

Induction of fatty liver by fasting in suncus

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Abstract We found that a fatty liver was easily induced in a novel experimental animal, *Suncus murinus* (suncus), by withholding food. Hepatic triglyceride content increased linearly for up to 24 h after fasting in these animals. Serum levels of neutral lipids are very low in the fed state compared with those in rats, and decreased significantly after 24 h fasting. On the other hand, serum free fatty acids, which are at the same level in fed animals as in rats, increased threefold in the fasting suncus. In order to learn whether the fatty liver induced by fasting is an unusual physiological state or a pathological on-going state in suncus, they were refed after 24 h fasting. Refeeding resulted in a decrease in hepatic triglyceride content to the level of fed animals. Serum lipid levels, which decreased with fasting, returned to those of fed animals. This evidence indicates that hepatic lipid secretion is impaired even in a physiological state to some extent and that starvation causes increasing influx of free fatty acid to the liver, which might be followed by esterification and result in triglyceride accumulation in the liver. ■ In conclusion, hepatic lipid and lipoprotein metabolism is unique to the suncus, which is a useful animal model for the study of intra-hepatic lipid transport. —Yasuhara, M., T. Ohama, N. Matsuki, H. Saito, J. Shiga, K. Inoue, K. Kurokawa, and T. Teramoto. Induction of fatty liver by fasting in suncus. *J. Lipid Res.* 1991. 32: 887–891.

Supplementary key words suncus • fasting • fatty liver • triglyceride • refeeding

Suncus murinus (suncus or house musk shrew) belongs to the family Soricidae of the order Insectivora, regarded as the direct ancestors of the Primates in the phylogenetic system (1). Recently, the suncus has been introduced as a novel experimental animal, and has proved to be a unique experimental animal model for emesis (2). Their daily rhythms of eating and activities are reported to be of the nocturnal pattern, though brief active periods of eating and drinking are observed intermittently at intervals of 1 and 2 h during daytime (3). They are characterized by large consumption of living materials and low resistance to fasting (4). Their life span is not well known, though it is thought that they usually live for 1–2 years and occasionally for 3 years (5).

We have shown previously that fatty droplets are often observed in the suncus liver in response to various

hepatotoxins (6). In these studies, we also noted fatty droplet accumulation in the liver of the 24-h fasted suncus. Such phenomena could not be observed in other experimental animals. Triglyceride, which is formed by esterification in the liver from fatty acid derived from plasma, is secreted in the form of very low density lipoprotein or stored as droplets when the synthetic rate is higher than the secretory rate.

In this study, we report that fatty liver is induced easily by fasting in this animal and that refeeding results in a rapid reversal of triglyceride accumulation in the liver.

MATERIALS AND METHODS

Animals

Male suncus (40–70 g body weight) and male Wistar rats (250–270 g body weight) were used. The animals were housed in temperature- and humidity-controlled rooms and fed with laboratory chow (special food for suncus, Central Institute for Experimental Animals, Tokyo, Japan; CE-2 for rats, CLEA Japan Inc., Tokyo, Japan).

Induction of fatty liver

The animals were fasted or refed for the indicated periods and given water ad libitum. Blood was collected from the inferior vena cava while the animals were under ether anesthesia. Serum was separated from the blood by centrifugation for biochemical analysis; the contents of serum triglyceride, total cholesterol, phospholipid, and free fatty acid were measured on an auto-analyzer (Hitachi). The livers were removed immediately after the animals were killed, and a portion was fixed in 10% neu-

Abbreviations: TLC, thin-layer chromatography; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PI, phosphatidylinositol; PS, phosphatidylserine; T-chol, total cholesterol; PL, phospholipid; TG, triglyceride; FFA, free fatty acid.

