Effects of Overnight Fasting on Lipid and Fatty Acid Profiles of Rats Fed cis- or trans-Fat

Cho, Young-Ja
Laboratory of Nutrition Chemistry, Faculty of Agriculture, Kyushu University

Ide, Takashi
Laboratory of Nutrition Chemistry, Faculty of Agriculture, Kyushu University

Sugano, Michihiro
Laboratory of Nutrition Chemistry, Faculty of Agriculture, Kyushu University

KYUSHU UNIVERSITY

Effects of Overnight Fasting on Lipid and Fatty Acid Profiles of Rats Fed *cis*- or *trans*-Fat*

Young-Ja Cho, Takashi Ide and Michihiro Sugano

Laboratory of Nutrition Chemistry, Faculty of Agriculture,
Kyushu University 46-09, Fukuokn 812

(Received June 12, 1984)

Male rats were fed semipurified diets containing partially hydrogenated corn oil (*trans*-fat) or olive oil (*cis*-fat) for 3 weeks and then fasted overnight (15 hr). The fatty acid compositions of dietary fats differed virtually only in the geometry of the octadecenoic acid. There were no detectable differences in concentrations of tissue lipids (cholesterol, triglyceride and phospholipid) between the dietary groups excepting considerably higher levels of intestinal mucosal triglyceride and adipose tissue cholesterol in rats fed the *cis*-fat. Overnight fasting caused lipid-dependent changes in the concentrations and the fasting-effect appeared to be more salient in the *cis*-fat group. Although fasting also caused characteristic changes in fatty acid compositions of several lipid classes from the different tissues, changes in the truns-octadecenoate were rather marginal compared to the cis-octadecenoate. The responses of the *trans*-octadecenoate did not necessarily parallel to those of the c&counterpart or saturated fatty acids. The results suggest the complexity of the metabolic fate of the truns-octadecenoate in relation to the cis-octadecenoate.

INTRODUCTION

The fatty acid compositions of animal tissues can be modified by the type of dietary fats. Thus, feeding of partially hydrogenated fats such as margarines and shortenings results in an accumulation of *trans*-fatty acids to a predictable extent (Ohlrogge *et al.,* 1981, Cho *et al.,* 1984). There is a respectable controversy regarding the fate of *trans*-fatty acids deposited in the tissues. The rate of oxidation of the *cis*- and *trans*-isomers appears to differ depending on whether it was measured in *vivo* or in *vitro* (Beare-Rogers, 1983; Ide and Sugano, 1984). Anyhow, the cessation of *trans*-fat feeding leads to the disappearance of *trans*-fatty acids from the tissues in a relatively short period (Alfin-Slater and Aftergood, 1979).

Since the fatty acid serves as an exclusive fuel in the fasted animals, the analyses of the profiles of concentrations and compositions of tissue lipids under this situation should give us a clue to understanding the fate of specific fatty acids. In rats, it has been shown that overnight fasting causes characteristic changes in the fatty acid profiles of tissue lipids (Swell and Law,

---

* Abbreviations used : TG ; triglyceride, PC; phosphatidylcholine, PE; phosphatidyletha-
nolamine.

1967 ; Rogers, 1971; Imaizumi *et al.*, 1972). The fasting effect is specific for in-dividual lipid components and is dependent on the essential fatty acid status of the animals (Sugano *et al.*, 1975 a, 1975 b).

The aim of the present study was to compare the effect that overnight fasting exerts on lipid parameters between rats fed fats in which the fatty acid compositions are comparable except the difference in the geometry of the octadecenoate. Thus, rats were fed diets containing partially hydrogenated corn oil or olive oil for 3 weeks and then fasted overnight.

MATERIALS AND METHODS

Animals and diets

Male, S. D. rats were purchased from Seiwa Experimental Animals, Yoshitomi, Fukuoka, housed individually and fed for 1 week the commercial laboratory chow (Oriental Yeast Co., Type NMF, Tokyo) in a temperature $(22 \pm 2^{\circ}C)$ and light (lights on 0800 to 2000 hours) controlled environment. The animals weighing average 107 g were then randomly divided into two groups of 13 rats each and were fed ad *libitum* one of the following two diets for 3 weeks. The experimental diets contained in percent: casein 20, fat 10, salt mixture 4, vitamin mixture 1 (the salt and vitamin mixtures according to Har-per, Oriental Yeast Co., Tokyo), choline chloride 0.2, cellulose powder 2, and sucrose to 100. Dietary fats were either partially hydrogenated corn oil (Su-gano *et al.*, 1983 a, 1983 b) or olive oil as *trans*- or *cis*-fat sources, respectively. By partially substituting, safflower oil (1.1 %) for the *trans*-fat, the content of linoleic acid in these diets was adjusted to be the same (2% of total energy) (Sugano *et al.*, 1983 b). The sole detectable difference in these fat diets was the proportion of the geometric isomers of the octadecenoate (Table 1). At the end of feeding, half of rats in each dietary group was fasted overnight (1800-0900 hours, fasted rats) and the remaining half was continued to access diets freely (fed rats).

Table 1. Fatty acid compositions of diets.

Groups	Fatty acids (%)					
	16 : 0	16 : 1	18 : 0	t-18 : 1	c-18 : 1	18 : 2
PHC	13. 1	t r	7.9	42.0	27. 2	9. 4
OL	'3.7	0. 9	3.4	tr	74.1	9. 4

PHC and OL: partially hydrogenated corn oil and olive oil. tr: trace.

