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Effects of medium-chain triglycerides on gluconeogenesis and ureagenesis in weaned rats fed a high fat diet

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ABSTRACT

We explored the effects of Medium-chain triglycerides (MCT) on gluconeogenesis and ureagenesis in the liver of weaned male rats fed high fat, carbohydrate-free diets. The rats of three experimental groups and control were fed for 10 days. The diets were high fat, carbohydrate-free diets consisting either of a corn oil or MCT, and high protein carbohydrate-free diet and a control (high carbohydrate) diet. The hepatic glucose-6-phosphatase (G6Pase) activity increased in the experimental groups. Despite the elevated G6Pase activity in these groups, hepatic activities of glutamic alanine transaminase (GAT), pyruvate carboxylase (PC) and arginase differed among the experimental groups. The HF-corn oil rats showed elevation of PC activity, but no elevation of GAT activity, and the lowest arginase activity among the three groups. The HF-MCT diet-fed rats showed higher GAT and arginase activities than the HF-corn oil group. In the HP diet-fed rats, GAT and arginase activities enhanced, PC did not.

1. Introduction

A number of studies [1,2] have suggested that intravenous lipids can stimulate gluconeogenesis in preterm infants by providing glycerol, a gluconeogenic precursor. However, the fatty acid composition of the supplied lipids could have important lipid-effects on glucose metabolism. Saturated fatty acids [3,4] had more potent effects on insulin release, glucose oxidation and glucose production than unsaturated fatty acids. Feeding medium-chain triglycerides (MCT) provide active gluconeogenesis in suckling newborn rats. Moreover, MCT are commonly used for the treatment of fat mal-absorption or to provide energy in situations such as preterm neonates [5,6]. According to the reports, hepatic gluconeogenesis was enhanced with a high protein, carbohydrate-free diet, and this type of diet elevated liver glucose synthesis by stimulating glucagon secretion [7]; however, gluconeogenesis promotion in the rat liver by high fat, carbohydrate-free diet feeding was not accompanied by this type of hormone secretion [1].

Our previous study [8] demonstrated that a low carbohydrate-high fat (corn oil) diet caused a reduction in urea formation and enhanced hepatic gluconeogenesis at a fixed dietary protein level in weanling as well as growing rats. It is not clear that an increase in gluconeogenesis would not necessarily accompany elevation of urea formation. Thus, the present study aimed to clarify whether MCT-rich diet feeding enhances hepatic gluconeogenesis and ureagenesis in comparison with corn oil-rich diet feeding or protein-rich diet feeding in weaned rats.

2. Methods

2.1. Animals and diets

Three-week-old male Wistar weaned rats (just after being weaned from suckling) weighing about 50 g were housed individually and bred with a standard chow diet (MF, Oriental Yeast Co., Ltd., Tokyo, Japan) for 3–4 days, while they became acclimated to their surroundings. Animals were then randomly divided into four groups and fed either a high protein, carbohydrate-free (HP) diet, a high fat, carbohydrate-free (HF-corn oil) diet, a high fat, carbohydrate-free (HF-MCT) diet or a control diet (high carbohydrate). The composition of the diets is shown in Table 1. Feed and

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Table 1
Composition of the diets.

Ingredient	Control	High-fat		High-protein (HP)
		Corn oil (HF-corn oil)	MCT (HF-MCT)	
Casein	20	20	20	85
Sucrose	65	0	0	0
Corn oil	5	35	2	5
MCT oil[a]			33	
Cellulose[b]	5	40	40	5
Salt mixture[c]	4	4	4	4
Vitamin mixture[d]	0.5	0.5	0.5	0.5
Choline chloride	0.5	0.5	0.5	0.5
Calorie (kcal/100 g)	385	395	395	385

[a] Medium-chain tryglycerides were generously supplied by Nissin Oil Manufacture, Yokohama, Japan.
[b] Cellulose was purchased from Toyo Roshi Co., Tokyo, Japan.
[c] NIN-93 Harper's salt mixture was purchased from Oriental Yeast Co., Tokyo, Japan.
[d] AIN-93 Vitamin mixture was purchased from Oriental Yeast Co., Tokyo, Japan.

water were given freely and rats were bred for 10 days. Individual rat had ad libitum access to synthetic diet and drinking water. The food intake of each animal was determined daily by monitoring the remained food in a feed vessel. Each animal was weighed at the start of the feeding period, after 4 days on the diets and prior to being sacrificed. Animals were sacrificed by decapitation and the livers were quickly removed. The blood serum and livers were collected. One part of the serum was used for determination of blood glucose within the day, and the remainder was stored at −20 °C until used for serum free fatty acid (non-esterified) measurement. After the liver was washed with chilled physiological saline, the extra moisture was wiped off with filter paper and total liver weight was measured. Half of the liver was used for enzyme determinations, and the other half was stored at −20 °C until used for glycogen determinations. The animal experiments were approved by the Committee on the Care and Use of Laboratory Animals of the Hamamatsu University and met the guidelines and regulations.

