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Altered Hepatic Metabolism of Free Fatty Acid in Rats Fed a Threonine-Imbalanced Diet

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Summary The effects of a threonine-imbalanced diet (8% casein supplemented with 0.3% methionine, TI) on the ketone body production and the secretion rate of lipids were examined in the isolated perfused rat liver. Feeding a TI diet compared to an 8% casein (C) diet resulted in an enlargement of liver, presumably due to 2–4-fold accumulation of triglyceride. Serum triglyceride likewise increased significantly in rats fed a TI diet. No significant difference was found in the other lipid components both in serum and liver. When the livers from rats fed C or TI diets were isolated and perfused in the presence of an exogenous oleate substrate, the TI diet decreased the ketone body production and conversely increased the secretion rate of triglyceride, suggesting an inverse relationship between rates of ketogenesis and triglyceride secretion. The proportion of oleate in the perfusate triglyceride obtained at the end of perfusion was comparable between the C and TI groups, whereas in the post-perfused liver it was higher in the former than in the latter, suggesting a stimulatory effect of the TI diet on the secretion of the oleate in the form of triglyceride. These results indicate that altered hepatic metabolism of long-chain free fatty acids between the pathways of oxidation and esterification is one of the causative factors for triglyceride accumulation in the liver produced by threonine imbalance.

Key Words threonine imbalance, fatty acid metabolism, ketone body production, triglyceride secretion, liver perfusion

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Threonine imbalance caused by feeding a diet containing 8% casein supplemented with 0.3% methionine (TI) has been characterized by the moderate accumulation of hepatic triglyceride in rats (*1–8*). It has been reported that the mechanisms responsible for fatty infiltration in the liver is either an increased hepatic lipogenesis *in situ* or an impaired secretion of triglyceride (*3–8*), or both. However, the fatty infiltration in the livers can already be seen at 3 days after feeding the TI diet with no accompanying increase in the activity of the key enzyme of hepatic lipogenesis, acetyl-CoA carboxylase, or the incorporation of [1-^{14}C]-acetate into hepatic lipid fractions *in vitro* and *in vivo* (*5–7*). On the other hand, Ogura *et al.* recently reported that the secretion of triglyceride was rather higher in the perfused livers *in situ* of rats fed the TI diet than in those fed the C diet (*8*). These results suggest that the mechanism whereby the fatty liver is induced in rats fed the TI diet could not be fully explained either by the stimulation of lipogenesis or by the impaired secretion of triglyceride. These observations led us to suggest that the remaining possible factor(s) involved in the development of this type of fatty liver is an altered hepatic metabolism of free fatty acids that enter the liver from the circulation, since a reciprocal relationship has been observed between the rates of ketogenesis and triglyceride secretion in the perfused rat livers under various hormonal and physiological conditions (*9–11*).

In the present study, we report that the fatty liver due to feeding the TI diet is partly caused by a reciprocal alteration of free fatty acids between rates of ketogenesis and esterification.

Male Wistar rats (Kyudo, Kumamoto) weighing approximately 85 g were housed individually in an air-conditioned room (22–24℃, lights on from 6 a.m. to 6 p.m.), and were fed the diets *ad libitum* for 7 days (Experiment I) or 7–9 days (Experiment II), respectively. The composition of the basal diet (C), expressed as weight percent, was as follows: vitamin-free casein, 8.0; sucrose, 77.85; corn oil, 5; mineral mixture, 4; vitamin mixture, 1; choline chloride, 0.15; and cellulose, 4.0. The mineral and vitamin mixtures were according to Harper. For the threonine-imbalanced diet (TI), methionine at the 0.3% level was added at the expense of sucrose. After feeding these diets, rats received intraperitoneal injection of Nembutal (5 mg/100 g body weight) around 9 a.m. Blood was obtained from the portal vein, and the liver was excised. The serum was separated by centrifugation at 3,000 rpm at 5℃.