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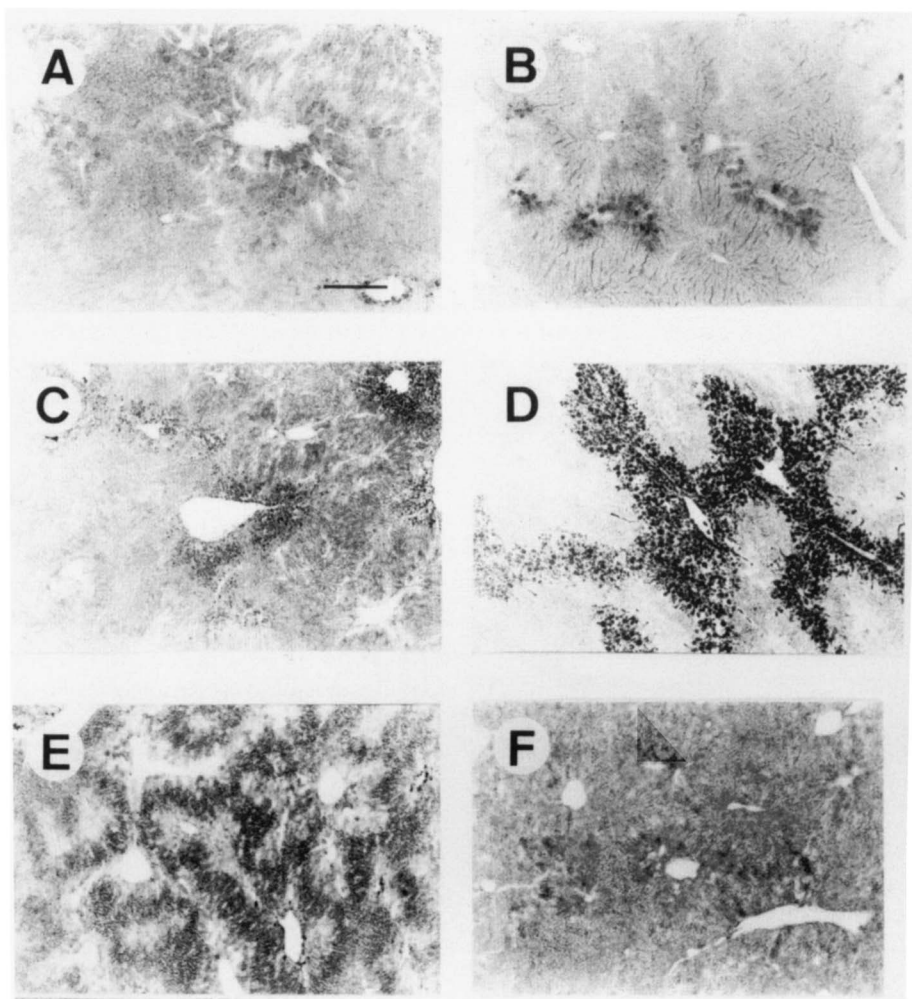
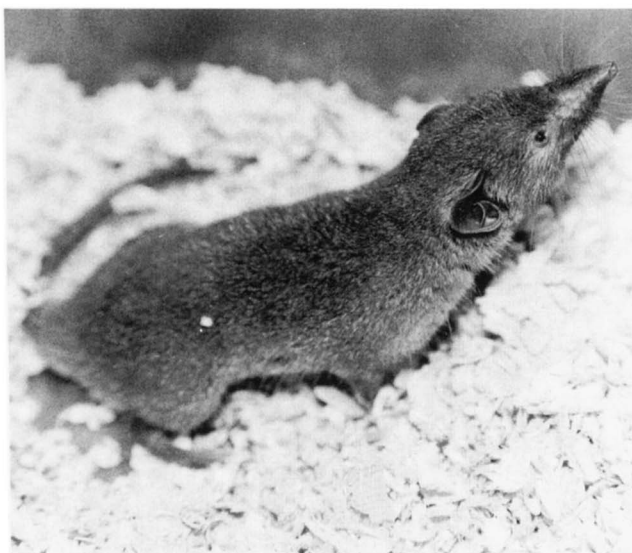


Fig. 1. Light microscopy of the suncus liver. The livers were stained with oil red O. Fasting period A: 0 h; B: 8 h; C: 16 h; D: 24 h; E: 32 h; F: refed for 1 day after 1-day fasting. The short bar in A represents 400 μm .



The house musk shrew, *Suncus murinus*.

tral formalin, sectioned, and stained with oil red O for assessment of liver morphology. Another part of the liver was homogenized in 0.1 M phosphate buffer (pH 7.5), and triglyceride content was measured with commercial kits (Wako, Osaka, Japan).

