GLYCEROLIPID METABOLISM IN CELLULAR AND SUBCELLULAR FRACTIONS OF FASTED RAT LIVER¹

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Summary The effect of overnight fasting on glycerolipids of the rat liver organelles was studied.

The concentration of microsomal phospholipids was markedly decreased by fasting, whereas that of mitochondria and whole liver increased significantly. The significant increase due to fasting in the activity of microsomal phosphorylcholine-glyceride transferase appeared to be responsible for the increased concentration of hepatic phospholipid. The incorporation by the liver slice of [1-¹⁴C] glycerol into PC³ in mitochondria was increased by fasting, while there was no difference in the microsomal fraction containing supernatant.

The significant decrease in the relative proportions of oleic and linoleic acids and a significant increase in stearic and arachidonic acids in phosphatidylcholine were observed in the intact liver as well as its subcellular fractions. The possible relationship of these changes after fasting to the similarity in the incorporation pattern of [1-¹⁴C] glycerol into the molecular species of these glycerolipids was demonstrated.

Considerable but equivocal information is available concerning the effect of fasting on the concentration of hepatic lipids in rats (1-6). In the previous experiments in which the effect of fasting was examined systematically, we have shown that overnight fasting produces a significant increase in hepatic PC and PE but not TG in the liver of male rats (7). An attempt to determine the fatty acid composition of hepatic subcellular fractions in fasted rats was done by ALLMAN *et al.* (3) and ROGERS (6). These investigators were, however, concerned only with total lipids or total phospholipids, and not with each class of glycerolipids in

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³ The following abbreviations are used in this paper: PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triglyceride; TLC, thin-layer chromatography.

the organelles. It is the purpose of the present study to determine this point precisely on the one hand, and to examine the effect of overnight fasting on the synthesis of glycerolipid at the cellular and subcellular levels in the liver on the other.

MATERIALS AND METHODS

Materials. [$1\text{-}^{14}\text{C}$] Glycerol (spec. act. 20 mCi/mmole) was purchased from the Radiochemical Centre, Amersham. Phospholipase C (*Clostridium welchii* toxin) was a product of the Sigma Chemical Co. CDP-choline was a generous gift of Takeda Chemical Industries, Osaka. Solvents of analytical grade were redistilled and deaerated with N_2 gas before use.

Animals. Male Wistar rats weighing 235–305 g and maintained on a standard laboratory chow (Oriental Rat Chow NMF, Oriental Yeast Co.) and water *ad libitum* were fasted overnight (from 6 p.m. to 9 a.m.). Control rats (fed rats) were given free access to the diet and water. The animals were killed by decapitation.

Preparation of subcellular fractions. Cell fractionation was performed according to the standardized procedure (8). Livers were homogenized in 9 volumes of 0.25 M sucrose in a Teflon homogenizer. The homogenate was centrifuged at $1,000\times g$ for 5 min to remove nuclei and cell debris. The supernatant was centrifuged at $20,000\times g$ for 10 min to sediment mitochondria. The pellet of crude mitochondria was resuspended in the sucrose solution and centrifuged similarly. This procedure was repeated twice. Microsomes were prepared by centrifuging the first mitochondrial supernatant at $104,000\times g$ for 60 min. The microsomal pellet was resuspended in the sucrose and centrifuged similarly. Washed microsomes were suspended in the sucrose solution. All manipulations were carried out at 0 to 4°C.

Liver slice experiment. Rat liver slices were prepared with a Stadie-Riggs slicer and 500 mg of the slices were incubated for selected periods in 5 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing [$1\text{-}^{14}\text{C}$] glycerol (2 μCi) at 37°C with constant shaking in an atmosphere of 5% CO_2 –95% O_2 . Following incubation, mitochondria were isolated from the slices in 0.25 M sucrose as described above. The resulting first supernatant was also collected and referred to as the microsomal fraction containing supernatant.

