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Effects of Dietary Fat Levels on Lipid Parameters and Eicosanoids Production of Rats under Fixed N-6/N-3 and P/S Fatty Acid Ratios

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The effects of dietary fat levels on lipid metabolism under fixed P/S (1.3) and n-6/n-3 (5.1) fatty acid ratios were examined in rats using palm oil, soybean oil and perilla oil. These ratios correspond to the recommended composition of dietary fat for humans. The range of dietary fat levels was 5-20% by weight (11.8-39.3% of total energy). The levels of dietary fat did not influence the concentrations of serum and liver cholesterol, whereas the level of triglycerides was gradually elevated with increasing levels of dietary fat, especially in the liver. The fatty acid composition of tissue phosphatidylcholine seemed to vary with the different levels of fat. The ratio of linoleic acid to arachidonic acid was increased more significantly in the heart than in the liver. In adipose tissue total lipids, the percentages of saturated and monounsaturated fatty acids decreased, whereas the percentage of polyunsaturated fatty acid increased, with increasing dietary fat levels. In addition, though the level of aortic prostacyclin was not uniformly affected by increasing dietary fat levels, thromboxane A2 production by platelets tended to increase with higher levels of dietary fat, suggesting an increased risk of thrombosis in this situation. Thus, even though dietary fat may have desirable compositions of fatty acids, these excessive consumption can produce unfavorable metabolic responses.

key words : dietary fat level, n-6/n-3 ratio, P/S ratio, thromboxane A_2, prostacyclin

INTRODUCTION

It is generally recognized that dietary fat level is positively correlated with obesity, atherosclerosis, coronary heart diseases (CHD)[1-3] and some types of cancers[4,5]. A number of studies on the effects of different levels of dietary fat on lipid metabolism have been reported in humans[6-10]. However, these studies focused mainly on plasma lipid and lipoprotein parameters, probably due to limitations in clinical studies. Differences in dietary energy source should influence not only lipid levels but also the fatty acid composition of tissue lipids and then eicosanoid production. The fatty acid composition of dietary fat is also an important factor in lipid-related diseases; for example, Oliver[1] suggested that the higher the polyunsaturated/saturated fatty acid (P/S) ratio, the less the incidence of coronary heart disease. In addition, the effects of dietary fat levels may be modified by different ratios of n-6/n-3 polyunsaturated fatty acid (PUFA) families, because even when the P/S ratio is at a desirable level, lipid parameters may readily be influenced by any differences in the n-6/n-3 ratio[11]. Therefore, the effects of different dietary fat levels can best be determined if the status of n-6/n-3 and P/S ratios remain fixed. Thus, in the present study, the effects of varying dietary lipid levels on lipid metabolism, including the fatty acid composition and production of prostacyclin (PGI_2) and thromboxan A_2 (TXA_2) that are closely related to the incidence of CHD, were examined in rats under fixed n-6/n-3 (5.1) and P/S (1.3) ratios that are recommended for the prevention of CHD in humans.[11]

ANIMALS AND METHODS

Animals and diets

Four-week old male Sprague-Dawley rats were divided into 4 groups (6 rats in each group) according to the four levels of total dietary fat (5-20% by weight, or 11.8-39.3% of total energy). The dietary fats used were palm oil (PLO), soybean oil (SBO) and perilla oil (PRO), and these were mixed as necessary to achieve the required constant n-6/n-3 and P/S ratios of total dietary fat for each of the four groups. PLO and SBO were obtained from Fuji Oil, Osaka, and Kishida Co., Osaka, Japan, respectively. PRO was a gift from Lion Co., Tokyo. The diets were formulated based on the AIN-76 recommendation,[12] and the composition is described in Table 1. The fatty acid composition of the combined

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dietary fats is shown in Table 2. Animals were housed individually in an air-conditioned room (20-23℃, lights on between 08:00 and 20:00 hours) and fed experimental diets ad libitum for 3 weeks; blood was withdrawn from the abdominal aorta of the rats under diethyl ether anesthesia. Aliquots of blood were clotted by incubating them at 37℃ for 30 minutes, for the measurement of the production of TXA_2 by the platelet-rich serum.[13),14)] The remaining portions of blood were clotted in ice and the serum was analyzed for lipids. The thoracic aorta was excised for the measurement of PGI_2 production and fatty acid composition. The liver, heart and epididymal adipose tissue were excised and frozen at -20℃. This study was approved by the Kyushu University Animal Care Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals at Kyushu University.