Lipid analyses

Rats were killed by decapitation. Serum and liver lipid were analyzed for cholesterol, phospholipid and TG (Nagata *et al.*, 1980). Lipids were also extracted from liver mitochondria and microsomes, epididymal adipose tissue and intestinal mucosa(separated from the small intestine by scraping according

to Dietschy and Siperstein, 1965). These lipids were also separated into choles-
terol ester, TG, PC and PE by thin-layer chromatography (Mangold, 1964).
The fatty acid composition of each lipid fraction was analyzed by gas-liquid
chromatography (DEGS and OV-275 columns) as described elsewhere (Suga-
no **et al., 1983 a,** 1983 b). Cholesterol in liver organelles, intestinal mucosa and
adipose tissue was determined by gas-liquid chromatography using an OV-17
column (Sugano *et al.,* 1976). Protein **was** determined by the Lowry method
(Lowry *et al.,* 1951). Liver homogenate (10 %) in 0.25 M sucrose-o. 1 mM
EDTA, pH 7.0 was centrifuged at 1,000 xg for 10 min to sediment the nuclei
and cell debris. The supernatant was centrifuged at 9, $000 \times g$ for 10 min to
sediment mitochondria. Microsomes were sedimented at 105,000 xg for 60 min.
These two organelles were washed by respinning.

Statistical analysis

Results were analyzed statistically by two way analysis of variance, fol
lowed by inspection of all differences between pairs of means (Yoneda **et al.,**
1981).

RESULTS

General observations

There were no demonstrable differences in body weight gain and food intake
between the *cis-* and *trans-*fat groups. The extent of reduction of body weight
after overnight fasting was also the same in two dietary regimens (approxi-
mately 5 %). However, although the liver weight of fed rats was comparable
(5. 21±0. 23 vs. 5.26t0.21 g/100 g body weight for the **tram-** and *cis-*groups),
somewhat more greater reduction of liver weight was observed in the *cis-*fat
group (4. 04±0. 37 vs. 3. 57±0. 08 g/100 g body weight).

Tissue lipid concentrations

As shwn in Table 2, the type of dietary fat did not influence on the serum
levels of TG, phospholipid and cholesterol. Overnight fasting caused a marked
reduction of serum TG and phosholipid. The reduction of cholesterol level
was moderate and statistically not significant.

Deitary fat-dependent differences in concentrations of liver lipids were
observed only on TG; it was significantly greater in rats fed the cis-fat, while
fasting reduced liver TG to a comparable level (Table 2). There was a trend
toward increasing the concentration of cholesterol and phospolipid after fast-
ing and this was more notable in rats fed the cis-fat. The fasting-induced
increase in the cholesterol content was also observed on liver mitochondria
(9.03t1.80 vs. 11. 4±1. 2 μg/mg protein for the trans-fat and 8. 36±0. 49 vs. 14.2
±1. 7 μg/mg protein for the *cis-*fat, $p < 0.05$) and microsomes (39. 8±3. 2 vs. 50. 5±
2. 2 μg/mg protein for the trans-fat, $p < 0.05$ and 39. 4±3. 4 vs. 51. 6±4. 5 μg/mg
protein for the *cis-*fat).

As shown in Table 3, the adipose tissue from rats fed the cis-fat contained

Table 2. Concentrations of serum and liver lipids.

Groups	Cholesterol	Triglyceride	Phospholipid
Serum (mg/dl)			
PHC fed	86.0±3.3	255 ±64[a]	179±17[a]
fasted	72.5±5.7	44.9± 4.8	103±11
OL fed	91.3±3.4	258 ±32[a]	201±12[a]
fasted	78.7±7.6	65.4±13.0	106±13
Liver (mg/g)			
PHC fed	2.88±0.19	27.1±3.2	33.4±1.3
fasted	3.53f0.33	22.6±1.7	36.3±2.1
OL fed	2.97±0.19	42.9-16.3[a]	29.9±0.6[a]
fasted	5.02±0.46	19.0±2.9	38.2±0.7

PHC and OL: partially hydrogenated corn oil and olive oil. Means±SE of 6 to 7 rats per group. "Values for fed rats are significantly different from those for the corresponding fed animals at $p < 0.05$.

Table 3. Concentrations of adipose tissue and intestinal mucosal lipids.

Groups	Cholesterol	Triglyceride	Phospholipid
Adipose tissue (mg/g)			
PHC fed	0.26 ±0.04	nd	nd
fasted	0.29±0.05	nd	nd
OL fed	0.41 ±0.05	nd	nd
fasted	0.26f0.02	nd	nd
Intestine (mg/g)			
PHC fed	2.01±0.17	5.92±1.27	10.6±1.0[a]
fasted	1.84±0.16	5.54±1.26	7.94±0.82
OL fed	2.19±0.12	8.56f1.89	12.5±0.7'
fasted	1.80±0.11	9.64± 1.84	9.43±0.54

PHC and OL: partially hydrogenated corn oil and olive oil. Means±SE of 6 to 7 rats per group. "Values for fed rats are significantly different from those for the corresponding fasted animals at $p < 0.05$. nd: not determined.

a markedly higher level of cholesterol compared to rats fed the *trans*-fat. The difference was, however, disappeared when the animals were fasted overnight; the concentration of cholesterol was uninfluenced in the *trans*-fat group, **while in rats** fed the cis-fat it reduced to a level of rats fed the *trans*-fat after fasting.

