Change in Plasma Total, Esterified and Non-esterified Capric Acid Concentrations during a Short-term Oral Administration of Synthetic Tricaprin in Dogs

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We studied change in the plasma total, esterified and non-esterified capric acid (FA10:0) and its effect on longer fatty acid concentrations during the short-term oral administration of synthetic tricaprin in dogs. We administered 150 and 1500 mg tricaprin/kg body weight per day orally to dogs for 7 consecutive days. Blood samples were collected at 0, 0.5, 1, 2, 4, 8, and 24 h on the 1st and 7th days for measuring the total-, esterified- and non-esterified-FA10:0. The total-FA10:0 concentration increased in a dose-dependent manner, reaching a peak at 1 h on the 1st day and at 2 to 4 h on the 7th day; it then mostly disappeared within 24 h. The mean esterified FA10:0 concentration was found to be 75.5 and 60.3% of total-FA10:0 in dogs fed 150 and 1500 mg of tricaprin/kg body weight, respectively. The plasma level of FA10:0 depends on the duration and dose of tricaprin administration, but are rapidly cleared from circulation within several hours.

Keywords Medium-chain triglycerides, medium-chain fatty acid, fatty acid, tricaprin, triglyceride deposit cardiomyovascularopathy

(Received May 4, 2017; Accepted August 9, 2017; Published November 10, 2017)

Introduction

Medium-chain triglycerides (MCT) are composed of medium-chain fatty acids (MCFA), mainly octanoic and capric acid (decanoic acid, FA10:0).¹ MCT are rapidly digested by gastric lipase and readily absorbed through enterocytes; they are therefore, used in the treatment of pancreatic insufficiency and fat malabsorption syndrome. Due to the unique pharmacokinetic properties and metabolic pathways compared to long-chain fatty acids (LCFA), MCT have been reported to reduce body weight and prevent obesity,²⁻⁹ alcohol-induced liver injury¹⁰⁻¹³ and non-alcoholic fatty liver diseases in animal models,^{14,15} liver cirrhosis,¹⁶ metabolic syndrome and insulin resistance,^{8,17-20} and to improve serum lipid profiles in humans.²¹

In recent years, MCT have been shown to be beneficial in concerning cardiac diseases.²² Triglyceride deposit cardiomyovascularopathy (TGCV),^{23,24} a cardiac phenotype of adipose tissue triglyceride lipase (ATGL) mutation, is characterized by a massive accumulation of triacylglycerol (TG) in coronary atherosclerotic lesions and the myocardium. ATGL is a rate-limiting enzyme that has profound effect on the

intracellular mobilization of TG to liberate fatty acids (FA) for energy substrate.^{25,26} Mutation in PNPLA2, the gene encoding ATGL or its activator-comparative gene identification-58 (CGI-58) impairs the mobilization of TG, leading to excessive fat deposition not only in adipose tissue, but also in ectopic sites in mice²⁷ and humans.^{28,29} A recent study revealed that peroxisome proliferated activator receptors (PPARs) and related genes are up-regulated in the myocardium of TGCV patients, leading to increased uptake of LCFA and its storage as neutral fat.³⁰ However, a deficiency of ATGL severely impairs the mobilization of cellular triglycerides in the myocardium, resulting in its profound deposition and ultimately requiring cardiac transplantation. Conversely, the overexpression of myocardial ATGL can reduce TG deposition in the heart.³¹

MCT can easily bypass the rate-limiting action of ATGL using an alternative lipase, such as hormone-sensitive lipase (HSL), for its hydrolysis and thus can provide an energy substrate for cellular respiration. In addition, MCT can be beneficial to minimize intracellular lipid accumulation, compared to long-chain fatty acid (LCFA), as MCFA are poorly esterified into cellular TG and are ineffective for lipid droplet formation.^{32,33} Cellular studies on the fibroblast of neutral lipid storage disease (NLSD) have shown that the *in situ* degradation of capric acid (FA10:0)-containing TG is not defective in contrast to the degradation of LCFA-containing TG, suggesting that MCT can

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be mobilized through the catabolic pathway independent to that containing LCFA.³⁴ Therefore, it appears that the oral administration MCT, particularly rich in FA10:0, can be beneficial in NLSD patients. However, dietary MCT are readily hydrolyzed into its constituent MCFA in the gastrointestinal tract, which are predominantly carried to the liver through portal circulation and completely metabolized.³⁵ There is a paucity of data about the systemic circulation of MCFA after MCT administration, and little is known about the effect of the oral administration of synthetic tricaprin (tri-esters of capric acids and glycerol; TG10:0/10:0/10:0) in the systemic circulation of esterified and non-esterified MCFA and other longer-chain fatty acids. A detailed study of the pharmacokinetics of MCFA is important to select an appropriate dose of MCT to be administered so that MCFA can be delivered to extra-hepatic tissues.