Table 1. Composition of experimental diets

Ingredients	Weight %
Casein	20
Fat	5 - 20
(energy %)	(11.8 - 39.3)
Vitamin Mix (AIN-TM[76])	1.0
Mineral Mix (AIN-TM[76])	3.5
Choline bitartrate	0.2
DL-methionine	0.3
Cholesterol	0.5
Na-cholate	0.125
Cellulose power	5
Corn starch	15
Sucrose	to 100

Table 2. Fatty acid composition of dietary fat

Dietary fats	Fatty acid (weight %)							
	14:0	16:0	18:0	18:1	18:2 (n-6)	18:3 (n-3)	P/S ratio	n-6/n-3 ratio
PLO:SBO:PRO								
50 : 45 : 5	0.57	28.0	3.57	31.5	29.9	5.82	1.27	5.14

PLO; palm, SBO; soybean oil, PRO; perilla oil.

Lipid analyses

Serum and liver lipids were extracted with a chloroform/methanol mixture (2:1, v/v). Cholesterol, triglycerides and phospholipids were measured from tissue extractions as described previously.[11)] Tissue phospholipids and triglycerides were separated by Thin Layer Chromatography and their fatty acid compositions were measured by GLC on a DEGS column.[15)] Cholesterol in the aorta and adipose tissue was extracted and analyzed by using GLC on a OV-17 column.[16)]

Measurement of eicosanoids

Preincubated serum was diluted 20 times with a 50mM phosphate buffer (pH 7.3), containing 0.1% gelatin and 0.01% thimerosal, and was then used for measurement of TXB_2 by radioimmunoassay (NEK-007, New England Nuclear, Boston, MA, USA).[17)] The 6-keto-$PGF_{1\alpha}$ produced during incubation of the thoracic aorta at 25℃ for 30 min was extracted and analyzed by radioimmunoassay (NEK-008, New England Nuclear, Boston, MA, USA).[17)]

Statistical analysis

Data were analyzed by a one-way analysis of variance followed by inspection of all differences between pairs of means by Duncan's multiple-range test.

RESULTS

Growth performance and liver weight

As shown in Table 3, although food intake was comparable among the groups, weight gain was significantly lower in rats fed the diet with 11.8% of total energy as fat compared to those fed diets containing higher levels of fat. Relative liver weight also tended to be higher with the increasing fat content of diets, but the differences were not statistically significant.

Table 3. Growth parameters and liver weight

Groups (energy % as fat)	Body weight		Food intake	Liver weight
	Initial	Gain		
	(g)	(g/3weeks)	(g/day)	(g/100g B.W)
A(11.8)	123±2	163± 7[a]	19.7±0.7	6.55±0.30
B(22.1)	123±3	192±10[b]	21.1±0.5	6.94±0.17
C(31.2)	123±4	203± 7[b]	20.8±0.6	7.17±0.13
D(39.3)	124±4	195± 4[b]	19.0±0.3	7.09±0.18

Mean±SE of 6 rats per group. Values not sharing a common superscript letter are significantly different ($p<0.05$).

