

KB productions in KT-5 astrocyte cells were measured according to the method of Le Foll *et al.*[17] with some modifications. Experimental treatments were begun when KT-5 cells were confluent in 12-well plates. KT-5 cells were washed once with assay solution (Ham's F12 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 4 mM HEPES, and 2% FBS) and then incubated with 200 μL of assay solution containing vehicle alone (dimethyl sulfoxide [DMSO]), oleic acid (C18:1), caprylic acid (C8:0), or lauric acid (C12:0) for 4 h. In preliminary experiments, we found that FBS contains a larger amount of KBs, which may mask the small changes in KB concentrations in culture media induced by fatty acid treatments, and that KT-5 cells can survive for at least 4 h in the assay solution. Thus, the FBS concentration of the assay solution was reduced to 2% so that the basal KB concentration could be minimized. Fatty acids were dissolved in DMSO and added to the assay solution to reach final concentrations of 50 and 100 μmol/L. Media (supernatant) were harvested after the 4-h treatment and total KB concentrations in the media were assayed as an index of the KB production of KT-5 cells.

2.4 Analytical procedures

2.4.1 Fatty acid composition of plasma lipid samples

The fatty acid composition of plasma samples was determined by gas chromatography of fatty acid methyl esters, with minor modifications of the method of Lepage and Roy[18]. Briefly, 45 μL of plasma and 1.9 mL of the stock solution containing 1.7 mL of methanol, 100 μL of acetyl chloride, and 100 μL of the internal standard solution (2.5 μg of 17:0 methyl ester) were combined in screw-capped glass tubes. Tubes were capped and heated at 100℃ for 60 min, then allowed to cool to room temperature before 0.25 mL of hexane was added. The tubes were then mixed for 30 s and the upper organic phase was collected with a Pasteur pipette. This extraction procedure was repeated as above to optimize lipid extraction. The combined hexane solution was transferred to GC vials and capped under nitrogen.

Fatty acid methyl esters were analyzed in the hexane extracts using a GC-2014 gas chromatograph (Shimadzu Co., Kyoto, Japan) equipped with a fused silica capillary column (TC-70), 60-m × 0.25-mm inner diameter (ID) × 0.25-μm film thickness (GL Sciences Inc.), a split/splitless injector, an AOC-20i automatic liquid sampler, and flame ionization detection. The helium was used as a carrier gas, with injector and detector temperatures of 250℃ and 260℃, respectively. The column temperature was held at 80℃ for 5 min and increased in a step-wise fashion to reach a plateau of 250℃. The gas chromatograph was calibrated using a standard mixture of fatty acids.

2.4.2 Biochemical assays

Total KB, TG, and FFA concentrations in rat plasma and culture media were analyzed using colorimetric assays (Triglyceride E-Test Wako, NEFA C-Test Wako, and Autokit Total Ketone Bodies, Wako Pure Chemicals, Osaka, Japan).

2.5 Statistical analysis

The data are presented as means ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). Whenever the ANOVA indicated significant effects, the Fisher's LSD test was used for post-hoc analysis. Statistical significance was defined as $p < 0.05$.

3 Results

3.1 Effects of oral lipid administrations on plasma KB, TG, and FFA concentrations in rats

The time course of changes in the plasma total KB concentrations after the oral administration of high-oleic sunflower, coconut, and MCT oils and their AUCs are shown in Fig. 1A and 1B, respectively. Although administration of MCT oil, which is exclusively composed of caprylic and capric acids, substantially increased the plasma total KB concentration at 2 and 4 h after the administration, no such increases in plasma total KB concentrations were observed in the Coconut and Sunflower groups at both time points (Fig. 1A). The plasma total KB AUC in the MCT group was significantly larger than those observed in the Coconut and Sunflower groups (Fig. 1B). No significant difference in the plasma total KB AUC was observed between the Coconut and Sunflower groups.

Plasma TG and FFA concentrations after oral administration are also shown in Fig. 1. The Coconut and Sunflower groups showed increases in plasma TG and FFA concentrations after oil administrations, whereas those concentrations were decreased in the MCT group (Fig. 1C and 1E). Plasma TG and FFA AUCs in the Coconut and Sunflower groups were significantly larger than those in the MCT group (Fig. 1D and 1F). Although the plasma TG AUC was significantly higher in the Sunflower group than in the Coconut group, there were no significant differences in the plasma FFA AUC between the Coconut and Sunflower groups (Fig. 1F).