It is of significance to monitor the plasma non-esterified and total FA10:0 concentration, and its association with clinical signs and symptoms in patients receiving dietary therapy with MCT, which would provide scientific evidence about the mechanism explaining the benefit of such therapy. We recently reported simple, highly sensitive and specific methods for the quantification of serum FA10:0 using high-performance liquid chromatography (HPLC) that can be useful to measure the FA10:0 level in order to monitor the efficiency of MCT intervention.³⁶ Our method is superior to other existing methods which was discussed in our previous report. Particularly, FA10:0 is absent or present in a very trace amount in normal serum, and is relatively volatile compared to LCFA, and also the methyl derivatization adds more volatility to FA10:0 resulting in low recovery during specimen processing for gas chromatography.³⁶ In this study, we applied the method to demonstrate the effects of the oral administration of synthetic tricaprin on the plasma concentration of esterified and non-esterified FA10:0. We also examined the alteration in the serum fatty acids profile after the short-term oral administration of synthetic tricaprin.

Experimental

Chemicals

2-Nitrophenylhydrazide of FA10:0, undecanoic (FA11:0), lauric (FA12:0), myristic (FA 14:0), palmitic (FA16:0), heptadecanoic (FA17:0), stearic (FA18:0), oleic (FA18:1), linoleic (FA18:2), linolenic (FA18:3), arachidonic (FA20:4), eicosapentaenoic (FA20:5; EPA), docosahexaenoic (FA22:6; DHA), arachidic (FA20:0), behenic (FA22:0), tricosanoic (FA23:0), lignoceric (FA24:0), and cerotic acid (FA26:0) were synthesized in our laboratory by the method that we previously described.³⁶ FA labeling reagents were obtained from YMC Co. (Kyoto, Japan). HPLC-grade methanol and water were from Wako Pure Chemical Industry (Osaka, Japan). Synthetic tricaprin was obtained from Yashiro, Osaka, Japan.

Specimens

A dog model was used to evaluate the toxicity of the oral administration of synthetic tricaprin. The dog model was selected since it is a choice of animal in pharmacokinetic studies.³⁷ The animal experiments were performed in Kowa Co. Ltd, Pharmaceutical Division, Tokyo, Japan. Ethical approval for animal experimentation was obtained from the Institutional Animal Care and Use Committee (IACUC) (SOP no. GM016). A total of six dogs were used in this study, and they were divided into 3 groups. In one group of dogs ($n = 2$), 150 mg/kg

Fig. 1 HPLC chromatograms of the 2-nitrophenylhydrazine derivatives of (a) mixture of fatty acids and (b) fatty acids from the plasma of a dog collected after 2 h of ingestion of 1500 mg/kg body weight of tricaprin. Peaks: (1) capric [FA10:0], (2) undecanoic [FA11:0; IS], (3) lauric [FA12:0], (4) myristic [FA14:0], (5) linolenic [FA18:3], (6) eicosapentaenoic [EPA; FA20:5], (7) linoleic [FA18:2], (8) arachidonic [FA20:4], (9) docosahexaenoic [DHA, FA22:6], (10) palmitic [FA16:0], (11) oleic [FA18:1], (12) heptadecanoic [FA17:0; IS], and (13) stearic acid [FA18:0].

body weight of the synthetic tricaprin was administered orally at 24 h intervals for 7 consecutive days. In another group ($n = 2$), a high dose of tricaprin, that is, 1500 mg/kg body weight, was used at the same time interval, while the remaining 2 dogs were fed a control diet without tricaprin. Blood samples were collected from each dog before the ingestion of tricaprin and at 0.5, 1, 2, 4, 8 and 24 h after ingestion of tricaprin on the first and seventh day. Blood samples were also collected from the control dogs at the same time intervals.

