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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