2.2. *Enzyme activity assays*

For glutamic alanine transaminase [EC2.6.1.2. GAT] enzyme determinations, 1 g of liver tissue was homogenized in 10 volumes of 100 mM Tris hydrochloric acid containing 0.25 M sucrose (pH 7.5) using a glass vessel and Teflon pestle. The 10% liver homogenate was centrifuged at 10,000× *g* for 15 min at 4 °C, and the resultant supernatant fraction was used for enzyme activity determinations. Next, approximately 2 g of liver tissue was homogenized in 10 volumes (vol/wt) of 0.25 M sucrose solution, followed by centrifugation at 700× *g* for 10 min at 4 °C. The upper phase of the resulting supernatant was withdrawn at 30% volume for the arginase [EC3.5.3.1.] activity assay. The residual supernatant fraction was re-centrifuged at 1000× *g* for 15 min; the resulting supernatant was then separated into a microsomal fraction and a soluble fraction, which was used for the glucose-6-phosphatase [EC3.1.3.9. G6Pase] activity assay. The microsomal pellet fraction was homogenized in five volumes (vol/wt) of cold distilled water and this homogenate was used for the pyruvate carboxylase [EC6.4.1.1. PC] activity assay. The G6Pase activity was measured by the procedure described by Segal and Washko [9], followed by measurement of the phosphorus produced as a result of the enzyme reaction. The GAT activity was measured by the procedure described by Hopper and Segal [10]. The PC activity was assayed by the method described by Utter and Keech [11]. Enzyme activities were determined by photometric assay, in which the absorbance decrease at 340 nm was used to estimate the amount of consumed NADH in a conjugated enzyme reaction system of GAT (from lactic acid dehydrogenase) and PC (from malic acid dehydrogenase). The arginase activity was measured according to the procedure described by Schimke [12]. The assays were based on the colorimetric determination of urea. Protein levels were determined using the method of Lowry et al. [13] with bovine serum albumin standard.

2.3. *Measurements of serum glucose and FFA, and hepatic glycogen and FFA*

Serum glucose was measured according to the glucose oxidase method using a glucostat enzyme assay kit (Fujisawa Pharmaceutical Co., Ltd., Tokyo, Japan). Serum FFA levels were measured by a previously described procedure [14]. Hepatic glycogen was measured by the modified method of Seifter et al. Briefly, about 0.5 g of liver tissue was dissolved in 30% KOH and a part of this tissue solution was subjected to glycogen extraction, which was extracted with 95% alcohol and 6% Na_2SO_4, and then subjected to colorimetric measurement. To measure the amount of hepatic FFA, 1 g of liver was homogenized in five volumes of 1% KCl solution containing 1 mM EDTA. FFA determinations were conducted on homogenates by the same method used for the serum FFA assay.

2.4. *Statistical analysis*

All results were subjected to one-way analysis of variance (ANOVA) using the SPSS 17 package for Windows (SPSS Inc.). Values are expressed in terms of mean ± standard error (SEM). Differences in mean values among groups were tested using Tukey's multiple range test and were considered to be significantly different at a p-value of less than 0.05.

3. Results

Weight gain and food intake of rats fed the control diet and the three experimental diets are presented in Table 2.

In the three experimental groups, serum glucose levels and liver glycogen levels were low compared with the control group (Table 3). Liver glycogen levels were significantly lower in the three experiment groups, especially in the two high-fat diet groups, with levels less than 1/3 of the control group. Serum FFA levels did not significantly differ among the three groups; however, the HP group showed a slight increasing tendency (Table 3). It appears that glucagon secretion might be increased due to the high protein diet, followed by a release of FFA from the adipose tissue. The liver FFA level was higher in the HF-corn oil group than any other group. FFA levels in the livers of the HF-MCT group were similar to those of the

Table 2
Initial body weight, weight gain and food intake of rats fed control, high fat and high protein diets.[a]

Groups	Initial body weight (g)	Weight gain[b] (g)	Food intake (g/day)
Control	45 ± 1a	46 ± 2a	9.3 ± 0.6a
HF-corn oil	45 ± 1a	41 ± 1[c]b	8.5 ± 0.4a
HF-MCT	44 ± 1a	17 ± 1c	5.2 ± 0.8b
HP	45 ± 1a	30 ± 2b	6.3 ± 0.4b

[a] Data are represented as means ± SEM for 8 rats in each group.
[b] Weight gain after 10 days on the diets.
[c] Labeled means in a column without common letter differ ($p < 0.05$). Differences among groups were analyzed by one-way ANOVA with Tukey's test.

