A Butter Diet Induces Higher Levels of n-3 PUFA and of n-3/n-6 PUFA Ratio in Rat Serum and Hearts than a Safflower Oil Diet

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Abstract

The effects of a 47-week diet of butter or safflower oil as fat in combination with casein or soy protein as protein were observed for the serum concentrations of lipids and fatty acid compositions in rat serum and heart. Serum total cholesterol (Chol) did not differ among the four experimental diet groups. In the butter groups, significantly higher low-density lipoprotein (LDL)-Chol and lower high-density lipoprotein (HDL)-Chol were observed than in the safflower oil groups ($p < 0.005$, respectively). Higher levels of α -tocopherol were found in the butter groups than in the safflower oil groups ($p < 0.05$) and in the casein groups than in the soy protein groups ($p < 0.01$). In comparison with the safflower oil groups, the butter groups showed higher n-3 polyunsaturated fatty acids (PUFA) contents and lower n-6 PUFA contents in serum and the hearts ($p < 0.005$). The ratios of n-3/n-6 PUFA in the butter groups in serum, 0.26 and 0.18, and in the hearts, 0.37 and 0.36, (butter-casein diet and butter-soy protein diet, respectively) were higher than those of the safflower oil groups of under 0.01 in serum and 0.02 and 0.03 in the hearts (safflower oil-casein diet and safflower oil-soy protein diet, respectively) ($p < 0.005$). In the soy protein groups, higher n-3 PUFA contents in the hearts were found than those of the casein groups ($p < 0.05$). This study suggested that the butter diet induces higher levels of n-3 PUFA and a higher n-3/n-6 PUFA ratio than the safflower oil diet in rat serum and hearts over a long feeding period.

Key words: dietary fat, n-3 PUFA, n-3/n-6 PUFA ratio, tocopherol, heart of rat.

Introduction

Serum cholesterol (Chol), which is considered to be a risk factor for coronary heart disease¹⁾, is influenced by dietary fat and protein^{2), 3)}. Tallow raises the serum Chol level in humans while vegetable oils lower it^{4), 5)}. The hypocholesterolemic effect of certain vegetable oils is due to their high polyunsaturated fatty acids (PUFA)^{6), 7)} and to their high dietary ratio of PUFA to saturated fatty acids (SFA) which lower the serum Chol^{3), 8)}. However, recently, it has been reported that n-6 PUFA, such as linoleic acid (LA, C18:2n-6) and arachidonic acid (AA, C20:4n-

6), enhance coronary vascular disease (CVD)^{9), 10)}. Cardiac disease mortality in groups treated according to nutritional recommendations to increase the dietary PUFA/SFA ratio was 2.4-fold higher than that in the control group¹⁰⁾.

The risk of CVD is known to be reduced by fish consumption^{11), 12)}, due to the high contents of n-3 PUFA such as eicosapentaenoic (EPA, C20:5n-3) and docosahexaenoic (DHA, C22:6n-3) acids. Those n-3 PUFA promote hypolipidemic effects^{13), 14), 15)}, anti-thrombogenesis¹⁶⁾ and anti-atherosclerosis¹⁷⁾ to prevent CVD^{11), 12), 18)}. The two families of n-3 PUFA and n-6 PUFA are not metabolically interconvertible and are direct precursors of a wide variety of bioactive compounds such as prostaglandin related thrombosis and atherosclerosis¹⁹⁾. Therefore, a high n-3 PUFA and a high ratio of n-3/n-6 PUFA are expected to play a role in the prevention of CVD.

Proteins derived from animal sources show a greater

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tendency to produce hypercholesterolemia than those from plant sources^{21, 3)} 20). Soy protein has been shown to lead to a reduction in serum Chol compared with casein^{20), 21), 22)}. However, most studies of normocholesterolemic subjects have shown little difference in the effects of dietary soy protein on serum Chol concentration. The reason that the effects of dietary soy protein on serum Chol concentration have not been consistent, may be due to the levels of Chol in the diet or the feeding periods. Most dietary studies are conducted over a few months, and the effects of long periods of consumption which can lead to hyperlipidemia have been less frequently studied.

Mortality from CVD and plasma levels of tocopherol (Toc) shows an inverse correlation²³⁾. From the hypothesis that low-density lipoprotein (LDL) oxidation is involved in the development of atherosclerosis²⁴⁾, Toc as an antioxidant may inhibit the oxidation of LDL and thus lower the risk of CVD.