The fatty acid compositions of plasma lipid are shown in Table 2. At both 2 and 4 h after the fat administrations, the proportions of caprylic acid (C8:0) and capric acid (C10:0) were slightly but significantly higher in the MCT group than in the Coconut and Sunflower groups. The Coconut

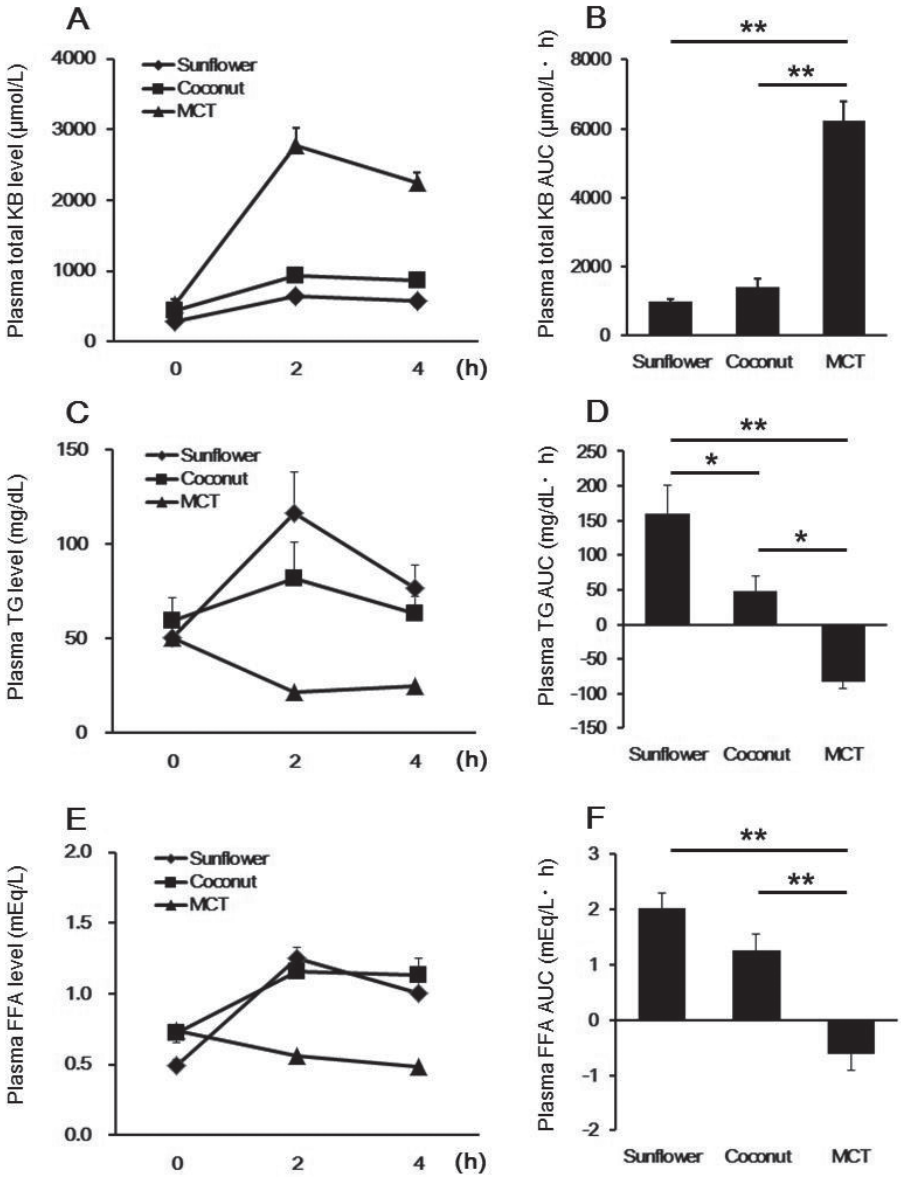

Fig. 1 Effects of oral lipid administrations on total KB, TG, and FFA plasma concentrations in rats. Values are means ± SEM (n = 5). Plasma total KB (A), TG (C), and FFA (E) concentrations after oral administrations of sunflower, MCT, or coconut oils (10 mg/g of body weight) in rats. The AUCs for plasma total KB (B), TG (D), and FFA (F) during the 4-h period after lipid administrations were calculated in accordance with the trapezoidal rule. *$p < 0.05$, **$p < 0.01$. Sunflower, high-oleic sunflower oil; MCT, medium-chain triacylglycerol oil; Coconut, coconut oil.

group had a substantial higher proportion of lauric acid (C12:0) than the MCT and Sunflower groups at both 2 and 4 h after the administrations, whereas the proportion of oleic acid (C18:1) in the Sunflower group was significantly higher than those in the MCT and Coconut groups.