HP group, and both the HF-MCT and HP groups' FFA levels were higher than those of the control group (Table 3).

3.1. Effects of high-fat diets on gluconeogenic enzyme activities

Possible differences might exist in gluconeogenesis between rats fed the high-fat diets and the high-protein diet. Thus, we assessed G6Pase (Fig. 1A), GAT (Fig. 1B) and PC (Fig. 1C) enzyme activities in the rat liver. The G6Pase activity in any of the three experimental groups was significantly higher, 1.3–1.5 times, than that of the control group. Moreover, G6Pase activity did not significantly differ among the three experimental groups.

The GAT activity increased remarkably in the HP and HF-MCT groups. The GAT activity in the control and HF-corn oil groups was low compared with the other groups. Interestingly, there were clear differences in aspects of GAT activity levels between the two high-fat diet groups. The gluconeogenesis activity in the HF-corn oil group was not accompanied by increased GAT activity, whereas enhanced GAT activity was observed the HF-MCT group, at almost the same level as in the HP group, suggesting that metabolic pathways for pyruvic acid synthesis from alanine might be active in the HF-MCT group.

The PC activity was significantly increased in the HF-corn oil group compared with the HP and the control groups (Fig. 1C). In the high fat-diet groups, no difference in PC activity was observed between the corn oil and MCT groups. In the HP group, the PC activity was almost the same as that of the control group (Fig. 1C).

3.2. Effect of the high fat diets on ureagenic enzyme activity

If dietary fats can affect gluconeogenesis, it is also possible that they may influence the relationship between gluconeogenesis and ureagenesis. Thus, we investigated arginase activity involved in urea formation (Fig. 2). The HP group showed the highest level of arginase activity among the three groups. The HF-corn oil group was significantly lower as compared with each of the other groups. While these two fat groups have similar diet conditions of high-fat (35% oil) and 20% casein diet conditions (Table 1), the arginase activity was obviously different (Fig. 2); specifically, arginase and GAT (Fig. 1B) activities in the HF-MCT group were higher than those in the HF-corn oil group.

4. Discussion

The results of our study showed differences in the metabolic influence of two dietary fats on hepatic arginase activity, indicating an increase in each group's G6Pase activity that was not accompanied by enhanced arginase activity in the rat liver. The metabolic changes during gluconeogenesis may differ according to the diet composition (Table 1); consequently, different relationships between gluconeogenesis and ureagenesis would exist among groups.

The 40% higher G6Pase activities of the experimental diet groups compared to the control group were attributed to the carbohydrate-free diet. Thus, we could use the accelerated gluconeogenesis condition of the animals to clarify relationships between high gluconeogenesis and ureagenesis, which may be enhanced by MCT feeding. In these three experimental groups, despite accelerated gluconeogenesis compared to the control, GAT and PC activities differed between the high fat groups and the high protein group. Specifically, these activities of the HF-corn oil group showed large differences from the HF-MCT and high protein groups.

Table 3
Effects of high fat and high protein diets on serum glucose and free fatty acids (FFA), and liver glycogen and FFA.[a]

Groups	Serum glucose (mg/dl)	Serum FFA (μEq/l)	Liver glycogen (g/100 g)	Liver FFA (μmol/g)
Control	187 ± 7a	281 ± 46a	6.5 ± 1.3a	137 ± 5a
HF-corn oil	157 ± 3[b]b	227 ± 12a	2.0 ± 0.3c	225 ± 10c
HF-MCT	105 ± 9c	291 ± 56a	1.5 ± 0.1c	156 ± 6b
HP	165 ± 5b	336 ± 36a	3.2 ± 0.4b	161 ± 3b

[a] Data are represented as means ± SEM for 8 rats in each group.
[b] Labeled means in a column without common letter differ ($p < 0.05$). Differences among groups were analyzed by one-way ANOVA with Tukey's test.