Lipid Concentration

As shown in Fig 1, the concentration of serum triglyceride tended to increase with increasing dietary fat levels, whereas the level of cholesterol was somewhat lower at fat levels above 21.2% of energy. The concentration of liver triglycerides increased with increasing fat levels, and the difference between the lowest and highest fat diets was significant (Fig 2). The hepatic cholesterol level was not influenced by dietary fat level. Levels of serum and liver phospholipids were also independent of the dietary fat level. The concentration of cholesterol in the adipose tissue tended to decrease with increasing dietary fat levels, and was significantly lower in rats fed a 39.3% fat energy diet than in those fed a 11.8% fat energy diet (Table 4). The concentration of aortic chole-

sterol was not influenced by differences in the fat content of the diet.

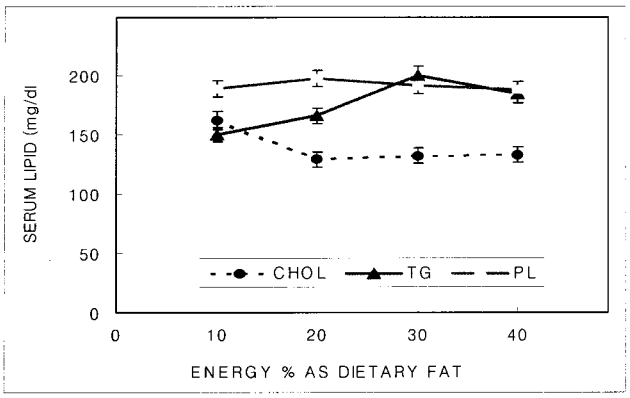

Fig 1. Effects of isocaloric diets with different fat levels on serum lipids in rats. Mean ± SE of 6 rats per group. CHOL; cholesterol, TG; triglyceride, PL; phospholipid.

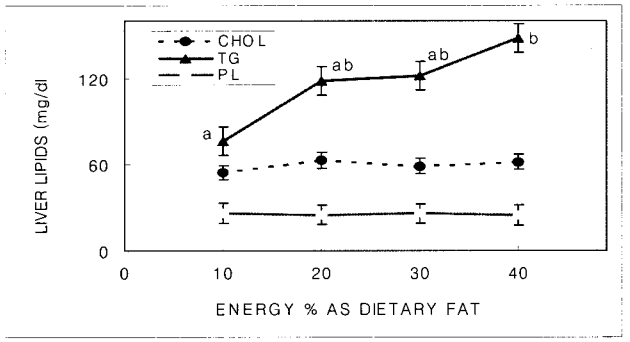

Fig 2. Effects of isocaloric diets with different fat levels on liver lipids in rats. Mean ± SE of 6 rats per group. CHOL; cholesterol, TG; triglyceride, PL; phospholipid. Values not sharing a common superscript letter are significantly different ($p<0.05$).

Table 4. Concentrations of cholesterol in adipose tissue and aorta

Tissues	Groups (energy % as fat)			
	A(11.8)	B(22.1)	C(31.2)	D(39.3)
	(mg/g tissue)			
Adipose tissue	0.77 ± 0.05^{a}	0.75 ± 0.02^{ab}	0.68 ± 0.03^{ab}	0.61 ± 0.05
Aorta	1.61 ± 0.02	1.62 ± 0.05	1.62 ± 0.02	1.57 ± 0.05

Mean ± SE of 6 rats per group. Values not sharing a common superscript letter are significantly different ($p<0.05$).

Fatty acid composition of tissue lipids

The fatty acid compositions of liver and heart phosphatidylcholine (PC) showed similar responses to diets each other (Tables 5 and 6). However, the percentage of linoleic acid (18:2 n-6, LA) remained unchanged in the liver, whereas in the heart it tended to decrease with an increasing dietary fat level. The percentage of arachidonate (20:4 n-6, AA) tended to increase in both the liver and the heart in response to an increase in the dietary fat level. Thus, the ratio of LA to AA was increased more clearly in the heart PC than in the liver PC. In the aorta PC, the proportion of LA increased, whereas the proportion of AA was not influenced by increasing dietary fat levels (Table 7). Consequently, LA desaturation decreased significantly with increasing dietary fat levels. In adipose tissue total lipids, the percentage of saturated fatty acids, mainly palmitate(16:0) and stearate(18:0), and monounsaturated fatty acid, mainly oleic(18:1) and palmitoleic acids(16:1), decreased significantly, whereas that of polyunsaturated fatty acids increased consistently with increasing dietary fat levels (Table 8).