Excepting cholesterol and phospholipid which were comparable between the two groups, the concentration of TG in the intestinal mucosa tended to be higher in rats fed the cis-fat than in those fed the Pans-fat (Table 3). Fasting did not alter the concentration of TG. The concentration of mucosal phospholipid and cholesterol tended to decrease after fasting in both groups of rats.

Fatty acid compositions

Serum *lipids*

Table 4 summarizes fatty acid compositions of serum lipid components. In TG, the composition was essentially comparable between the two fat groups,

except the presence of trans-octadecenoate and a somewhat lower percentage of total octadecenoate in rats fed partially hydrogenated corn oil. Linoleic acid percentage was the same each other. The response of fatty acid profile to fasting was comparable in the two groups of rats; a decrease in the percentage of oleic acid and an increase in linoleic and arachidonic acids. In addition, there was a significant decrease in the trans-octadecenoate in the *trans*-fat group.

The fatty acid composition of serum PC was roughly comparable between the two fat groups, but there were slight but detectable differences; the percentage of total octadecenoate was somewhat higher while that of stearic acid was lower in rats fed a trans-fat diet. In addition, the proportion of linoleic to arachidonic acids was elevated on feeding the trans-fat although the total amount of polyunsaturated fatty acids were in no way changed. Fasting caused increases in palmitic and arachidonic acids and a decrease in linoleic acid in both groups of rats. The percentage of oleic acid was reduced in rats fed olive oil.

Fatty acid composition data for serum cholesterol ester were also comparable beween the two fat groups, except for a considerable difference in the percentage of linoleic and arachidonic acids. This fraction contained a very low level of the *trans*-octadecenoate compared to the other lipid components. The characteristic changes commonly observed after overnight fasting were an increase in arachidonic acid and a decrease in linoleic acid. The percentage of oleic acid tended to reduce only when rats were fed the hydrogenated fat.

Liver lipids

(a) *Whole liver:* In liver TG, the fatty acid composition was essentially the same between the two fat groups excluding the octadecenoate (Table 5). The percentage of *trans*-octadecenoate was considerably lower than that for the serum counterpart. The most obvious changes produced by overnight fasting was a striking increase in linoleic acid and a reduction of palmitic acid.

In liver PC, there were detectable differences in fatty acid compositions between rats fed two types of dietary fats; a higher percentage of linoleic acid and a lower percentage of arachidonic and stearic acids in the trans-fat group. The incorporation of the trans-octadecenoate was apparently counterbalanced by the decrease in stearic and palmitic acids since the percentage of oleic acid was comparable. Although there were somewhat different responses to fasting between the two fat groups, the common phenomena were an increase in arachidonic and palmitic acids and a decrease in linoleic acid. The trans-octadecenoate tended to increase after fasting.

The fatty acid composition of liver PE was also comparable with respect to major components. In contrast to PC, the fasting effect was the least in PE, no changes were observed on polyunsaturated fatty acids except docosahexaenoic acid. There was, however, a significant elevation of the *trans*-octadecenoate after fasting.

(b) *Mitochondrial phospholipids*: As shown in Table 6, the fatty acid com-

Table 4. Effect of overnight fasting on fatty acid compositions of serum lipid.

Groups			Fatty acids (%)		
	16 : 0	*t-16* : 1	c-16 : 1	18 : 0	*t-18* : 1
Triglyceride					
PHC fed	25.9±0.8	1.5±0.1	5.0±0.2[a]	2.8±0.2·4	11.0±0.1[a]
fasted	23.5±0.7	1.3±0.1	4.0±0.2	2.9±0.3	8.2f0.5
OL fed	21.5±0.6	tr	4.4±0.4[a]	2.1±0.1	tr
fasted	23.4f0.7	tr	3.2±0.2	2.7±0.2	tr
Phosphatidylcholine	18.0±0.5[a]	0.5F0.0	1.4±0.1	16.7±0.4	9.1±0.3
PHC fasted	18.2 2.8f1.2	0.4±0.1	1.5f0.4	15.3±0.4	7.9±0.5
OL fed	20.1±0.6[a]	tr	0.7±0.2	21.9±0.8	tr
fasted	25.2±0.9	tr	1.1±0.5	19.8±0.9	tr
Cholesterol ester					
PHC fed	9.9±0.4	0.7±0.1	5.4±0.4[a, A]	1.9±0.2	1.1±0.1
fasted	9.2±0.5	0.6±0.1	3.4±0.2[A]	1.6±0.1	0.8±0.1
OL fed	8.9±0.6	tr	4.2±0.4”	2.1Iko.5	tr
fasted	8. 1i0.4	tr	2.2±0.2	2.0±0.2	tr

PHC and OL: partially hydrogenated corn oil and olive oil. Means? SE of 6 to 7 rats
per group. [a]Values for fed rats are significantly different from those for the corre-

Table 5. Effect of overnight fasting on fatty acid compositions of liver lipids.