In experiment II, the liver was isolated and perfused with 120 ml of recirculating Krebs-Hensleit medium (pH 7.4), which contained 25 mM glucose, 1.5% (w/v) bovine serum albumin (fraction V, Boehringer Mannheim), and 25% (v/v) washed bovine erythrocytes, at the rate of 15 ml/min at 37℃. At the beginning of recirculation, 5 ml of 20 mM potassium oleate (100 μmol) was added and the solution was continuously infused at the rate of 4.5 ml/h (90 μmol/h). At 1-h intervals, 13–15 ml of perfusate was removed for analyses of ketones and lipids. The same amounts of fresh perfusion medium were added at each removal. The perfusions were continued for 4 h. Detailed conditions for liver perfusions were

described previously (*10, 11*).

Acetoacetate and β-hydroxybutyrate were measured enzymatically in a deproteinized sample of liver perfusate as described previously (*10, 11*).

The lipids in serum, liver, erythrocyte-free perfusate, and post-perfused liver were extracted and purified (*12*). Cholesterol and triglyceride were measured by the methods described elsewhere (*5–7*). The fatty acid composition of the triglyceride fraction in serum, liver (Experiment I), perfusate at the end of perfusion, and post-perfused liver (Experiment II) was analyzed by gas-liquid chromatography after separation of triglyceride by thin-layer chromatography with a solvent mixture of *n*-hexane/diethyl ether/glacial acetic acid, 80/20/1 (v/v/v) (*13*).

The data were analyzed for statistical significance using Student's *t*-test (*14*).

Table 1 summarizes growth parameters, liver weight, as well as the concentrations of lipids in serum and liver. The responses of the growth parameters and lipid concentrations to the diets in Experiments I and II were comparable, although weight and lipid concentrations of liver in Experiment II were measured at the end of the perfusion. Rats fed the TI diet were significantly heavier than those fed the C diet due to an increased food consumption. The relative liver weight was significantly higher in the TI group than in the C group. Serum and liver triglyceride in the TI-fed rats elevated about 2- and 4-folds, respectively, in both experiments. On the other hand, neither the concentration of serum circulating free fatty acids, which is presumed to be an important source of triglyceride in hepatic

Table 1. Growth parameters and the lipid concentrations in serum, liver, and post-perfused liver.
Numbers in parentheses indicate the number of animals and the results were expressed as mean±SEM.

	Experiment I		Experiment II	
	C (7)	TI (7)	C (5)	TI (5)
Food intake (g/day)	6.7±0.3	10.0±0.3**	7.1±0.2	10.8±0.5**
Body weight (g)				
Initial	93±2	90±1	86±2	88±1
Final	89±2	104±3**	82±2	111±7**
Liver weight (g/100 g BW)	5.6±0.1	6.1±0.1**	6.1±0.1	7.0±0.2**
Serum lipid (μmol/dl)				
Triglyceride	55.9±6.5	119.0±9.4**	74.6±7.4	179.8±7.6**
Cholesterol	180.2±15.6	227.4±16.0	149.9±7.5	196.7±17.9
Free fatty acid	11.7±0.7	13.1±1.1	—	—
Liver lipid (μmol/g)[a]				
Triglyceride	18.4±2.8	76.8±13.6**	22.5±2.7	40.2±2.4**
Cholesterol	11.1±1.3	10.9±0.6	9.5±1.2	10.0±0.5

[a] Values for Experiment II were from post-perfused liver. ** Significantly different from the C group at $p < 0.01$.

synthesis, nor the concentration of cholesterol in serum and liver were influenced by the TI diet. These results agree well to those reported previously (*1–8*).