Table 5. Fatty acid composition of liver phosphatidylcholine

Fatty acids	Groups (energy % as fat)			
	A (11.8)	B (22.1)	C (31.2)	D (39.3)
	(weight %)			
16:0	20.5 ± 0.2^{a}	21.8 ± 0.7^{ab}	22.8 ± 0.2^{b}	22.2 ± 0.3^{b}
16:1	3.43 ± 0.11^{a}	3.08 ± 0.22^{ab}	2.76 ± 0.17^{ab}	2.47 ± 0.28^{b}
18:0	18.1 ± 0.9^{a}	16.7 ± 0.8^{ab}	15.2 ± 0.3^{b}	15.4 ± 0.7^{b}
18:1	14.1 ± 0.6^{a}	11.6 ± 0.4^{a}	11.6 ± 0.4^{b}	10.1 ± 0.3^{b}
18:2(n-6)	12.1 ± 0.9	13.1 ± 0.9	13.5 ± 0.9	12.6 ± 0.5
18:3(n-3)	0.25 ± 0.04^{a}	0.64 ± 0.14^{ab}	0.86 ± 0.09^{b}	0.71 ± 0.10^{b}
20:3(n-6)	2.70 ± 0.09^{a}	2.22 ± 0.26^{ab}	1.83 ± 0.09^{bc}	1.57 ± 0.15^{c}
20:4(n-6)	18.9 ± 0.4^{a}	20.3 ± 1.4^{ab}	20.6 ± 1.1^{ab}	24.0 ± 0.7^{b}
20:5(n-3)	0.83 ± 0.09^{a}	0.57 ± 0.06^{b}	0.38 ± 0.3^{b}	0.35 ± 0.04^{ab}
22:5(n-3)	0.68 ± 0.06^{a}	1.33 ± 0.08^{b}	1.66 ± 0.12^{b}	1.82 ± 0.20^{b}
22:6(n-3)	5.33 ± 0.29	5.43 ± 0.26	5.47 ± 0.39	5.28 ± 0.10
20:3+20:4/18:2	1.87 ± 0.22	1.63 ± 0.15	1.98 ± 0.23	2.06 ± 0.13

Mean ± SE of 6 rats per group. Values not sharing a common superscript letter are significantly different ($p<0.05$).

Table 6. Fatty acid compositions of heart phosphatidylcholine

Fatty acids	Groups (energy % as fat)			
	A (11.8)	B (22.1)	C (31.2)	D (39.3)
	(weight %)			
16:0	17.4 ± 0.3	17.8 ± 0.3	18.1 ± 0.3	17.6 ± 0.5
16:1	1.04 ± 0.06	0.83 ± 0.11	0.81 ± 0.21	0.43 ± 0.26
18:0	22.4 ± 0.2^{a}	23.5 ± 0.4^{ab}	23.5 ± 0.5^{ab}	24.8 ± 0.3^{b}
18:1	12.7 ± 0.2^{a}	10.7 ± 0.4^{b}	9.35 ± 0.22^{c}	8.85 ± 0.28^{c}
18:2(n-6)	13.7 ± 0.9^{a}	11.5 ± 1.3^{ab}	8.92 ± 0.76^{b}	9.09 ± 0.52^{b}
18:3(n-3)	0.27 ± 0.04	0.09 ± 0.01	0.15 ± 0.06	0.24 ± 0.07
20:3(n-6)	0.67 ± 0.06^{a}	0.51 ± 0.07^{ab}	0.36 ± 0.04^{b}	0.33 ± 0.02^{b}
20:4(n-6)	24.4 ± 0.8^{a}	26.0 ± 1.3^{ab}	$26.8\pm0.9a^{b}$	28.1 ± 0.7^{b}
22:5(n-3)	1.39 ± 0.07^{a}	2.25 ± 0.14^{b}	2.53 ± 0.13^{b}	3.16 ± 0.24^{c}
22:6(n-3)	3.95 ± 0.30	4.42 ± 0.29	4.85 ± 0.18	4.46 ± 0.20
20:3+20:4/18:2	1.92 ± 0.16^{a}	2.49 ± 0.33^{ab}	3.18 ± 0.33^{b}	3.22 ± 0.33^{b}