Groups			Fatty acids (%)		
	16 : 0	*t-16* : 1	c-16 : 1	18 : 0	*t-18* : 1
Triglyceride					
PHC fed	35.2±0.5	1.6±0.1	6.6±0.3	3.0±0.1[A]	4.3±0.4
fasted	28.5±0.8	1.4f0.2	5.0±0.3	2.8±0.4[A]	4.7f0.7
OL fed	31.3±0.8	tr	7.5±0.8[a]	1.5±0.1	tr
fasted	26.7±0.7	tr	5.2±0.4	2.1±0.1	tr
Phosphatidylcholine					
PHC fed	16.9±0.7[a, A]	0.6±0.0	1.9±0.2[a]	15.7±0.6[A]	8.3±0.6[a]
fasted	20.7±0.9	0.5±0.1	1.0±0.2	13.8±0.6[A]	9.9±0.3
OL fed	19.5±0.8[a]	tr	1.9±0.2”	20.9±0.9	tr
fasted	22.1±0.3	tr	0.8±0.4	20.1±0.6	tr
Phosphatidylethanolamine PHC fed	15.1±0.9[A]	0.3±0.1	1.2±0.1	19.5±0.5	7.4±0.2[a]
PHC fasted	15.0±0.8	0.2±0.1	0.8±0.1	18.9±0.6	10.7±0.5
OL fed	18.0±0.7[a]	tr	1.8±0.3”	22.1±0.5	tr
fasted	15.6±0.6	tr	0.9 10.3	24.8±0.5	tr

PHC and OL: partially hydrogenated corn oil and olive oil. Means±SE of 6 to 7 rats
per group. “Values for fed rats are significantly different from those for the corre-

position of mitochondrial PC differed to some extent between two types of
dietary fats; a markedly higher percentage of linoleic acid and a lower per-
centage of stearic and arachidonic acids in rats fed the *trans*-fat compared to
rats fed the *cis*-fat. However, the fasting effect was comparable in the two
groups and was essentially similar to that observed on whole liver PC; a de-

c-18:1	$18:2	cc-18:2	20:4	20:5	22:6
41.9±0.6	1.0±0.2	6.6±0.5a	0.3±0.1	tr	tr
38.740.8	1.0±0.1	12.5±0.6	2.5±0.4	tr	tr
62.9±0.8	tr	6.1±0.2a	0.4±0.0	tr	tr
49.3±1.1	tr	11.5±0.3	5.6±0.5	tr	tr
15.6±0.2	0.7±0.1	15.2±0.4	9.9±0.4	1.8±0.1	2.5±0.1
16.1±0.2	0.5±0.2	11.7±0.3	12.3f1.4	1.6±0.2	3.0f0.4
19.4±1.1		5			
15:3±0.6	trtr	12.7±0.7,8±0.8	14.9±1.911.4±1.0	1.4±0.21.3±0.2	2.7±0.4 3.8k0.6
15.8±0.7	1.0±0.1a	16.5±0.5	40.3±1.2a	tr	tr
12.4±0.4A	0.4±0.1	11.1±0.8	52.5±1.6	tr	tr
18.0±0.9	tr	11.9±0.7	47.3±3.3a	tr	tr
17.8±1.6	tr	8.0i0.9	56.3±3.2	tr	tr

sponding fasted animals at $p<0.05$. [A]Values for PHC groups are significantly different from those for the corresponding OL gorups at $p<0.05$. tr: trace.

c-18:1	$\frac{ct}{tc}$-18:2	cc-18:2	20:4	20:5	22:6
41.4±0.6A	0.7±0.1	2.9±0.1a	0.3±0.0a	tr	tr
42.7±1.2A	0.9±0.2	7.3±0.4A	1.1±0.1	tr	tr
54.3±1.1	tr	1.9±0.5a	0.2±0.0a	tr	tr
54.6±1.4	tr	5.8±0.3	1.0±0.1	tr	tr
13.1±0.6	0.4±0.1		16.2±0.8a,A	2.1±0.2	3.6±0.2
12.1±0.7	0.5±0.1	10.0±0.4a,A	24.3±1.8A	1.8±0.2	4.5±0.5
14.9±0.6	tr	7.7±0.5a	21.3±1.0a	2.1±0.3	4.7±0.1
11.7±0.5	tr	5.0±0.2	28.4±0.7	1.5±0.1	5.3±0.5
7.7±0.4A	0.1±0.0	4.140.2	25.3±0.8	4.1±0.3	9.3±0.3a
6.2±0.7	0.2±0.0	3.5±0.3	26.2±1.4	3.8±0.4	12.0±1.3
10.1±1.2	tr	3.3±0.6	24.3±1.5	3.4±0.6a	9.9±0.6a
8.2±0.6	tr	2.9±0.4	25:8±0.9	2.6±0.3	14.7±0.6

sponding fasted animals at $p<0.05$. [A]Values for PHC groups are significantly different from those for the corresponding OL groups at $p<0.05$. tr: trace.

crease in the percentage of stearic, oleic and linoleic acids and an increase in the percentage of arachidonic and clocosahexaenoic acids.