3.2 Effects of treatments with LCFAs and MCFAs on KB production in KT-5 astrocyte cells

Figure 2 shows the total KB concentrations in the cell culture supernatant, which has been used as an index of KB production from cells, after 4-h treatment of KT-5 astrocyte cells with 50 and 100 μM fatty acids. Although there were no significant differences between the vehicle- and 50 μM oleic acid-treated cells in the total KB concentrations of the culture media (Fig. 2A), treatment with a higher concentration of oleic acid (100 μM) significantly increased the KB concentration (Fig. 2B). At both 50 and 100 μM concentrations, the caprylic acid- and lauric acid-treated KT-5 cells had significantly higher total KB concentrations in the supernatant than the vehicle- and oleic acid-treated cells (Fig. 2A and 2B). Furthermore, the total KB concentrations in the media collected from the cells

Table 2 Fatty acid composition of plasma lipid in rats (g/100 g total fatty acids).

	0 h	2 h			4 h		
		Sunflower	Coconut	MCT	Sunflower	Coconut	MCT
8:0[$]	ND	ND[a]	0.5 ± 0.2[a]	4.8 ± 0.9[b]	ND[a]	0.7 ± 0.2[a]	2.7 ± 0.5[b]
10:0	ND	0.1 ± 0.1[a]	1.4 ± 0.2[b]	2.2 ± 0.3[c]	ND[a]	1.5 ± 0.5[b]	2.5 ± 0.3[c]
12:0	ND	0.4 ± 0.3[a]	14.7 ± 3.3[b]	ND[a]	ND[a]	14.8 ± 3.0[b]	0.2 ± 0.2[a]
14:0	1.7 ± 0.3	1.0 ± 0.3[a]	5.8 ± 1.1[b]	ND[a]	1.0 ± 0.1[a]	6.9 ± 1.1[b]	0.9 ± 0.2[a]
16:0	41.6 ± 1.7	29.7 ± 1.9[a]	29.6 ± 0.8[a]	28.6 ± 1.3[a]	29.0 ± 2.1[a]	26.1 ± 2.0[a]	31.4 ± 2.1[a]
18:0	11.3 ± 0.7	10.9 ± 0.7[a]	11.2 ± 1.3[a]	16.5 ± 1.0[b]	10.9 ± 0.5[a]	9.7 ± 0.6[a]	16.2 ± 1.1[b]
18:1	10.1 ± 0.4	28.2 ± 2.8[a]	7.1 ± 0.2[b]	6.9 ± 0.6[b]	29.8 ± 1.4[a]	13.5 ± 6.3[b]	5.9 ± 1.0[b]
18:2	24.7 ± 0.8	19.7 ± 0.5[a]	17.9 ± 1.2[a]	20.0 ± 0.8[a]	17.6 ± 0.3[ab]	15.4 ± 1.5[a]	20.1 ± 1.5[b]
18:3	10.6 ± 0.9	10.0 ± 1.1[a]	11.8 ± 2.1[a]	21.0 ± 1.8[b]	11.7 ± 3.1[a]	11.4 ± 2.3[a]	20.1 ± 2.5[b]

Values are means ± SEM (n = 5). Within the same row at the same time point, different superscript letters indicate significant differences between the groups at a level of $p < 0.05$. Sunflower, high-oleic sunflower oil; MCT, medium-chain triacylglycerol oil; Coconut, coconut oil. [$]Numbers are carbon atoms:double bonds.

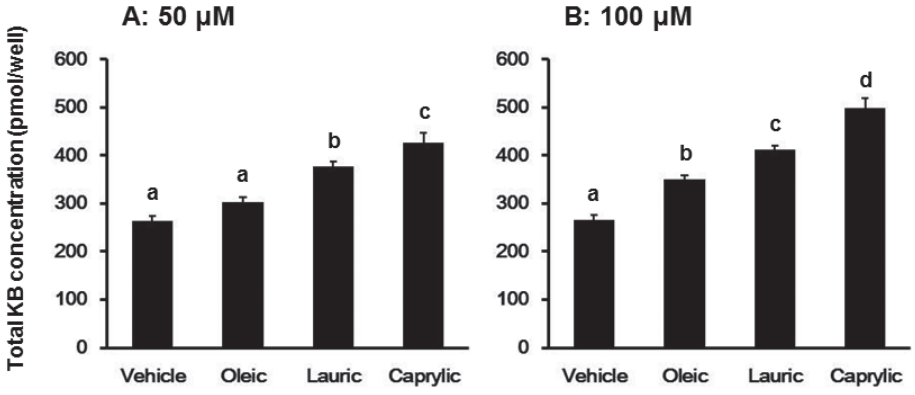

Fig. 2 Effects of 4-h treatments with 50 (A) and 100 μM (B) fatty acids on KB production in KT-5 astrocyte cells. Values are means ± SEM (n = 8). Values not sharing a common letter within the figure are significantly different at a level of $p < 0.01$. Sunflower, high-oleic sunflower oil; MCT, medium-chain triacylglycerol oil; Coconut, coconut oil.