Preparation of radioactive 1,2-diacyl-*sn*-glycerol. Radioactive PC was prepared from liver slices which had been incubated with [$1\text{-}^{14}\text{C}$] glycerol. PC purified by TLC (9) was hydrolyzed with phospholipase C according to the methods of LANDS *et al.* (10). Hydrolysis was almost complete (>95%). Hydrolyzed products were chromatographed on a thin-layer plate of Silica gel G containing 4% boric acid (solvent; petroleum ether-diethyl ether-formic acid, 50:50:2, by vol.) and the 1,2-diacyl-*sn*-[$1\text{-}^{14}\text{C}$] glycerol was extracted with chloroform-methanol (2:1, by vol.). Radioactivity of the 1,2-diacyl-*sn*-glycerol preparation was detected solely in the

glycerol portion. 1,2-Diacyl-*sn*-glycerol was prepared on the day of the enzyme experiment and used immediately.

Preparation of 1,2-diacyl-sn-glycerol emulsion. Following evaporation of the solvent under N₂ stream, 1,2-diacyl-*sn*-glycerol was suspended in appropriate amounts of 1% Tween 20 in 0.1 M Tris buffer (pH 7.2) by sonicating twice for 60 sec at 0°C with a Tominaga UR-150 Sonicator (10 Kc, 100 w, Tominaga Co.). The emulsion obtained was almost clear. An aliquot was taken for measurement of the radioactivity in order to determine the amounts of 1,2-diacyl-*sn*-[1-¹⁴C] glycerol actually employed for incubation.

Measurement of phosphorylcholine-glyceride transferase activity. Liver microsomes were prepared similarly as described above. The buffered sucrose solution with Tris to pH 7.2 was used as a homogenizing medium. The microsomal pellet was suspended in appropriate amounts of 0.02 M Tris-0.125 M KCl buffer (pH 7.2).

Phosphorylcholine-glyceride transferase [EC 2.7.8.2] was determined essentially by the method of DE KRUYFF *et al.* (11). The reaction mixture contained radioactive 1,2-diacyl-*sn*-glycerol (equivalent to 0.74 or 0.86 μ moles of the original PC), 200 nmoles of CDP-choline (free form), 20 μ moles of MgCl₂, 8 μ moles of GSH, 140 μ moles of Tris (pH 7.2), 0.4 mg of Tween 20, and 1.1 to 1.4 mg of microsomal protein in a final volume of 2.0 ml. Incubation was carried out at 37°C.

Lipid analysis. Lipids from the liver slices and the subcellular fractions were extracted with 20 volumes of chloroform-methanol (2: 1, by vol.) and purified according to FOLCH *et al.* (12).

Fractionations of lipids were performed by TLC as described elsewhere (7). The fatty acid composition of the individual lipid classes was determined by gas-liquid chromatography (7). Phosphorus determination of individual phospholipid components was carried out according to the method of ROUSER *et al.* (13). Protein was determined by the procedure of LOWRY *et al.* (14).

Radioactivity measurements. The zones corresponding to each lipid component on a thin-layer plate were scraped directly into scintillation vials after detection with iodine vapor. Radioactivity was counted with a Beckman LS-250 liquid scintillation system in 10 ml of a scintillation solution (2 g of 2,5-diphenyl-oxazole and 50 mg of 1,4-bis-(5-methyloxazolyl-2) benzene per liter of toluene) (15). The molecular species of PC, PE and TG were separated by argentation TLC (7), eluted from Ag⁺-gel and submitted to radioactivity counting.

RESULTS

Concentration of phospholipid

The effect of overnight fasting on the concentration of protein and phospholipid in the hepatic mitochondria and microsomes is shown in Table 1. Protein concentration of the intact liver from fasted rats was slightly higher when compared to that from fed rats (235 ± 1.3 vs 214 ± 4.5 mg/g liver, $p < 0.05$). However, no

Table 1. Effect of overnight fasting on the protein and phospholipid contents in liver mitochondria and microsomes.^a

Components		Mitochondria		Microsomes	
		Fasted	Fed	Fasted	Fed
Protein	mg/g liver	36.6±2.6	36.5±0.7	16.0	18.4
Phospholipid-P	μg/g liver	184.0±3.6*	103.0±6.6	222.0	328.0
	μg/mg protein	5.0±0.3**	2.8±0.4	13.9	17.8

^a Each value for mitochondria represents the mean±S.E. of 4 animals, and that for microsomes the mean of duplicate analyses of the pooled microsomes.

* $p \leq 0.01$ Significance from the fed rat.