Mean ± SE of 6 rats per group. Values not sharing a common superscript letter are significant different ($p<0.05$).

Table 7. Fatty acid compositions of aorta phosphatidylcholine

Fatty acids	Groups (energy % as fat)			
	A (11.8)	B (22.1)	C (31.2)	D (39.3)
	(weight %)			
16:0	35.6±0.9	34.6±0.98	36.2±0.58	35.5±0.58
16:1	3.05±0.06^{a}	2.64±0.13^{b}	2.49±0.11^{bc}	2.18±0.09^{c}
18:0	12.1±0.2	12.6±0.5	12.6±0.2	13.0±0.3
18:1	16.3±0.4^{a}	15.5±0.3^{ab}	14.4±0.6^{bc}	13.5±0.1^{c}
18:2(n-6)	4.89±0.20^{a}	5.89±0.20^{ab}	6.99±0.57^{bc}	7.43±0.43^{c}
18:3(n-3)	0.63±0.06	0.52±0.04	0.53±0.06	0.48±0.02
20:3(n-6)	1.24±0.06	1.30±0.06	1.22±0.06	1.19±0.03
20:4(n-6)	18.7±0.4	20.1±0.6	18.5±0.6	20.0±0.4
20:5(n-3)	0.32±0.02	0.37±0.01	0.31±0.02	0.34±0.02
22:6(n-3)	1.61±0.08	1.63±0.07	1.42±0.10	1.49±0.06
20:3+20:4/18:2	4.12±0.17^{a}	3.66±0.17^{ab}	2.94±0.30^{b}	2.90±0.16^{b}

Mean±SE of 6 rats per group. Values not sharing a common superscript letter are significantly different ($p<0.05$).

Table 8. Fatty acid compositions of adipose tissue total lipids

Fatty acids	Groups (energy % as fat)			
	A (11.8)	B (22.1)	C (31.2)	D (39.3)
	(weight %)			
14:0	1.61±0.07^{a}	1.29±0.03^{b}	1.12±0.05^{b}	1.04±0.05^{c}
16:0	27.0±0.6^{a}	25.3±0.5a^{b}	24.6±0.4^{b}	23.9±0.6^{b}
16:1	10.4±0.3^{a}	7.05±0.2^{b}	4.91±0.48^{c}	4.27±0.4^{c}
18:0	3.13±0.11^{a}	2.86±0.07^{ab}	2.94±0.10^{ab}	2.80±0.06^{b}
18:1	38.1±0.3^{a}	36.7±0.2^{b}	35.6±0.2^{bc}	35.0±0.3^{c}
18:2(n-6)	16.3±0.8^{a}	22.0±0.7^{b}	25.3±0.9^{bc}	27.0±0.9^{c}
18:3(n-3)	2.60±0.14^{a}	3.76±0.16^{b}	4.41±0.15	4.68±0.12^{c}
20:4(n-6)	0.22±0.04^{a}	0.30±0.02^{ab}	0.37±0.06	0.40±0.05^{b}

Mean±SE of 6 rats per group. Values not sharing a common superscript letter are significantly different ($p<0.05$).

Eicosanoid production

Although the responses of aortic PGI_2 and platelet TXA_2 production were variable, TXA_2 production tended to increase with increasing dietary fat levels (Fig 3).