Figure 1 shows the time course of the ketone body production (A) and the secretion rate of triglyceride (B) on the basis of the whole liver. As shown in Table 2, the uptake of oleate by the livers during 4-h perfusion, as calculated from the amounts infused and the amounts of oleic acid present in the perfusate at the consecutive time intervals, was comparable in the C and TI groups. This equivalent uptake appears to correspond to the observation in Experiment I that the serum level of free fatty acids remained unchanged. Therefore, the altered partition of oleic acid between the pathways of ketogenesis and esterification in the livers of rats fed the TI diet compared to those fed the C diet was a consequence of altered intracellular metabolism. The TI diet caused a 25% decrease in the rate of ketone body production compared to the control diet. The difference in the ketone body

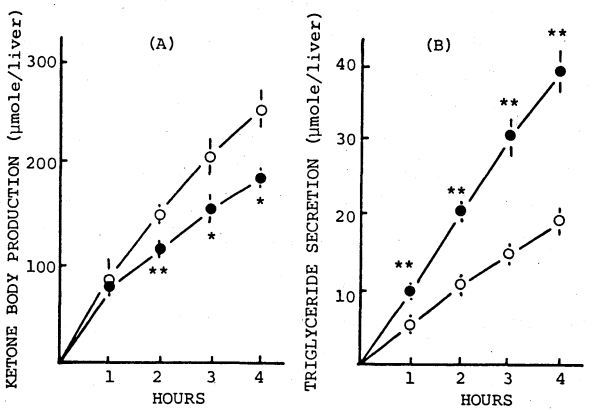

Fig. 1. Production of ketone bodies (A) and the secretion rate of triglyceride (B) by the isolated perfused rat liver. Each point represents mean±SEM of 5 rats. ○, C group; ●, TI group. ***Significantly different from the C group at $p<0.05$ and $p<0.01$, respectively.

Table 2. Uptake of oleic acid substrate by the prefused liver.
Numbers in parentheses indicate the number of animals and the results were expressed as mean±SEM. Livers were perfused with constant infusion of oleate (90 μmol/h) after an initial priming dose (100 μmol). The uptake of oleate was calculated from the amounts infused and the amounts of oleic acid present in the perfusate at the consecutive time intervals.

Time (h)	C (5)	TI (5)
	(μmol/liver)	
1	142.5±5.8	152.4±5.8
2	234.8±6.2	246.8±4.5
3	324.5±6.1	334.9±3.1
4	401.1±9.3	408.7±4.9

production became larger and significant at the entire perfusion period when expressed as per gram of liver, since liver weight of rats fed the TI diet was significantly higher than that of rats fed the C diet. The ratio of β-hydroxybutyrate: acetoacetate in the perfusate at the consecutive time intervals was comparable; 1.03–0.90 in the C-fed rats and 0.91–0.87 in the TI-fed rats, indicating that the TI diet did not affect the metabolic sequence to produce reduced pyridine nucleotide or to maintain phosphorylation of the adenine nucleotide (*15*).

The secretion rate of triglyceride was inversely related to the ketone body production; rats fed the TI diet compared to those fed the C diet secreted 2.2-fold more triglyceride, in good agreement with the result of Ogura *et al.* (*8*). The difference was significant at 2 h and thereafter of the perfusion even when expressed as per gram of liver. Thus, it is plausible that increased secretion is responsible for an increase in the serum triglyceride level on feeding the TI diet. These results also suggest that the production of this type of fatty liver is not attributed to the inhibition of triglyceride secretion by the liver. Ogura *et al.* (*4*) reported that oxidation of [1-^{14}C]palmitate to $^{14}CO_2$ by the liver slice was comparable between the imbalanced and control rats whereas incorporation of ^{14}C-carbon into triglyceride was significantly higher in the former. It seems therefore reasonable to propose that the decrease in the hepatic oxidation of fatty acid as reflected by the decrease in ketone body production in rats fed the TI diet diverts fatty acids to the esterification pathway and in turn to increase triglyceride synthesis and its accumulation. Consequently the alteration in the fatty acid metabolism appears to be an important causative factor for the fatty livers. Such a reciprocal response has also been observed in the rat liver perfused with 2-tetradecylglycidate, a specific and potent inhibitor of carnitine acyltransferase, the rate-limiting enzyme of mitochondrial fatty acid oxidation (*10*) or in the liver of genetically obese Zucker rats compared to their lean littermates (*11*). In these situations there were either fatty livers or hypersecretion of triglyceride as observed in threonine-imbalanced rats.