All of the plasma was separated from the samples within 30 min of collection, and then stored at -80°C until used.

Assay of FA in plasma

The plasma FA10:0 concentration was measured by a method that we previously reported.³⁶ Briefly, 50 μL of plasma was mixed with 100 μL of KOH (0.3 M KOH in ethanol) and heated at 80°C for 30 min for the saponification of triglycerides in the samples. The FA in the samples was then labeled with 2-nitrophenyl hydrazine (2-NPH) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride and pyridine.³⁸ Derivatization of the carboxylic group containing analytes including FA and phytanic acid with 2-NHP is a simple way for its HPLC measurement.³⁹ FA11:0, FA17:0, and FA23:0 were used as internal standards for measurements of MCFA, LCFA, and VLCFA, respectively. For determining non-esterified

FA (fFA), 100 μL of the plasma was mixed with 5 μL of FA11:0 (1 nmol) and 20 μL of FA17:0 (4 nmol). The FA present in the samples was directly labeled with 2-NPH without undergoing saponification. The labeled FA is extracted in a phosphate buffer (pH 4.6)/hexane mixture. After complete evaporation of the hexane extract, the residue was dissolved in 200 μL of methanol, and 10 μL of the filtrate was injected into a C₄Mightysil reversed-phase column [150×4.6 mm (i.d.); particle size 5 μm] (Cica Reagent, Kanto Chemical Co., Inc, Tokyo, Japan), maintained at 35°C in Shimadzu Prominence LC-20AD HPLC (Shimadzu Seisakusho, Kyoto, Japan) equipped with a Shimadzu Model CTO-10A injector and the SPD-M20A photo diode array detector. Gradient elution was performed with water (pH adjusted to 4.0 with 1% [v/v] trifluoroacetic acid (Solvent A) and methanol (Solvent B), at a flow rate of 1.0 mL/min. The absorbance was monitored at 400 nm. Typical chromatograms of a mixture of standards and a plasma dog after administration of tricaprin are shown in Fig. 1.

The concentrations of esterified FA were calculated by subtracting the corresponding fFA from total FA (tFA). The concentrations of saturated LCFA were calculated by adding up the concentration of FA14:0, FA16:0 and FA18:0. The concentration of VLCFA were calculated by adding up the concentration of FA20:0, FA22:0, FA24:0 and FA26:0.

The concentration of plasma triglycerides and total cholesterol (TC) were measured by enzymatic methods (Kyowa Medex Co, Ltd., Tokyo, Japan) in a fully automated Hitachi 7170 (Tokyo, Japan) chemistry analyzer.

Results and Discussion

Dietary MCT therapy is currently an integral part in the treatment of various clinical conditions. Although the mechanism of action of MCT-containing diets in cardiac metabolism remains largely unknown, interest in using MCT to benefit patients with cardiac diseases has increased in recent years.²² Several distinguishing features make MCT metabolism unique to that of other LCFA containing oils and fats. These features include the rapid hydrolysis of MCT into MCFA in the gastrointestinal tract, which are primarily transported through the portal vein in the non-esterified state following absorption into the enterocytes, and are then rapidly metabolized within the liver.³⁵ In addition, the entry of MCFA into mitochondria bypasses the rate-limiting carnitine transport system, resulting in rapid and unregulated utilization. Therefore, the plasma concentration of FA10:0 in fasting state is below the detection limit and negligible in non-fasting human plasma.³⁶

In this study, however, we demonstrated that the administration of tricaprin, a synthetic MCT, is characterized by a sharp increase in plasma FA10:0 in dogs, as shown in Fig. 2. The concentration of FA10:0 in plasma is proportional to the amount of tricaprin administered. In general, the plasma FA10:0 attains the peak value at around 1–4 h of tricaprin ingestion, and then undergoes a progressive decline to the baseline within 8–24 h. Although some studies have evaluated the change in the serum FA composition after the administration of MCT,^{40,41} our study provides the first precise description of the time-associated change in the plasma esterified and non-esterified FA10:0 level following tricaprin administration for up to 7 days. Furthermore, this is the first animal study to use pure tricaprin as MCT. The therapeutic use of MCT with the mixture of FA8:0 and FA10:0 can be found in numerous literatures. In this study, we fed tricaprin with a high degree of purity (>99.0%) and examined