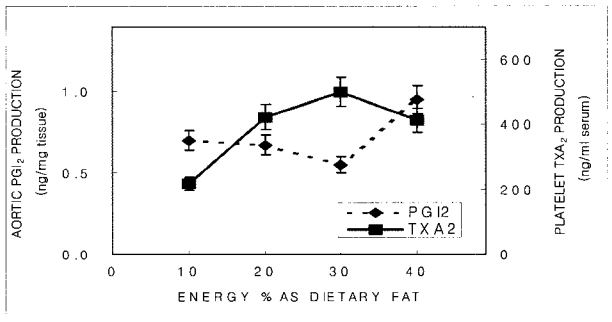

Fig 3. Effects of isocaloric diet with different fat levels on eicosanoid productions in rats. Means±SE of 6 rats per group. PGI_2; prostacyclin, measured as a 6-keto-$PGF_{1\alpha}$, TXA_2; thromboxane A_2, measured as a thromboxan B_2.

DISCUSSION

In this study, the concentrations of serum and liver triglycerides tended to increase in response to increased dietary fat levels, as demonstrated in the studies of Richard and Jacotot,[9] and Benyon et al.[18] Grundy[19] suggested that prolonged use of a low-fat diet lowers the plasma triglyceride level.

The concentration of cholesterol did not change, both in the liver and serum, with changes in dietary fat levels, in agreement with several studies.[9,20] In contrast, other reports showed an increase of total and LDL-cholesterol in the plasma with an increasing dietary fat level.[8,21] Watanabe et al[22] indicated that serum and liver cholesterol levels of rats remained unchanged at dietary fat levels of between 5 and 20%, when cholesterol-enriched diets were given under similar conditions to the present study. In cholesterol-free diets, serum cholesterol increased significantly in the 20% fat diet compared to the 5% fat diet.[22] Thus, exogenous cholesterol severely influences cholesterol metabolism, probably through the feedback inhibition of cholesterol synthesis.[23] Cholesterol in adipose tissue has the kinetic characteristics of a slowly exchangeable pool, and net cholesterol mobilization from this tissue occurs after acute starvation.[24] The cholesterol level appears to be positively correlated to the fat cell size, which was shown to increase with sucrose feeding.[25] Thus, the significant decrease in adipose tissue cholesterol in rats fed a high-fat (39.3% of total energy) and low sucrose diet, compared with those fed a low-fat (11.8% of energy) and high sucrose diet, can at least be attributed to the relative changes in sizes of fat cells. The fatty acid compositions of tissue PC and adipose tissue total lipids changed with differing dietary fat levels, particularly in the latter. The percentage of saturated and monounsaturated fatty acids decreased with increasing dietary fat levels, and vice versa for PUFA. Dietary fat depresses lipogenesis as a result of the increase in availability of long-chain fatty acids or acyl-CoA.[23] Under these circumstances, there is a possibility that the percentage of PUFAs increases. Accordingly, the linoleic acid desaturation index in tissue PC tended to increase with increasing dietary fat, except for aortic PC which showed a decreasing trend.

Although the production of aortic PGI_2 did not respond uniformly to increasing dietary fat levels as in a previous study,[26] platelet TXA_2 production tended to increase with increasing dietary fat levels. Lahoz et al[27] suggested that dietary fat level is also significantly related to the extent of platelet aggregation. Broughton and Wade[28] reported that elevated total fat intake reduced PGI_2 production. Thus, a high dietary level of fat may enhance the risk of thrombosis due to unbalanced production of TXA_2 and PGI_2.

In conclusion, in this study increases in dietary fat levels did not appear to influence serum cholesterol levels, but resulted in increased triglyceride levels. The metabolism of fatty acids in tissue lipids seems to be influenced by dietary fat; particularly, the increment of TXA_2 production in platelets after consuming a high level of dietary fat may enhance the risk of thrombosis. The present study thus suggests that even fats with the recommended P/S and n-3/n-6 ratios may produce unfavorable effects on lipid metabolism, when consumed in excess.

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