Although the regulatory factor(s) responsible for the partition of free fatty acids between oxidation and esterification remained obscure, the level of cellular malonyl-CoA is considered to be one of the major determinants (*16,17*). Thus, it has been shown that the rate of fatty acid synthesis correlates positively with the cellular malonyl-CoA level, and that both of these parameters are inversely related to the rate of fatty acid oxidation. Accordingly, the alteration in the fatty acid metabolism is closely related to the increase in the triglyceride synthesis and its accumulation in the liver (*9,16*). In this respect, the enhancement of hepatic acetyl-CoA carboxylase activity at 7 days after feeding the TI diet compared to C diet (*7*), which could be associated with an increase in the cellular malonyl-CoA level, favors this assumption. Threonine imbalance did not influence the secretion rate of cholesterol (data not shown).

Table 3 summarizes the fatty acid composition of triglyceride in serum, liver, perfusate, and post-perfused liver. Although there was no significant difference in the fatty acid composition of serum triglyceride, the TI diet caused a significant

Table 3. Fatty acid composition of triglyceride.
Numbers in parentheses indicate the number of rats and the results were expressed as mean±SEM.

	16:0	16:1	18:0	18:1	18:2
			(weight %)		
Experiment I					
Serum					
C (7)	24.1±1.7	5.3±0.8	5.2±0.8	39.4±0.8	26.0±2.9
TI (7)	27.2±1.5	6.8±0.8	3.4±0.2	39.8±0.3	22.9±2.3
Liver					
C (7)	27.6±1.3	7.1±1.1	3.1±0.3	45.6±0.4	16.5±2.8
TI (7)	34.7±0.8**	11.0±1.4	2.2±0.2	37.2±1.1**	14.9±1.6
Experiment II					
Perfusate					
C (5)	11.0±0.1	3.5±0.4	2.4±0.5	77.8±1.0	5.4±0.9
TI (5)	14.3±0.5	4.6±0.3	1.8±0.2	74.3±1.0	4.9±0.3
Post-perfused liver					
C (5)	13.7±0.7	3.9±0.4	2.0±0.1	70.4±1.3	9.7±1.5
TI (5)	26.0±2.4**	7.8±1.1*	2.3±0.2	54.0±1.4**	10.0±2.2

*,** Significantly different from the C group at $p<0.05$ and $p<0.01$, respectively.

decrease in the proportion of oleate and conversely an increase in palmitate in the hepatic triglyceride. When the isolated liver was perfused in the presence of oleate as an exogenous substrate, a considerable increase in the proportion of oleate in perfusate triglyceride was observed and to a somewhat lesser extent in post-perfused liver triglyceride. Threonine imbalance did not modify the fatty acid profile of triglyceride secreted into perfusate, whereas in post-perfused liver triglyceride it increased the proportion of palmitate and decreased that of oleate. These alterations in the fatty acid profiles were similar to those observed in the liver, indicating that an increase in the secretion rate of triglyceride by the perfused liver from rats fed the TI diet is ascribed to the concomitant change in the metabolism of free fatty acids. The results also suggest that the free fatty acid supplied exogenously during the perfusion period is incorporated in hepatic triglyceride and is actively secreted in the form of triglyceride-fatty acid. However, the secretion rate of oleate in the liver of rats fed the TI diet was more than that in the control counterpart when considered as the content of oleate present in the perfusate at the end of perfusion.

In conclusion, an increase in triglyceride synthesis and its accumulation in the liver of threonine-imbalanced rats is likely to be the consequence of a selective decrease in the ketone body production of long-chain fatty acid by the liver. However, more studies are needed to confirm the relationship between the alteration in the free fatty acid metabolism and the fatty infiltration in the liver.

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