Analysis of liver lipid

Hepatic lipids were extracted by the method of Bligh and Dyer (7). Phospholipids, diacylglycerol, triacylglycerol, cholesterol, and cholesteryl ester were separated by thin-layer chromatography (TLC) (Merck) using n-hexane-diethyl ether-acetic acid 70:30:1 (v/v) as a developing solvent. After development, the plates were sprayed with a 0.002% primuline solution in acetone-water 5:1 (v/v), and lipid bands were visualized under UV light and identified by comparison with authentic standards. The spots were scraped and the lipids were eluted from silica gel with chloroform-methanol 1:2 (by volume). The amounts of triacylglycerol and diacylglycerol were determined from the quantity of fatty acyl moieties. Fatty acids

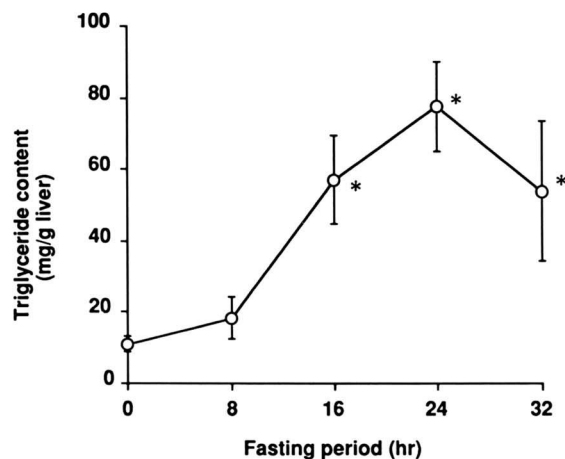


Fig. 2. Effect of fasting on triglyceride content in the suncus liver. Suncus were fasted for the periods indicated. The livers were removed immediately after the animals were killed; a part of the liver was homogenized in 0.1 M phosphate buffer (pH 7.5), and triglyceride content was measured. Each point represents mean \pm SE of four animals.

were analyzed in the form of methyl esters by gas-liquid chromatography. Free cholesterol and cholesteryl ester contents were measured by enzymatic fluorescence (8).

Individual phospholipids were separated by two-dimensional TLC with the first solvent system of chloroform-methanol-acetic acid 65:25:10 (v/v) and the second solvent system of chloroform-acetone-methanol-acetic acid-water 50:20:10:10:5 (v/v). The phospholipids were extracted from the silica gel by the method of Bligh and Dyer, and lipid phosphorus was estimated as described previously (9).

Statistical analysis

The statistical significance or difference among the groups was evaluated using Duncan's multiple range test.

RESULTS

Induction of fatty liver

The light photomicrographs of suncus livers after fasting are shown in **Fig. 1(A-E)**; they reveal that fatty metamorphosis occurred after 8 h of fasting and progressed up to 32 h. In order to estimate the fatty deposits quantitatively, we measured the triglyceride contents of the liver. As shown in **Fig. 2**, liver triglyceride contents of suncus fasted for 0, 8, 16, 24 and 32 h increased up to 24 h in a time-dependent manner, and declined thereafter. On the other hand, there was no significant difference in liver triglyceride contents of the fed and 24-h fasted rats, which were 11.4 ± 1.1 mg/g liver and 12.7 ± 1.0 mg/g liver, respectively.

The lipid compositions of the livers from fed and fasted suncus are given in **Fig. 3** in comparison with those of

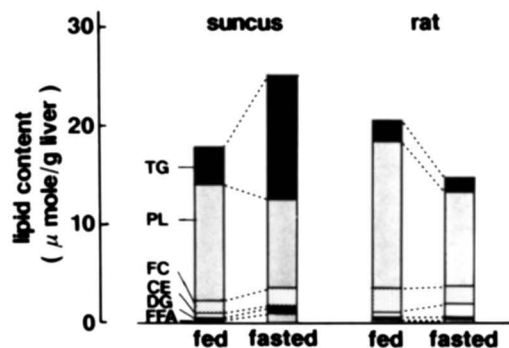


Fig. 3. Lipid composition of livers from animals fed or fasted for 24 h. Lipids were extracted and separated by TLC. Each lipid was determined as described in the text.

rats. Although the lipid composition of the livers from fed suncus was comparable with that of the livers from fed rats, 24 h of fasting led to an increase in triglyceride of the suncus liver, in contrast to the rats, in which 24-h fasting induced a slight decrease in triglyceride. In order to investigate whether choline deficiency in the suncus exists or not, the effect of fasting on liver phospholipid composition was investigated, as shown in **Table 1**. No decrease in the hepatic phosphatidylcholine content was observed after fasting.