In PE, the pattern of the dietary fat-dependent difference in the fatty acid composition resembled that of PC, although PE was much more highly unsaturated than PC. However, the fasting effect was different from that ob-

Table 6. Effect of overnight fasting on fatty acid compositions of liver

			Fatty acids (%)			
Groups		16 : 0	t-16 : 1	c-16 : 1	18 : 0	t-18 : 1
Phosphatidylcholine						
	PHC fed	17.3±0.4[A]	0.4±0.1	2.1±0.1	15.8±0.8[a,A]	6.9±0.3
	fasted	19.0±0.9[A]	0.5±0.1	2.0±0.2	12.2±0.2[A]	7.1±0.2
	OL fed	19.5±0.8[a]	tr	2.4±0.2	20.7±0.8[a]	tr
	fasted	23.2±0.7	tr	2.1±0.2	18.6±0.7	tr
Phosphatidylethanolamine						
	PHC fed	17.3±1.6[a]	0.2±0.1	2.1±0.4	19.7±0.7[A]	6.6±0.5"
	fasted	12.6±0.5	0.2±0.1	2.0io.2	18.8±0.9[A]	8.0±0.3
	OL fed	14.6±0.5	tr	1.7±0.1	23.9±0.4	tr
	fasted	13.3±0.5	tr	1.6±0.2	23.9±0.7	tr

PHC and OL: partially hydrogenated corn oil and olive oil. Means±SE of 6 to 7 rats per group. [a]Values for fed rats are significantly different from those for the corre-

Table 7. Effect of overnight fasting on fatty acid compositions of liver

			Fatty acids (%)			
G r o u p s		16 : 0	t-16 : 1	c-16 : 1	18 : 0	t-18 : 1
Phosphatidylcholine						
	PHC fed	20.0±0.6[A]	0.4±0.1	2.4±0.2	17.9±0.6[A]	8.9±1.1
	fasted	23.3±0.7	0.5±0.0	1. oio. 1	17.6±0.2[A]	11.4±0.2
	OL fed	25.9±2.0	tr	1.8±0.2	29.1±2.4	tr
	fasted	25. Otl. 4	tr	1.3±0.1	25.3±0.9	tr
Phosphatidylethanolamine						
	PHC fed	18.3±0.7	0.1±0.1	1.9io.3	19.6±0.5[a,A]	8.7±0.4[a]
	fasted	12:9±0.6	0.1±0.0	0.9±0.1	22.9±0.5[A]	13.4±0.3
	OL fed	20.4±0.7	tr	1.9±0.3	26.1±0.9[a]	tr
	fasted	20.9±0.6	tr	2.7io.4	30.8± 1.6	tr

PHC and OL: partially hydrogenated corn oil and olive oil. Means±SE of 6 to 7 rats per group. "Values for fed rats are significantly different from those for the corre-

served on PC. The percentage of linoleic and arachidonic acids remained almost unchanged while eicosahexaenoic acid increased detectably. The effect of fasting on PE fatty acids appears to be much more moderate than the PC counterparts.

(c) *Microsomal phospholipids*: As shown in Table 7, the difference essentially similar to those observed on mitochondrial PC were found in microsomal PC. However, the percentage of stearic acid differed more markedly and that of arachidonic acid was the same between the two dietary fat diets, although the level of the latter was considerably low compared to that of mitochodrial PC. The common fasting effect was only an increase in arachidonic acid. In rats fed the *trans*-fat, the percentage of the *trans*-octadecenoate tended to increase while that of the cis-octadecenoate showed a trend toward decreasing.

mitochondrial lipids.

c-18 : 1	$^{ct}_{tc}$–18 : 2	cc-18 : 2	20 : 4	20 : 5	22 : 6
16.0±0.4	0.4±0.1	12.3±0.4[a,A]	16.7±0.6[a,A]	2.2±0.1	3.7±0.2[a,A]
14.0±0.5	0.5±0.0	10.1±0.4[A]	21.7±1.1[A]	2.0±0.2	4.7±0.3[A]
15:0±0.7[a]	tr	7.3±0.5[a]	21.9±0.8[a]	2.2±0.3	4.8±0.2[a]
12.9±0.8	tr	4.8±0.4	25.2±1.5	1.6±0.2	5.9±0.3
10.9±0.7	0.3±0.0	7.6±0.9	19.2±1.0	3.0±0.3	6.2±0.6[a,A]
9.0f0.3	0.4tr0.0	10.0±1.4[A]	22.2±1.2	3.3±0.4	9.0±0.5"
10.2±0.4	tr	5.1±0.1	26.5±0.3	3.61kO.5	9.9±0.4[a]
10.3±0.7		5.3±0.7	23.6±0.9	2.5kO.2	12.2±1.1

sponding fasted animals at $p<0.05$. [A]Values for PHC groups are significantly different from those for the corresponding OL groups at $p<0.05$. tr: trace.

microsomal lipids

c-18 : 1	$^{ct}_{tc}$–18 : 2	cc-18:2	20:4	20:5	22:6
16.0±0.8	0.7±0.1	10.2±1.1[A]	11.7±0.3[a]	1.1±0.1	1.6±0.2"
13.8±0.6[A]	0.6±0.0	8.4±0.4[A]	16.9k1.2	1.0±0.1	2.3±0.1
16.5±1.4	tr	6.7±1.1	11.4±1.8"	1.0±0.2	1.8±0.2
17.7±0.8	tr	6.0±0.3	16.1tl.3	0.6±0.1	2.3±0.3
10.4±0.3[a,A]	0.3±0.1	5.4±0.2[A]	18.7±0.7	3.3±0.2	6.1±0.3
7.9±0.4[A]	0.4±0.1	4.8±0.2[A]	21.7± 1.0	2.8±0.3[A]	7.6k0.6
14.0±0.7	tr	4.1Iko.3	18.3±1.1	2.5±0.3[a]	6.0±0.6
13.2±0.8	tr	4.0±0.3	14.5±1.0	1.1±0.1	5.3±0.6

sponding fasted animals at $p<0.05$. [A]Values for PHC groups are significantly different from those for the corresponding OL groups at $p<0.05$. tr: trace.