treated with 50 and 100 μM caprylic acids were significantly higher than those from the lauric acid-treated cells (Fig. 2A and 2B).

4 Discussion

The major findings of this study were that (1) oral administration of coconut oil markedly increased the plasma FFA concentration and lauric acid content, but not the KB content, and (2) treatments with 50 and 100 μM lauric acid stimulated KB production in the KT-5 astrocyte cell line in vitro.

MCFAs such as caprylic and capric acids are mostly absorbed by the liver via the portal vein, bypassing peripheral circulation[8, 9]. MCFAs are more easily oxidized in the liver because their intramitochondrial transport does not require a CPT-I system, which is a rate-limiting step in mitochondrial β-oxidation[8, 9]. These characteristics make MCFAs a more ketogenic substrate than LCFAs in the liver. In this study, we confirmed that oral administration of MCT oil, which is mainly composed of caprylic and capric acids, substantially increased the plasma total KB concentration in rats (Fig. 1A and 1B). In contrast, coconut oil intake significantly elevated the plasma FFA concentration (Fig. 1E and 1F) and lauric acid content (Table 2), which is the major fatty acid constituent of coconut oil, without a remarkable increase in total KB concentration. These results were what we expected because previous work has shown that the portal vein absorption of lauric acid is less than one-fourth of the lymphatic absorption[11].

Astrocytes work in close contact with neurons and play several important roles in the brain, such as synapse remodeling, ion homeostasis, and synaptic transmission[19, 20]. Because astrocytes have high glycolytic capacity, they release large amounts of lactate into the extracellular space[21, 22]. This lactate is then taken up by neighboring neurons and serves as an energy source, forming the astro-

cyte–neuron lactate shuttle[23, 24]. Recent studies have shown that primary astrocytes can also oxidize fatty acids and generate KBs, suggesting that such central production of KBs in astrocytes may compensate for a diminished energy supply from glucose in nearby neurons[13, 17].

Consistent with these finding that primary astrocytes can generate KBs, in this study, we observed that treatment with a higher concentration of oleic acid significantly increased KB production in KT-5 cells (Fig. 2B), providing evidence that KT-5 astrocyte cells, as well as primary astrocytes, can generate KBs from fatty acids. Recently, Thevenet *et al*. have reported that caprylic acid treatment activated ketogenesis in induced pluripotent stem (iPS) cell-derived human astrocytes[25]. The present finding that treatment with caprylic acid induced a larger increase in KB production in the KT-5 astrocyte cell line (Fig. 2) is consistent with those results and provides further evidence that MCFA can potently promote the astrocyte–neuron KB shuttle system. Another major finding in the present study was that lauric acid as well as caprylic acid was able to stimulate KB production in astrocytes more strongly than the LCFA oleic acid (Fig. 2), although we need to confirm this finding using primary astrocyte cells or human iPS cells in future studies.

Indeed, the lauric acid-induced increase in KB production in KT-5 cells was significantly smaller than that observed in caprylic acid-treated cells (Fig. 2). However, as mentioned above, the oral administration of coconut oil markedly increased the FFA concentration and lauric acid content in peripheral plasma, whereas the FFA and TG concentrations were decreased and the proportions of caprylic and capric acids in the plasma lipid were somewhat smaller after MCT administration (Table 2). This is because most of the caprylic and capric acids ingested were absorbed via the portal vein and oxidized in the liver without entering the peripheral circulation. It is therefore more likely that in vivo astrocytic ketogenesis after coconut oil administration would be higher, to compensate for a diminished energy supply from glucose in nearby neurons, whereas the MCT oil would have little effect on astrocyte ketogenesis despite a marked ketogenic response in hepatocytes. Future studies are required to elucidate whether coconut oil intake actually increases the local KB production in the brain in vivo despite lower hepatic ketogenesis.

5 CONCLUSION

Coconut oil intake markedly increases the plasma FFA concentration and lauric acid content, which has the capacity to potently stimulate KB production in astrocytes and may provide neighboring neurons with fuel.

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