Fig. 2 Time-dependent changes in mean concentration of capric acid (FA10:0) in the plasma of dogs after ingestion of 0 mg/kg (●, dashed line), 150 mg/kg (■, solid line) and 1500 mg/kg (△, solid line) body weight of medium-chain triglycerides (MCT) per day. (a) and (b) for total capric acid following 1 and 7 days of MCT ingestion, respectively; (c) and (d) for esterified capric acid following 1 and 7 days of MCT ingestion, respectively; (e) and (f) for non-esterified capric acid following 1 and 7 days of MCT ingestion, respectively.

the plasma level of FA. We demonstrated that the plasma levels of both the total and non-esterified FA10:0 are dose- and time-dependent (Fig. 2).

Total capric acid concentration

The mean concentration of the total FA10:0 (tFA10:0) and the total saturated LCFA in the plasma of control dogs collected at various times for 2 days ($n = 28$) were 0.7 ± 0.2 , (mean $\pm$ SD), and 2307.3 ± 128.5 $\mu\text{mol/L}$, respectively. Tricaprin administration is associated with a drastic increase in plasma tFA10:0. The tFA10:0 ranged from 0.6 to 53.0 $\mu\text{mol/L}$ and 2.0 to 150.3 $\mu\text{mol/L}$ in the plasma of dogs fed with 150 mg/kg body weight of MCT for 1 day and 7 consecutive days, respectively, while it increased up to 103.4 and 446.4 $\mu\text{mol/L}$ at day-1 and -7, respectively, in the plasma of dogs fed with 1500 mg/kg body weight of tricaprin. The trends of change in the mean tFA10:0 ($n = 2$) observed after the ingestion of tricaprin with relation to time are elucidated in Figs. 2(a) and 2(b). The mean area under curves of 0, 150 and 1500 mg group is, respectively, 27.9, 141.3 and 664.7 $\mu\text{mol/L}$ for day-1 and 13.4, 293.1 and 2599.1 $\mu\text{mol/L}$ for day-7 (Table 1). The concentration of tFA10:0 increased persistently soon after tricaprin ingestion, reaching the peak at 1 h in dogs fed with 150 mg/kg body weight of tricaprin. In dogs fed with 1500 mg/kg body weight of tricaprin, the concentration of tFA10:0 reached the peak at 1 h after tricaprin ingestion on the first day (Figs. 2(a) and 2(b)). The rate of increase in plasma tFA10:0 after the ingestion of tricaprin was significantly higher on the 7th day compared to

Table 1 Area under curve (AUC) of a chart plotted with the concentration of plasma capric acid ($\mu\text{mol/L}$) against time after the administration of tricaprin

Tricaprin administration/mg		Sample-1		Sample-2		Mean	
		Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Total capric acid	0	26.6	11.9	29.1	14.8	27.9	13.4
	150	122.4	382.0	160.2	204.2	141.3	293.1
	1500	900.7	4215.0	428.7	983.3	664.7	2599.2
Esterified capric acid	0	11.3	7.9	17.6	14.8	14.5	11.4
	150	53.7	336.6	117.8	157.0	85.8	246.8
	1500	486.0	3845.6	143.6	639.8	314.8	2242.7
Non-esterified capric acid	0	4.9	4.0	0.0	0.0	2.5	2.0
	150	68.7	45.4	42.4	47.3	55.6	46.4
	1500	414.7	369.4	285.1	343.5	349.9	356.5

the first day of tricaprin administration, and remained maximum for up to 4 h, after which it progressively decreased to the baseline.