The fatty acid composition of microsomal PE from rats fed either partially hydrogenated corn oil or olive oil was comparable except a lower percentage of stearic and oleic acids in the former group, no differences were found on polyunsaturated fatty acids. The effect of fasting was more marked with respect to the inverse response pattern of the cis- and *trans*-octadecenoates compared to mitochondrial PE or microsomal PC.

Adipose tissue lipids

Table 8 shows fatty acid composition of adipose tissue lipids. The type of dietary fats did not exert any effects on the composition excepting an introduction of the *trans*-octadecenoate in rats fed hydrogengated corn oil, the level of the total octadecenoate being the same. Fasting showed no detectable effects on the composition.

Y. *Cho., T. Ide and M. Sugano*

Table 8. Effect of overnight fasting on fatty acid compositions of adiopse

	Fatty acids (%)			
Grous	16 : 0	t-16 : 1	c-16 : 1	18 : 0
To& lipids				
fed	10.8±1.4	1.1±0.1	7.1±0.3^A	1.3±0.2^A
fasted	9.3±0.8^A			
OL fed	14:6±2.2	1.0±0.tr 1	6.9±0.4^A 6.1±0.3	1.2±0.1^A 1.7±0.2
fasted	15.5±1.8	tr	5.9-to.3	1.9±0.2

PHC: and OL: partially hydrogenated corn oil and olive oil. Means±SE of 6 to 7 rats per group. *Values for PHC groups are significantly different from those for the co-

Table 9. Effect of overnight fasting on fatty acid compositions of intestinal

	Fatty acids (%)					
Groups	16 : 0	*t-16 : 1*	c-16 : 1	18 : 0	*t-18 : 1*	c-18 : 1
Triglyceride						
PHC fed	27.0±1.6^A	0.6±0.1	4.9zko.4	5.3±0.6^A	18.5±1.6	34.9±1.2^A
fasted	28.7±0.6^A	0.7±0.1	5.0f0.4	4.6±0.6^A	15.5±0.7	35.6±0.7^A
OL fed	23.8±0.5	tr	4.7±0.3	3.1±0.1	tr	61.4±0.5
fasted	22.6±0.7	tr	4.8f0.4	2.5±0.1	tr	62.7±1.0
Phosphatidylcholine						
PHC fed	18.5±0.6	0.7±0.1	2.3±0.2	16.2±1.4	8.8±0.9	19.5±0.8
fasted	18.8±2.1	0.4±0.2	1.9±0.4	21.0±1.1	7.8±0.5	20.2±1.1
OL fed	19.0±0.6	tr	1.5±0.2	22.3±0.9	t r	25:1±0.2
fasted	21.3±0.6	tr	1.6±0.2	20.4±0.4	tr	22.3±0.9
Phosphatidylethanolamine						
PHC fed	8.5±0.6"	tr	1.5±0.1	**25.**5±3.1	9.1±0.6^a	21.5±1.0
fasted	13.6±1.7	tr	1.5±0.2	26.8±3.1	6.7k0.5	22.8±1.6
OL fed	8.6±0.8	tr	1.4±0.1	28.8±2.3	tr	26.9±1.2
fasted	10.0±1.3	tr	1.5±0.2	25.6±1.9	t r	23.1±1.4

PHC and OL: partially hydrogenated corn oil and olive oil. Means±SE of 6 to 7 rats per group. [a]Values for fed rats are significantly different from those for the corre-

Intestinal mucosal lipids

TG fatty acid composition of the intestinal mucosa was the same between the two fat groups, excepting the type of the octadecenoates (Table 9). A considerable portion of the *cis*-octadecenoate was replaced by the *trans*-counterpart, thus the percentage of the total octadecenoate being comparable. Fasting caused no detectable changes in the fatty acid profiles.

There were considerable differences in the fatty acid compositions of mucosal PC between the two dietary fat groups; rats fed the hydrogenated fat in relation to those fed olive oil contained less stearic and arachidonic acids. The intestinal phospholipids contained a relatively high level of the eicosamono-enoate (identification tentative) in rats fed the trans-fat diet. Fasting caused an increase in a proportion of stearic and arachidonic acids and a decrease in linoleic acid, the *trans*-fatty acid perecentage was unaltered by fasting.

tissue lipid.

t-18 : 1	c–18 : 1	$\frac{ct}{tc}$-18 : 2	cc-18 : 2	20 : 4
17.0±0.6	46.2±1.2[A]	1.9±0.1	9.7±0.3	2.5±0.1
15.8±0.9	48.9±1.3[A]	2.5±0.4	9.5±0.7	2.6±0.1
tr	66.5±1.7	tr	8.3k0.7	0.4±0.0
tr	65.9±1.6	tr	8.1f0.5	0.4±0.0

rresponding OL groups at $p<0.05$, tr: trace.

mucosal lipids.