** $p \leq 0.05$ Significance from the fed rat.

Table 2. Phospholipid composition in liver mitochondria and microsomes.^a

Phospholipids	Distribution (%)			
	Mitochondria		Microsomes	
	Fasted	Fed	Fasted	Fed
PE	21.6	21.9	20.9	24.6
PI+PS	9.2	9.1	17.9	15.2
PC	44.4	45.7	48.0	48.7
SPH	16.8	15.2	7.8	7.6
LPC	4.0	5.1	5.4	3.9
Others	4.0	3.0	—	—

^a Phospholipid was separated into the following components by TLC: phosphatidylethanolamine (PE), phosphatidylinositol (PI)+ phosphatidylserine (SE), phosphatidylcholine (PC), sphingomyeline (SPH), lysophosphatidylcholine (LPC), and unidentified phospholipid (others). Each value represents the mean of duplicate analyses of pooled samples from 4 rats.

fasting effect was observed on the concentration of mitochondrial and microsomal proteins. Alterations due to fasting of phospholipid concentration differed between mitochondria and microsomes; increase in mitochondria and decrease in microsomes. Nevertheless, the proportion of individual phospholipids remained unchanged in both organelles (Table 2).

Fatty acid composition

Fatty acid composition of TG in the hepatic nuclei fraction (containing cell debris), mitochondria and microsomes is shown in Table 3. The significant increase in linoleic acid and the decrease in oleic acid were common variations due to fasting of all these three cell organelles. In addition, fasting caused a significant increase in the proportion of arachidonic acid in the nuclei and microsomal fractions, and a significant decrease in palmitic acid in the latter. Overnight fasting also affected the relative proportions of fatty acids of PC in the subcellular fractions (Table 4). The principal changes were significant increases in the propor-

Table 3. Fatty acid composition of triglyceride in subcellular fraction of liver.^a

Fatty acids	Nuclei+cell debris		Weight % Mitochondria		Microsomes	
	Fasted	Fed	Fasted	Fed	Fasted	Fed
16:0	24.6±1.5	26.9±2.0	24.4±0.6	27.3±2.2	24.8±0.1**	29.7±1.0
16:1	3.5±0.9	4.6±0.8	3.8±0.5	5.5±1.2	4.3±0.6	5.5±0.7
18:0	2.8±0.9	3.7±0.8	2.8±0.5	4.9±1.4	3.4±0.2	2.7±0.1
18:1	15.9±0.3*	22.9±0.4	18.5±1.7*	22.0±0.8	16.3±0.7*	21.9±1.8
18:2	39.9±1.7**	33.1±1.8	36.2±1.0**	30.5±2.8	38.4±0.8*	30.7±2.2
18:3	1.1±0.1	1.3±0.1	1.2±0.1	1.3±0.1	1.2±0.1	1.3±0.2
20:4	2.9±0.2**	1.6±0.2	2.0±0.3	1.5±0.4	3.7±0.5*	1.8±0.1
unknown-1	1.7±0.1	0.8±0.2	1.2±0.2	0.6±0.1	2.0±0.1	1.1±0.2
unknown-2	0.5±0.0	0.4±0.1	0.5±0.0	0.5±0.0	0.3±0.1	0.3±0.1
22:5	2.1±0.3	1.6±0.1	2.0±0.1	1.6±0.1	1.1±0.3	1.6±0.3
22:6	5.7±0.8	4.0±0.5	6.6±0.6	4.7±0.7	4.8±0.6	3.6±0.4

^a All determinations represent the mean±S.E. of 4 rats.* $p \leq 0.01$ Significance from fed rat. ** $p \leq 0.05$ Significance from fed rat.Table 4. Fatty acid composition of phosphatidylcholine in subcellular fraction of liver.^a

Fatty acids	Nuclei+cell debris		Weight % Mitochondria		Microsomes	
	Fasted	Fed	Fasted	Fed	Fasted	Fed
16:0	20.8±0.4	21.8±0.9	18.5±0.9	20.4±1.0	16.9±0.8*	22.0±1.4
16:1	1.6±0.2	1.3±0.1	1.0±0.1	2.3±0.6	1.3±0.2	1.4±0.2
18:0	23.8±0.8*	20.5±0.7	24.5±1.0*	19.4±0.7	25.0±0.9*	21.7±0.8
18:1	4.6±0.3*	6.5±0.1	4.3±0.0*	7.4±0.5	5.3±0.5*	7.1±0.4
18:2	14.5±0.7*	21.2±0.3	14.4±0.7*	20.7±0.4	14.3±0.5*	22.5±0.7
20:4	29.2±1.2**	23.3±1.0	30.6±0.6**	23.2±0.4	32.1±0.4*	20.1±2.1
22:5	0.8±0.1	0.9±0.1	0.6±0.1	0.9±0.3	0.5±0.0	1.4±0.1
22:6	4.9±0.7	5.2±0.5	5.3±0.2	5.7±0.6	4.8±0.5	3.7±0.4