Esterified capric acid concentration

The majority of plasma FA10:0 are present in esterified form in control dogs. Tricaprin administration is associated with a decrease in the percentage content of esterified FA10:0, indicating an increase in the non-esterified (free) fraction. On the average, more than 87% of plasma FA10:0 is present in the esterified form, while the mean content of esterified FA10:0 was found to be 75.5 and 60.3% in dogs fed with 150 and 1500 mg of tricaprin/kg body weight. Therefore, a considerable amount of FA10:0 is also circulated in the non-esterified (free) form and is readily available to extra-hepatic tissues for its utilization. The pattern of the change in the concentration of esterified FA10:0 after the administration of tricaprin is similar to that of the total FA10:0 (Figs. 2(c) and 2(d)). The mean area under the curve of 0, 150 and 1500 mg group is, respectively, 14.1, 85.7 and 314.8 $\mu\text{mol/L}$ for day-1 and 11.4, 246.8 and 2242.7 $\mu\text{mol/L}$ for day-7 (Table 1). The esterified FA10:0 attained the maximum concentration within 1 h following the ingestion of 150 mg/kg body weight of tricaprin, after which it decreased to the baseline by 4–8 h. In the group of dogs fed with 1500 mg/kg body weight of tricaprin, the concentration of esterified FA10:0 attained the maximum within 2 to 4 h and, then declined to the baseline within 24 h following tricaprin ingestion. Interestingly, in dogs fed with 1500 mg tricaprin/kg body weight, the peak timing is not similar between esterified and non-esterified FA10:0. The peak of esterified FA10:0 was observed 1 h after the fFA10:0 attend maximum (Figs. 2(e) to 2(f)).

Our data suggest that the majority of FA10:0 in plasma exists in the esterified form. FA10:0 is more likely to be esterified into triglycerides because FA10:0 esterified to phospholipids or cholesterol has not been detected in plasma.^{40,42,43} This finding supports the hypothesis that during the oral loading of MCT, the hepatic clearance of MCFA remains incomplete. Therefore, unmetabolized MCFA may be esterified to triglycerides, packed into VLDL and released into the systemic circulation, as MCFA containing VLDL has also been detected following the MCT diet.⁴⁴ Furthermore, esterified FA10:0 found in the plasma may also be contributed by chylomicrons, since possible integration of dietary MCFA into chylomicrons cannot be excluded.^{45–49} Nonetheless, these MCFA-containing triglyceride-rich lipoproteins can be important substrates for lipoprotein lipase in the capillaries of adipose tissues, skeleton, and cardiac muscles. The MCFA that are taken up by adipose tissues can be stored and subsequently released into the circulation, depending upon

the feed/fast cycle.⁵⁰ This phenomenon may be particularly advantageous in ATGL-defective individuals, since MCFA-containing triglycerides can bypass the rate-limiting action of ATGL. Hilaire *et al.*³⁴ demonstrated that MCFA incorporates into the cellular lipids at a lower rate than LCFA, and induce a lower accumulation of triglycerides in the NLSD cells. In addition, it appears that cytoplasmic triglycerides are degraded through two separate pathways, one being specific to long-chain triglycerides and the others one specific to MCT. Therefore, the *in situ* degradation of FA10:0 containing triglyceride is not defective in NLSD.³⁴

Non-esterified capric acid concentration

The mean concentration of non-esterified FA10:0 and non-esterified saturated LCFA in the plasma of control dogs collected at different times for 2 days ($n = 28$) was 0.1 ± 0.1 and 121.8 ± 85.2 $\mu\text{mol/L}$, respectively. The maximum concentration of fFA10:0 observed in the plasma of dogs following ingestion of 150 and 1500 mg/kg body weight of tricaprin were 31.4 and 61.4 $\mu\text{mol/L}$, respectively. The changes of fFA10:0 levels also follow a similar pattern to that of tFA10:0, reaching the maximum at 1 h after the ingestion of 150 mg/kg body weight of tricaprin and 1–2 h after ingestion of 1500 mg/kg body weight of tricaprin (Figs. 2(e) and 2(f)). The mean area under the curve of 0, 150 and 1500 mg group is, respectively, 2.5, 55.5 and 349.9 $\mu\text{mol/L}$ for day-1 and 2.0, 46.3 and 356.4 $\mu\text{mol/L}$ for day-7 (Table 1). These non-esterified or free FA10:0 can function as an energy substrate for fat-utilizing tissues, including muscles. Therefore, it appears that the dietary tricaprin administration in TGCV patients can be a good strategy for promoting the use of MCFA as an energy source in cardiomyocytes without significant accumulation of triglycerides.