Serum lipids

The serum lipid levels in fed and 24-h fasted suncus were assayed and compared with those in rats. As shown in **Table 2**, total cholesterol and phospholipid levels in fed suncus were lower than those in fed rats, and the triglyceride level was much lower than that of rats. Serum levels of total cholesterol, phospholipid, and triglyceride were decreased in the suncus after fasting, in contrast to the rat, in which no significant change in these values was observed. Although the values of free fatty acid in fed suncus were almost the same as those in fed rats, the levels of free fatty acid after 24-h fasting in the suncus were about three times those of fed animals, and those in rats were double those of fed animals.

TABLE 1. Composition of liver phospholipids from animals fed or fasted for 24 h

Class	Suncus		Rat	
	Fed	Fasted	Fed	Fasted
PC	54.6 \pm 2.1	53.1 \pm 1.7	58.8 \pm 0.7	55.7 \pm 2.1
PE	25.0 \pm 0.3	27.4 \pm 0.9	20.3 \pm 1.6	19.4 \pm 1.3
PI	7.8 \pm 1.1	4.4 \pm 0.2	7.4 \pm 0.6	6.0 \pm 1.8
SM	4.6 \pm 0.2	6.6 \pm 0.4	5.3 \pm 0.7	7.2 \pm 1.0
PS	2.9 \pm 0.4	2.7 \pm 0.3	3.4 \pm 0.1	5.5 \pm 3.6
Others	4.1 \pm 0.5	5.8 \pm 0.5	5.1 \pm 1.6	6.2 \pm 0.5

The data are presented as mole % of total phospholipids (mean \pm SE; n = 3).

TABLE 2. Concentration of serum lipids in animals fed or fasted for 24 h

Serum Constituents	Suncus		Rat	
	Fed (n = 4)	Fasted (n = 4)	Fed (n = 5)	Fasted (n = 5)
T-chol (mg/dl)	32 ± 1	18 ± 2*	59 ± 5	63 ± 8
PL (mg/dl)	100 ± 18	58 ± 4**	114 ± 6	106 ± 7
TG (mg/dl)	31 ± 9	15 ± 1	96 ± 24	71 ± 16
FFA (μ Eq/l)	550 ± 42	1629 ± 95***	506 ± 41	1021 ± 137**

The data are presented as mean ± SE; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Effects of refeeding

The light microscopy of suncus liver after 1-day refeeding is shown in Fig. 1(F); it revealed that fatty deposits decreased after 1-day refeeding and that the appearance of the hepatocytes was almost normal except for many particles which seemed to be glycogen. As shown in Fig. 4, hepatic triglyceride contents of suncus that were refed after 1-day fasting decreased to the level of fed animals.

The serum lipid levels of animals that were fed, fasted for 1 day, and refed for 1, 2, and 7 days are shown in Table 3. The levels of total cholesterol, triglyceride, and phospholipid decreased after 1-day fasting and increased to the levels of fed animals after 1-day refeeding and remained unchanged thereafter.