^a All determinations represent the mean±S.E. of 4 rats.* $p \leq 0.01$ Significance from fed rat. ** $p \leq 0.05$ Significance from fed rat.

tion of stearic and arachidonic acids and significant decreases in the oleic and linoleic acids. Though no change was apparent in palmitic acid in the mitochondria and nuclei fractions, the proportion of this acid in microsomes decreased significantly. Similar alterations were also observed with PE (Table 5). On the other hand, the response of fatty acids in the microsomal phosphatidylinositol fraction (which was contaminated with phosphatidylserine) to overnight fasting was not so evident.

Formation of phosphatidylcholine from 1,2-diacyl-sn-glycerol

The activity of phosphorylcholine-glyceride transferase was measured under

Table 5. Fatty acid composition of microsomal phosphatidylethanolamine (PE) and phosphatidylinositol (PI) containing phosphatidylserine (PS).^a

Fatty acids	Weight %			
	PI+PS		PE	
	Fasted	Fed	Fasted	Fed
16:0	10.8	8.6	22.0	26.0
16:1	3.6	1.2	1.2	1.4
17:0	0.7	0.5	0.9	0.6
18:0	46.0	42.4	28.6	20.7
18:1	5.5	7.0	4.2	6.7
18:2	6.1	5.3	10.7	13.9
20:2	—	0.6	0.6	0.6
20:3	—	1.3	0.6	—
20:4	25.7	27.5	22.6	20.0
20:5	—	0.7	1.3	1.1
22:5	—	0.9	1.3	2.8
22:6	2.8	3.9	8.0	6.2

^a Each value represents the mean of duplicate analyses of pooled samples. See also Tables 3 and 4.

Table 6. Relative activity of phosphorylcholine-glyceride transferase in liver microsomes.^a

Animals	nmoles phosphatidylcholine formed/min/mg protein
Expt. I	
Fed	0.137±0.052
Fasted	0.341±0.084**
Expt. II	
Fed	0.433±0.071
Fasted	0.966±0.164*

^a Expt. I with 0.74 μ moles of 1,2-diacyl-*sn*-[1-¹⁴C]glycerol (spec. act. 4,800 cpm/ μ mole) prepared from the fasted rats and Expt. II with 0.86 μ moles of 1,2-diacyl-*sn*-[1-¹⁴C] glycerol (spec. act. 5,800 cpm/ μ mole) prepared from the fed rats. Each value represents the mean±S.E. of 4 rats.

* $p \leq 0.02$ Significance from the fed rat.

** $p \leq 0.05$ Significance from the fed rat.

the condition where the linear and maximum rate of the reaction can be determined: 0.4–1.2 μ moles of 1,2-diacyl-*sn*-glycerol and 1–2 mg of microsomal proteins both from fasted and fed rats at 37°C up to 60 min.

The influence of fasting on the relative specific activity (nmoles PC formed per min/mg microsomal protein) of phosphorylcholine-glyceride transferase is shown in Table 4. The activity of the transferase measured by using 1,2-diacyl-*sn*-glycerol prepared from the liver of a fed rat (Expt. II) was approximately threefold that of a fasted rat (Expt. I). However, regardless of the sources of the substrate, the enzyme activity of fasted rats was significantly higher than that of fed rats.

Fig. 1. Incorporation of $[1-^{14}\text{C}]$ glycerol into phosphatidylcholine in mitochondria and microsome fraction containing the supernatant in liver slices. These two subcellular fractions were prepared from the fasted (○—○) and fed (●—●) rat liver slices. Each point represents the mean of duplicate analyses of pooled samples from 4 rats.