We observed that prolonged ingestion of tricaprin results in a prominent elevation of the total and esterified FA10:0, but not to non-esterified FA. In other words, there is noticeable increase in the concentration of total and esterified FA10:0 on day-7 compared to day-1 (Fig. 2). For instance, the area under the curve for the dog group fed with 150 mg of tricaprin is 141.3 $\mu\text{mol/L}$ for day-1 and 293.1 $\mu\text{mol/L}$ for day 7 (2 times increase), and for dog group fed with 1500 mg tricaprin is 664.7 $\mu\text{mol/L}$ for day-1 and 2599.1 $\mu\text{mol/L}$ for day-7 (3.9-times increase). However, no such increase was observed for non-esterified FA10:0 (55.5 vs. 46.3 $\mu\text{mol/L}$ for the 150 mg group and 349.9 vs. 356.4 $\mu\text{mol/L}$ for the 1500 mg group). Our result implies that the prolonged use of tricaprin promotes the transportation of FA10:0 primarily in the esterified form, which is most likely to be carried as either chylomicron or VLDL,

or both. We also observed that the peak of esterified FA10:0 is delayed by 1 h compared to non-esterified FA10:0 when a high dose of tricaprin is administered (Fig. 2). This may indicate that a considerable amount of digested FA10:0 that is transported through the portal system escape the hepatic utilization, and is promptly released into the systemic circulation. While the remaining molecules are esterified to form MCT and delivered to systemic circulation as VLDL. The measurement of both esterified and non-esterified FA10:0 is another novel aspect of this study. The existence of esterified and non-esterified FA10:0 in the circulation signifies different metabolic aspects associated with tricaprin administration. Non-esterified FA10:0 in the plasma is freely circulated, and readily available for utilization. This may imply that the non-esterified fraction of FA10:0 are directly absorbed through the gastrointestinal (GI) tract. Whereas, esterified form mainly indicates either FA10:0 that are absorbed through the formation of chylomicrons through GI tract or that present in VLDL secreted from the liver. Further studies are needed to elucidate the detail metabolic pathways of MCT utilization and its mode of transportation.

Plasma FA10:0 levels became maximum at 1 h following the ingestion of 150 mg tricaprin/kg body weight. In dogs fed with 1500 mg/kg body weight tricaprin, the FA10:0 levels became maximum at 1–2 h at day-1 and 2–4 h at day-7. The levels decline almost by half after 2 h, and return to the baseline by 4 h following 150 mg/kg body weight tricaprin, while in dogs fed with 1500 mg/kg body weight of tricaprin, both the non-esterified and total FA10:0 decline to almost half by 8 h, and then reach the baseline by 24 h. This finding suggests that both the esterified and non-esterified FA10:0 are completely taken up by fat utilizing cells, and cleared from circulation within 24 h. Therefore, this implies that in order to keep the concentration of FA10:0 within the therapeutic target level, tricaprin should be administered at least 2–3 times a day. We believe that our data of kinetics of plasma FA10:0 will be helpful in selecting the appropriate dose of tricaprin for therapeutic purposes.

Concentration of other fatty acids, triglycerides, and total cholesterol

We also investigated the effect of the oral administration of synthetic tricaprin to the concentration plasma FA species, triglycerides, and TC. The change in the pattern of the mean concentration of total FA14:0, FA16:0, FA18:0, FA18:1, FA18:2, FA18:3, FA20:4, FA20:5, FA22:6, and VLCFA is shown in Fig. S1 (Supporting Information). We observed an increase in the concentrations of FA16:0, FA18:0, FA18:1, FA18:2, FA18:3, FA20:5, and VLCFA at 8 h of tricaprin administration both on day-1 and day-7. No such increase was observed in dogs fed with the control diet. The effect of the tricaprin diet in the plasma concentration of total FA, triglycerides, and TC is shown in Fig. 3. The concentration of total FA moderately increased within 8 h of tricaprin administration in both groups. Plasma triglycerides also elevated to the maximum level at 8 h of tricaprin administration at day-1. In the dogs fed with 1500 mg tricaprin/kg body weight for 7 days, plasma triglycerides markedly increased between 2 to 8 h. No significant change was seen in the plasma TC level. The concentration of total FA was significantly correlated with the triglyceride ($r = 0.461$, $p < 0.001$) and TC ($r = 0.420$, $p < 0.001$) level.