$\frac{ct}{tc}$-18 : 2	cc-18 : 2	20 : 1	20 : 4	20 : 5	22 : 6
1.1±0.1	4.6±0.2	1.1±0.2[A]	0.3±0.1	tr	tr
1.2±0.1	4.5f0.3	1.2±0.1[A]	0.4±0.2[A]	tr	tr
tr	5.0±0.2	0.6±0.1	0.1±0.0	tr	tr
tr	5.2±0.2	0.6±0.1	0.1±0.0	tr	tr
0.5±0.1	14.3±1.7[a]	8.6±2.1[A]	3.7±0.3[A]	0.4±0.1	0.4±0.1
0.3±0.1	8.5±0.8[A]	4.5±0.8	5.8±1.6[A]	1.0±0.2[A]	1.7±0.3
tr	16.7±0.4[a]	3.2-t0.3	6.9±0.4[a]	0.4±0.1	0.8±0.1
tr	13.2±0.5	3.1±0.3	11.4±0.7	0.4±0.1	1.0±0.1
0.5±0.1	10.5±0.7	3.4±0.5	10.9±0.6	1.1±0.1	2.1±0.2
0.3±0.1	7.1rt0.8	4.2±0.7[A]	7.8±1.0[A]	0.6±0.2	1.9±0.3[A]
tr	8.2±0.7	4.3±0.9	13.2±1.2	1.0±0.1	2.4±0.3
tr	9.5±0.6	7.7±1.6	13.2C1.5	1.1±0.1	3.0±0.4

sponding fasted animals at $p<0.05$. *Values for PHC groups are significantly different from those for the corresponding OL groups at $p<0.05$, tr: trace.

In PE, the fatty acid profile was comparable between the two fat groups. After fasting, the characteristic responses could be found. Thus, the proportion of arachidonic acid was unchanged or even tended to decrease and that of palmitic acid tended to elevate. The *trans*-octadecenoate was reduced.

DISCUSSION

Overnight fasting caused characteristic changes in concentrations of tissue lipids. The line of changes was generally consistent with that reported previously (Sugano *et al., 1975* a, 1975 b). Although TG was commonly decreased in each tissue tested, the changes in phospholipid were quite diverse; it decreased in serum and intestinal mucosa in both dietary fat groups, while increased in the liver of rats fed the *cis*-fat but not *trans*-fat diet. The response of cholesterol to fasting also varied depending on the type of fat ingested;

cholesterol tended to decrease in the mucosa and serum, while it increased in the liver, in particular in the *cis*-fat group. Also, decrease in cholesterol in the adipose tissue was observed only in rats fed a *cis*-fat diet. These results suggest that the effect of overnight fasting on the tissue lipid profile was more marked with the *cis*-fat rather than the *trans*-fat.

Although we have previously shown that lipid changes after overnight fasting are dependent on the essential fatty acid status of the rats (Sugano *et al.*, 1975 a, 1975 b), this factor can be ignored in the present study since both diets supplied the same and adequate level of linoleic acid. Thus, changes observed may be ascribed to the difference in the geometry of the fatty acids.

The extent of incorporation of the *trans*-octadecenoate differed considerably depending on the tissues and lipid fractions tested as demonstrated previously (Kummerow, 1979; Sugano *et al.*, 1983 a). TG from the intestinal mucosa and adipose tissues contained the highest level of the *trans*-octadecenoate (Sugano *et al.*, 1984) followed by serum TG, while in the liver the percentage was evidently higher in the phospholipids than in TG. In serum, apparently the same extent of incorporation was observed between TG and PC. Serum cholesterol ester contained only marginal amounts of the trans.acids implying that this isomer is exclusively incorporated into the l-position rather than the Z-position of PC since the lecithin : cholestrol acyltransferase reaction seems to be the major source for serum cholesterol esters (Glomset, 1968). This has been actually substantiated (Sugano *et al.*, 1984). In addition, since the percentage of the metabolites of the *trans*-octadecenoate such as the *trans*-hexadecenoate or *trans*, *cis*- or *cis*, *trans*-octadecadienoate was very low, it seems that the oxidation of the *trans*-octadecenoate proceeds rapidly (Ide and Sugano, 1984) or the isomer is not so significantly desaturated as in the *cis*-counterpart (Kinsella *et al.*, 1981).

From the relatively same level of incorporation of the *trans*-acid into phospholipids from different tissues irrespective of the tissue specificity of TG incorporation, there appears to be some kind of the mechanism to avoid excessive deposition of the *trans*-acid into the specific position of glycerophosphatides (Kummerow, 1979 ; Lands, 1979). In addition, the deitary *trans*-monoene elevated the ratio of the diene to tetraene in phospholipids. This was presumably the reflection of interference of the *trans*-acid with desaturation of the cis-diene (Kinsella *et al.*, 1981; Beare-Rogers, 1983).

The response of the *trans*-octadecenoate to overnight fasting differed characteristically and was tissue-dependent. In TG, there was a trend toward decreasing the *trans*-isomer in each tissue tested except the liver after fasting, although the magnitude was diverse. This tendency agreed with that of the cis-octadecenoate (Sugano *et al.*, 1975 a, 1975 b). In phospholipids, the fasting effect on the *trans*-acid differed markedly depending not only on the phospholipid species but also on the tissues analyzed. In general, however, it tended to increase in liver phospholipids while decrease in serum and intestinal phospholipids. These responses were in contrast with those observed with the &counterpart.