$[1-^{14}\text{C}]$ Glycerol incorporation into subcellular phosphatidylcholine

The incorporation of $[1-^{14}\text{C}]$ glycerol into PC in mitochondria and the microsomal fraction containing supernatant prepared after incubating liver slices with this label are shown in Fig. 1 as a function of the incubation periods. The incorporation (relative specific activity) of $[1-^{14}\text{C}]$ glycerol into the microsomal PC appeared to be the same between fasted and fed rats whereas that into mitochondrial PC seemed to be higher in fasted rats than in fed rats.

Incorporation of $[1-^{14}\text{C}]$ glycerol into molecular species of glycerolipids

The effect of fasting on the incorporation of radioactive glycerol into the molecular species of PC and PE and TG in liver slices is shown in Tables 7 and 8, respectively. The data are tabulated as a mean of the values obtained at several incubation periods (15, 30, 60, 90 and 120 min), since the percentage distribution of the radioactivity in each species was essentially the same irrespective of the incubation periods.

On fasting, the percentage distribution of $[1-^{14}\text{C}]$ glycerol in the species of PC appeared to increase slightly in the tetraenoic (04) and hexaenoic (06) species at the expense of the dienoic (02) species. Net incorporation of $[1-^{14}\text{C}]$ glycerol into the dienoic PC species during 120 min of incubation was similar between fasted and fed rats (1.20 vs 1.33%) and appeared to increase by fasting in the tetraenoic and hexaenoic species (0.625 vs 0.408% and 0.277 vs 0.145%, respectively). Decrease in the percentage distribution of the radioactivity in the dienoic species and increase

Table 7. Percentage distribution of [$1\text{-}^{14}\text{C}$] glycerol among newly synthesized species of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) in liver slices.^a

Molecular species	PC		PE	
	Fasted	Fed	Fasted	Fed
01*	12.8	9.6	1.1	2.3
02	54.9	61.6	38.3	55.4
04	24.9	20.1	16.5	18.9
06	14.1	8.6	44.1	23.3

^a PC and PE were separated by argentation TLC into four fractions, according to the degree of unsaturation.

* The figure represents the number of double bonds of the 1- and 2-position of the molecule, respectively.

in the hexaenoic species due to fasting were much more predominant in PE. Percentage distribution of [$1\text{-}^{14}\text{C}$] glycerol among the TG species was also altered by fasting (Table 8); an increase in the polyunsaturated species (≥ 5) of TG and decrease in the 012 and 022 species.

Table 8. Percentage distribution of [$1\text{-}^{14}\text{C}$] glycerol among newly synthesized species of triglyceride in liver slices.^a

Molecular species	Fasted	Fed
0 0 0*	3.3	2.4
0 0 1	0.5	2.3
0 1 1	4.4	4.7
0 0 2	1.5	1.1
0 1 2	4.9	10.2
1 1 2	1.0	2.1
0 2 2	9.5	14.0
5 $\leq$	75.2	63.2

^a Triglyceride was separated by argentation TLC into eight fractions, according to the degree of unsaturation.

* The figure represents the number of double bonds at the 1- and 2- and 3-position of the molecule.

DISCUSSION

The values in Table 6 were derived from the experiment where the rate of phosphorylcholine-glyceride transferase reaction was proportional to the amount of microsomal enzyme protein and of the substrate, 1,2-diacyl-*sn*-glycerol. Though application of 1,2-diacyl-*sn*-glycerol to the reaction system still remains to be improved, the enzymic activity was increased by fasting whichever the substrates from fasted or fed rats were used. The previous paper (16) showed that there was no significant difference in the incorporation by liver of labelled long chain fatty acids into PC between fasted and fed rats, and glycerol incorporation into it rather

increased by fasting, though the utilization of both precursors in TG synthesis was remarkably depressed by fasting. VAVRECKA *et al.* (17) reported that fasting caused a decrease in the activity of acyl-CoA glycerol phosphate acyltransferase in liver homogenates, and an increase in the activity of phosphatidate phosphohydrolase in the supernatant fraction of the liver. Though the phosphatidate phosphohydrolase activity may play a key role in the regulation of endogenous TG formation under such conditions as an accelerated liver TG formation (hepatoectomy (18), high carbohydrate intake (19–21)), the observation in the fasting state is difficult to interpret since most investigators have reported a decrease in neutral lipid formation under this situation (7, 21). Therefore, control of PC biosynthesis in fasted rats by the phosphorylcholine-glyceride transferase could be expected and, consequently, the activity of this enzyme could affect the synthesis of liver TG in fasted rats, if this enzyme was rate-limiting. However, it is not apparent if the increase due to fasting in the concentration of liver PC would simply be attributable to the elevation of the activity of phosphorylcholine-glyceride transferase, because the PC content in hepatic microsomes seemed to decrease in fasted rats though the extent of phospholipid output from liver to plasma was reduced (22).