DISCUSSION

We have found that suncus livers are very susceptible to fatty liver when food is withheld. The fatty liver is charac-

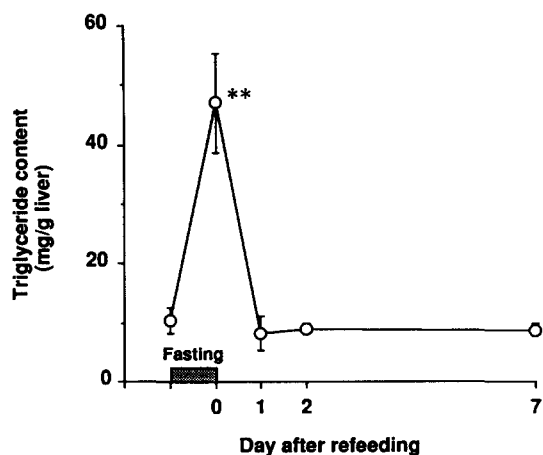


Fig. 4. Effect of fasting and refeeding on triglyceride content in the suncus liver. Suncus were fasted 24 h and refed for the periods indicated. The livers were removed immediately after the animals were killed; a part of the liver was homogenized in 0.1 M phosphate buffer (pH 7.5), and triglyceride content was measured. Each point represents mean ± SE.

terized to be induced by a short period of starvation and to be reversible simply by refeeding.

Fatty liver falls into two categories. The first type is associated with elevated levels of serum fatty acids resulting from mobilization of fat from adipose tissue or from the hydrolysis of lipoprotein triglyceride. Increasing amounts of free fatty acids are taken up by the liver and esterified. The production of plasma lipoproteins does not keep pace with the influx of free fatty acids, allowing triglyceride to accumulate, causing a fatty liver. The second type of fatty liver is usually due to a metabolic block in the production of plasma lipoprotein. Theoretically, the lesion may be due to a block in lipoprotein apoprotein synthesis, a failure in provision of phosphatidylcholine, which is necessary in order for VLDL to be secreted (10), or a failure in the secretory mechanism itself.

In general, plasma levels of free fatty acids increase as a result of fasting, and this was observed in the suncus. The ability to secrete VLDL is also impaired, causing increased amounts of triglyceride in the liver. In suncus, however, a very short period of starvation, e.g., 8 h, induced a striking accumulation of triglyceride in the liver, suggesting that there is an unusual mechanism unique to these animals.

The plasma levels of total cholesterol, triglyceride, and phospholipids were very low and decreased significantly after 1-day fasting, suggesting that the fatty liver could be induced by the metabolic block in the production of plasma lipoproteins.

There was no difference in the phosphatidylcholine contents in the liver after fasting (Table 1), suggesting that the fatty liver observed in this animal was not the result of choline deficiency. There was no evidence of morphological derangement of microfilaments or microtubulus, which play a role in the VLDL secretion (11). The fatty liver was completely reversible simply by refeeding, suggesting that the fatty liver is an unusual physiological state in the suncus but not an on-going pathological process. These observations indicate that intrahepatic assembly of VLDL may be impaired in the suncus.

Apolipoprotein B is essential for the secretion of lipoprotein particles and has very high affinity for lipids. Deficiency of apolipoprotein B results in an impaired

TABLE 3. Serum composition of fasted or refed suncus

Serum Constituents	Normal (n = 4)	Fasted 1 Day (n = 4)	Refed		
			1 Day (n = 3)	2 Day (n = 3)	7 Day (n = 3)
			<i>mg/dl</i>		
T-chol	32 ± 1	18 ± 2*	26 ± 6	35 ± 3	41 ± 6
PL	100 ± 8	58 ± 4**	95 ± 7	127 ± 10	124 ± 18
TG	31 ± 9	15 ± 1	57 ± 8	39 ± 17	47 ± 14

The data are presented as mean ± SE; *, $P < 0.05$; **, $P < 0.01$ compared with normal animals.

secretion of the lipids from the liver. In abetalipoproteinemia, which is a human hereditary disorder characterized by the absence of VLDL and LDL from plasma, triglyceride accumulation occurs in the liver to some extent. In studies on a few patients to date, both apoB mRNA and protein have been identified within hepatocytes, suggesting defective processing or secretion of the gene product. In the suncus, plasma lipid levels were very low and decreased significantly after 1-day fasting, suggesting that the transporting mechanism of the lipids from liver may be impaired. Apolipoprotein B metabolism of this animal will be discussed in another paper (12).

The fatty liver was not observed in the fed state. In starvation, increased influx of fatty acids into the liver plays a major role in triglyceride accumulation in the liver, as well as impairing the mechanism for transporting lipids from the liver. ■

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