The significance of fasting-induced changes in the trans-octadecenoate is obscure at the present time. In many cases the changes were neither paralleled to those of the cis-octadecenoate nor saturated fatty acids of comparable acyl-chains such as octadecanoate or hexadecanoate. From the similarity of the fatty acid composition of adipose TG before and after fasting, there seems no specific restriction for the use of the *trans*-fatty acid as an energy source. Although the reduction of the percentage of the trans-octadecenoate in serum TG as in the case of the cis-octadecenoate implies metabolic similarity of these fatty acids, the results obtained currently at least suggest the specific feature of the metabolism of the *trans*-fatty acids in *vivo.*

ACKNOWLEDGEMENT

REFERENCES

Alfin-Slater, R. B. and L. Aftergood 1979 Nutritional role of hydrogenated fats (in rats). *In* "Geometrical and Positional Fatty Acid Isomers", ed. by E. A. Emken and H. J. Dutton, American Oil Chemists' Society, Champaign, Illinois, pp. 53-74

Beare-Rogers, J. C. 1983 *Trans-* and positional isomers of common fatty acids. *Adu. Nutr. Res., 5: 171-200*

Cho, Y.-J., G. Ishibashi, T. Koga, K. Imaizumi and M. Sugano 1984 *Trans* fatty acid contents of human blood serum and adipose tissue and a regular meal of university refectory. *J. Jpn. Soc. Nutr. Food Sci.,* 37: *31-35*

Dietschy, J. M. and M. D. Siperstein 1965 Cholesterol synthesis by the gastrointestinal tracts: localization and mechanism of control. *J. Clin. Invest.,* 44: 1311-1327

Glomset, J. A. 1968 The plasma lecithin: cholesterol acyltransferase reaction. *J.* Lipid Res., 9: 155-167

Ide, T. and M. Sugano 1984 Oxidation and esteriiication of cis- and *trans-* isomers of octadecenoic and octadecadienoic acids in isolated rat liver. *Biochim. Biophys. Acta, 794: 281-291.*

Imaizumi, K., M. Sugano and M. Wada 1972 Nutritional regulation of lipid metabolism in rats. Part I. The effect of overnight fasting on the concentration and composition of liver and its microsomal lipids. *Agric. Biol. Chem., 36: 234-241.*

Kinsella, J. E., G. Bruckner, J. May and S. Shimp 1981 Metabolism of *trans* fatty acids with emphasis on the effects of *trans, trans*-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview. *Am J. Clin. Nutr., 34: 2307-2323.*

Kummerow, F. A. 1979 Effect of isomeric fats on animal tissue, lipid classes, and atherosclerosis. *In* "Geometrical and Positional Fatty Acid Isomers", ed. by E. A. Emken and H. J. Dutton, American Oil Chemists' Society, Champaign, Illinois, pp. 151-180

Lands, W. E. M. 1979 Selective recognition of geometric and positional isomers of fatty acids. *In* "Geometrical and Positional Fatty Acid Isomers", ed. by E. A. Emken and H. J. Dutton, American Oil Chemists' Society, Champaign, Illinois, pp. 181-212

Lowry, O. H., N. J. Rosenbrough, A. L. Farr and R. J. Randall 1951 Protein measurement

with Folin phenol reagent *J. Biol. Chem.,* 193: 265-275

Mangold, H. K,. 1964 Thin-layer chromatography of lipids. *J.* Am. *Oil Chem. Soc.,* 41: 762-773.

Nagata, Y., K. Imaizumi and M. Sugano 1980 Effects of soya-bean protein and casein on serum cholesterol levels in rats. *Brit. J. Nutr.,* 44: 113-121

Ohlrogge, J. B., E. A. Emken and R. M. Gulley 1981 Human tissue lipids: occurrence of fatty acid isomers from dietary hydrogenated oils. *J. Lipid Res., 22: 955-960.*

Rogers, C. G. 1971 Fatty acid composition of liver mitochondria and microsomes in fed and fasted rats. *Nutr. Rep. Int.,* 4: 351-362.

Sugano, M., T. Ide, M. Kohno, M. Watanabe and Y.-J. Cho 1983a Billiary and fecal steroid excretion in rats fed partially hydrogenated soybean oil. *Lipids,* **18:** *186-192.*

Sugano, M., K. Imaizumi and F. Kamo 1975 a Effect of overnight fasting on plasma lipids of rats fed different levels of essential fatty acids. *Nutr. Rep. Int.,* **11:** 177-183

Sugano, M., K. Imaizumi and F. Kamo 1975 b Effect of overnight fasting on liver lipids of rats fed different levels of essential fatty acids. *Nutr. Metab.,* 19: 65-72.

Sugano, M., F. Kamo, I. Ikeda and H. Morioka 1976 Lipid lowering activity of phytostanols in rats. *Atherosclerosis, 24: 301-309.*

Sugano, M., K. Ryu and T. Ide 1984 Cholesterol dynamics in rats fed *cis-* and *trans-*octadecenoate in the form of triglyceride. *J. Lipid Res., 25: 474-485.*

Sugano, M., M. Watanabe, M. Kohno, Y.-J. Cho and T. Ide 1983 b Effect of dietary *trans-*fat on biliary and fecal steroid excretion and serum lipoproteins in rats. *Lipids,* **18** : *375-381.*

Swell, L. and M. D. Law 1967 Heterogeneity in the composition of cholesterol esters synthesized by liver cell fractions of fasted and fed rats. *Biochem. Biophys. Res. Commun., 26* : *206-210.*

Yoneda, K., Y. Taga and T. Mori 1981 *Application and Exercise of Statistics,* Dobunshoin, Tokyo, pp. 95-99 (In Japanese).