Capric acid/long chain fatty acid ratio

In order to normalize the effect of change in the concentration of longer chain FA on the concentration of FA10:0, the ratio of FA10:0 to LCFA can provide a better indicator to monitor the tricaprin intervention. Trends in the change in the ratio of

Fig. 3 Time-dependent changes in the mean concentration of (a) total fatty acids, (b) triglycerides, (c) total cholesterol in the plasma of dogs after ingestion of 0 mg/kg (●, dashed line), 150 mg/kg (■, solid line) and 1500 mg/kg (△, solid line) body weight of tricaprin per day.

tFA10:0 to the total LCFA, fFA10:0 to non-esterified LCFA and esterified FA10:0 to esterified LCFA observed following ingestion of tricaprin are depicted in Figs. 4(a) and 4(b), 4(c) and 4(d), 4(e) and 4(f), respectively. Within 1 h following the ingestion of 150 mg/kg body weight of tricaprin, the ratios peaked and slowly declined to the baseline by 4–8 h. In the case of dogs fed with 1500 mg/kg body weight of tricaprin, the ratios elevated to the maximum within 1–2 h, and then declined to the baseline by 24 h (Fig. 4).

Another interesting result that we observed in this study, is the influence of tricaprin administration on the plasma level of FA14:0, FA16:0 and FA18:0, LCFA. There is a nearly reciprocal change between plasma FA10:0 and LCFA. Following tricaprin ingestion, as the plasma level of both non-esterified and total FA10:0 increases, the level of LCFA starts to decline. This inverse relationship between plasma FA10:0 and LCFA can be explained by the influence of the tricaprin diet toward insulin secretion. MCT therapy is associated with an increase in insulin production reaching the maximum after 1–2 h.¹⁷ Increase in insulin suppresses the mobilization of FA from adipose tissue leading to a decrease in plasma LCFA, and the plasma FA10:0 can be taken up by fat utilizing tissue including cardiac muscle. This phenomenon is particularly important in ATGL mutated cells, since FA10:0 containing triglycerides can be utilized without resulting in its accumulation.³⁴ This inverse relationship of FA10:0 with LCFA can be utilized for monitoring the FA10:0 levels in terms of ratio to LCFA (Fig. 4).

Small number of sample is the major limitation of this study.

Fig. 4 Time-dependent changes in ratio of capric (FA10:0) to long-chain fatty acid (LCFA) in the plasma of dogs after ingestion of 0 mg/kg (●, dashed line), 150 mg/kg (■, solid line) and 1500 mg/kg (△, solid line) body weight of medium-chain triglycerides per day. (a) and (b) the ratio of total FA10:0 to total LCFA at day 1 and 7; (c) and (d) the ratio of non-esterified FA10:0 to non-esterified LCFA at day 1 and 7; and (e) and (f) the ratio of esterified FA10:0 to esterified LCFA at day 1 and 7, respectively.

However, our data provide important insights into the systemic availability of FA10:0 following tricaprin ingestion. It is our future target to conduct animal experiments on a large scale in normal and ATGL knockout mice to evaluate how tricaprin is metabolized, and its role to improve the clinical outcome. However, this is the first study to demonstrate the dose- and time-dependent change in the concentration of both esterified and non-esterified FA10:0 following tricaprin administration. We believe that our finding will be valuable in formulating therapeutic use of tricaprin.

Conclusion

Oral ingestion of tricaprin is associated with a significant release of FA10:0 in the circulation, both in esterified and non-esterified forms. The plasma level of FA10:0 depends on the duration and dose of tricaprin administration. The circulatory FA10:0 is rapidly utilized by non-hepatic tissues and cleared up from plasma within 24 h. The determination of total and non-esterified FA10:0 can provide valuable information concerning the monitoring of the therapeutic target of tricaprin. Any actual benefits of tricaprin administration and monitoring plasma concentration of FA10:0 in TGC patients is yet to be determined.

Acknowledgements

This study was financially supported by Research Grant for Rare and Intractable Disease from the Ministry of Health, Labor, and Welfare of Japan, partially supported by a Grant-in-Aid for Scientific Research from the Japan Society for Promotion of Sciences, Japan and partly by the Regional Innovation Strategy Support Program, Sapporo Health Innovation “Smart-H”, of The Ministry of Education, Culture, Sports, Sciences and Technology, Japan.

Supporting Information

Figure S1: Change in the concentration of various fatty acid species in the plasma after oral administration of 0 mg/kg (●, dashed line), 150 mg/kg (■, solid line) and 1500 mg/kg (△, solid line) body weight of medium-chain triglycerides (MCT) per day at day 1 and day 7.

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