The concentration of phospholipid in hepatic mitochondria and microsomes was found to be differently affected by overnight fasting. It is quite difficult to explain clearly why the concentration of microsomal phospholipid remarkably decreases and that of mitochondria increases significantly after fasting. SCHULTZE *et al.* (23) observed significant reduction of microsomal phospholipid in rats fasted for 48 hr. The different effects of fasting on the incorporation of [1-¹⁴C] glycerol into PC in the liver mitochondria and microsomal fraction containing supernatant may provide a clue to this phenomenon. There was no difference in the specific activity of PC in the microsomal fraction between liver slices from fasted and fed rats, whereas that from mitochondria appeared high in fasted rats (Fig. 1). From the fact that *de novo* synthesis of PC and PE occurred only in microsomes, but not in mitochondria (24–26), and that the phospholipids in the latter are originated from interexchanges of them between these two organelles (27), it is likely that these exchange reactions are influenced specifically by fasting. Concerning the heterogeneous exchange of molecular species of phospholipid, TANIGUCHI and co-workers (28) recently showed that there is a slight difference in the extent of exchange among molecular species of PC and PE; the more unsaturated species exchanged more rapidly. Therefore, it is conceivable that the metabolic rate of phospholipids in these two organelles is modified specifically by fasting.

The percentage of palmitic acid in microsomal PC and TG significantly decreased by fasting, whereas the alteration of this acid was insignificant in mitochondria. On the other hand, the change after overnight fasting in the relative proportions of other major fatty acids in subcellular PC and TG was commonly seen in all of the organelles as could be observed in the intact liver (7).

The results with synthesis *de novo* of each molecular species of PC and PE

from [1-¹⁴C] glycerol and endogenous acyl molecules in liver slices are comparable with those concerned with the changes in fatty acid composition in cellular and subcellular phospholipids. The preceding paper (16) indicated that fasting in no way influenced the specificity in the esterification of [1-¹⁴C] palmitate, stearate and linoleate with glycerophosphate by the liver slices. ELLINGSON *et al.* (29) also stated that the changes due to fasting in the fatty acid composition in PC could not be attributable to the altered specificity of the acyl-CoA: 1-acyl glycerylphosphorylcholine acyltransferase. Thus, the fasting-induced alterations in the fatty acid patterns may rather be attributable to changes in the availability of acyl-CoA(s) in the liver. This rationale is especially appropriate for oleate, which is produced within the cells by a desaturation of stearate, the activity of the enzyme being reduced by fasting (30). In spite of the depression after fasting of the conversion of linoleate to arachidonate *in vitro* (31) and *in vivo* (32), the percentage of arachidonate in hepatic phospholipids was increased. The authors predict this phenomenon as a reflection of a decrease in turnover of arachidonic acid compared to that of linoleic acid in phospholipids of the liver cells (32).

The percentage incorporation of [1-¹⁴C] glycerol into the polyunsaturated species of TG in liver slices was increased by fasting. SUGANO *et al.* (33) showed that forty percent of total TG mass consisted of this fraction and linoleic acid occupied one-third of the fatty acids of this fraction. Thus, it is reasonable that the relative increase in linoleic acid in cellular and subcellular TGs in fasted rats is reflected either by the increased synthesis or the decreased turnover of this polyunsaturated species of TG. Kinetic heterogeneity in the synthesis and turnover of liver TG is reported by ÅKESSON *et al.* (34) and BAKER (35).

The reason why phospholipids in the cellular and subcellular fractions are specifically modified by overnight fasting is not apparent at present. Available information (36) shows that the concentration and composition of plasma and hepatic glycerolipids do not change as such in rats previously fed a diet containing a high level of linoleic acid. Thus, it is suggested that the structure and function of the biological membranes are at least maintained so as to correspond to the nutritional changes due to fasting in the cells, because phospholipids are the major structural components of cell membrane.

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