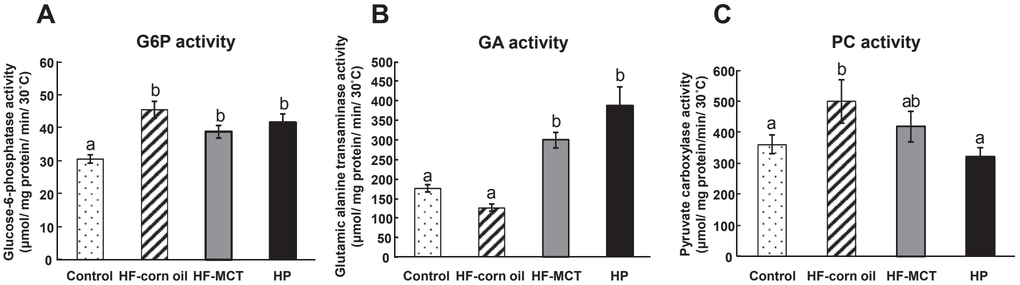

Fig. 1. Effects of high fat and high protein diets on glucose-6-phosphatase (G6P) (A), glutamic alanine transaminase (GA) (B) and pyruvate carboxylase (PC) (C) activities in the rat liver. All data are presented as the means ± SEM for eight rats. Differences among groups were analyzed by one-way ANOVA with Tukey's multiple-comparison test. Labeled means without a common letter differ ($P < 0.05$).

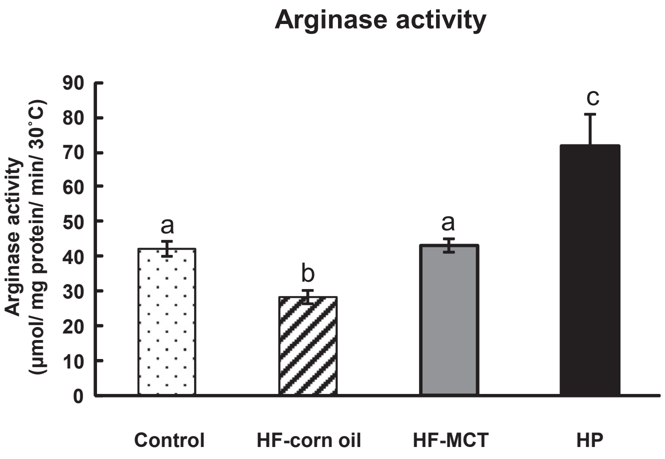

Fig. 2. Effects of high fat and high protein diets on arginase activity in the rat liver. Values are presented as the means ± SEM for eight rats. Differences among groups were analyzed by one-way ANOVA with Tukey's multiple-comparison test. Labeled means without a common letter differ ($P < 0.05$).

It was reported [7] that high protein diet-fed animals exhibited high levels of blood glucagon and liver GAT synthesis. According to Rémésy and Démigné [15], in rats fed a high protein, carbohydrate-free diet, the decrease in the amount of liver pyruvic acid resulted in a rise of GAT activity through enhanced glucagon secretion. In the high-fat diet groups, depending on the fat source (i.e., long chain fatty acids or medium chain fatty acids), significant differences were observed in gluconeogenic enzyme activities (Fig. 1A–C). The PC activity of the HF-corn oil group was statistically higher than those of the control and the HP, this high activity of the HF-corn oil group might be related to the high level of liver FFA levels (Table 3). Thus, gluconeogenesis-related metabolic pathways might differ among groups depending on the dietary composition. The interaction occurring in vivo between gluconeogenesis and ureagenesis are poorly known.

In the present study, hepatic arginase activity was assessed (Fig. 2). In the HF-corn oil diet group, the arginase activity was very low despite the increased gluconeogenic enzyme activity. However, an accompanying elevation in the amount of liver non-esterified fatty acids was observed (Table 3). In the HF-MCT group, the arginase activity increased significantly compared with the HF-corn oil group (Fig. 2). Therefore, the relationship between ureagenic enzyme activity and accelerated gluconeogenesis in the HF-MCT fed group is clearly different from that of the HF-corn oil group. While the reason for this discrepancy is poorly understood, it appears that differences in the metabolism of these two fats may be involved. The results of the present study suggest that gluconeogenesis-related metabolic pathways in rats fed the HF-corn oil diet might be different from those of the HF-MCT or HP diet groups. Additional experiments are necessary for detailed examination of the mechanism regulating enhanced urea formation by MCT feeding.

5. Conclusions

Feeding of the MCT-rich diet resulted in enhanced gluconeogenesis and increased ureagenesis in the weaned rat liver. The elevated urea nitrogen exclusion might lead to a negative precarious nitrogen-balance in body protein of young animals. This MCT feeding may require to modulate it by dietary protein supplement. The mechanism of enhanced ureagenesis by MCT feeding requires elucidation.

These MCT feeding data in this study can be beneficial information in a clinical aspect of hypoglycemia problems of preterm infants. Currently, reported by Van Kempen et al. that the hypoglycemia occurs after birth in preterm infants, in which infants, an intravenous lipid can stimulate gluconeogenesis to supply blood glucose [2].

Authors' contribution

All Authors participated in the conception and study design. CS and ST carried out preparation of the manuscript drafting. KNA and SM carried out the animal work. CS, SM, AM, and ST preformed enzyme activity assay and data analysis. All authors had done approval of final manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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