In this study we examined the effects of butter as tallow and safflower oil as vegetable oil and the effects of casein as animal protein and soy protein as plant protein on the CVD risk factors related to lipids and Toc in serum and the levels of n-3 PUFA and n-6 PUFA in serum and heart after a long period of feeding.

Methods

Experimental animals and diets

Male Fisher rats aged 6 weeks (Clea Japan Inc., Tokyo, Japan) were assigned to four dietary treatment groups, butter-casein diet (BuCa), butter-soy protein diet (BuSo), safflower oil-casein diet (SaCa) and safflower oil-soy protein diet (SaSo), with 5 rats per group and fed an experimental diet ad libitum for 47 weeks. After the rats had fasted for 17-20 hours, they were anesthetized with diethyl ether, and blood was collected through the abdominal aorta with a syringe and then centrifuged to separate the serum. The basal diet consisted of 10% fats, butter (Snow Brand Milk Products, Tokyo, Japan) or safflower oil (Yamakei Ind., Ltd., Osaka, Japan), 20% protein, casein-vitamin free (Wako Pure Chemical Ind. Ltd., Osaka, Japan) or soy protein (Clea Japan Inc., Tokyo, Japan), 4% mineral mixture (AIN-93G formulation), 1% vitamin mixture (AIN-93 formulation), 5% cellulose powder, 0.25% L-cystine, 0.25% choline hydrogen tartrate and sucrose to 100%. The fatty acid composition of butter and safflower oil are shown in Table 1.

Analysis of lipids and fatty acids

Serum total cholesterol (Chol) and high-density lipoprotein (HDL)-Chol, triglycerides (TG) and phospholipids (PL) were analyzed with commercial kits (Chol and TG reagent sets, Daiichi Pure Chemicals, Tokyo, Japan, and PL reagent set, Wako Pure Chemical Ind. Ltd., Osaka, Japan). Serum α -tocopherol (Toc) was extracted by the methods of Abe et al.²⁵⁾, followed by separation by high performance liquid chromatography (HPLC) on Wakosil 5NH₂ (4.0 mm i.d. x 250 mm) (Wako Pure Chemical Ind. Ltd., Osaka, Japan) with 0.8% isopropanol in hexane as the mobile phase²⁶⁾. The amounts of α -Toc were measured fluorometrically (Ex 298nm, Em 325nm) and calculated from recovery of the added internal standard of β -Toc (Eisai Co. Ltd., Tokyo, Japan).

Fatty acids of serum and homogenized heart were methylated and measured by gas liquid chromatography (GLC) using a

capillary column HR-SS-10 (0.25mm x 50m) (Shinwa Chemical Ind. Ltd., Kyoto, Japan). The temperature at the injection port and at the hydrogen flame-ionization detector was 250 °C. The initial column temperature was 170 °C and the rate of increase was 2 °C/min until a final temperature of 220 °C was reached. The amounts of fatty acids were calculated from the recovery of the added internal standard of heptadecanoic acid (C17:0) (NU-Chek-Prep Inc., Minnesota, USA) for fatty acids.

Statistical analysis

The results were expressed as mean $\pm$ standard deviation (SD) for 5 rats per group. Data were analyzed by two-way analysis of variance (ANOVA).

Results

Food intake and body and heart weights

As shown in Table 2, food intakes were larger in the butter groups than the safflower oil groups ($p < 0.005$) and in the soy protein groups than the casein groups ($p < 0.05$). Butter feeding resulted in more gain in body weight than safflower oil feeding ($p < 0.05$), possibly due to greater food intake. With casein diets, the rats showed more gain in body weight than those fed soy protein despite a lower food intake ($p < 0.05$). This indicates the efficiency of the animal protein, casein, for body weight gain in comparison with plant protein. Heart weights were not significantly different among the four groups. There was no interaction between dietary fat and protein on the food intake, body weight gain or heart weight.

Effects of dietary fat and protein on cholesterol concentration in serum

The effects of dietary fat as polyunsaturated fat, safflower oil, and as saturated fat, butter, on cholesterol (Chol) levels in serum lipoprotein were studied. As shown in Table 2, there were no significant differences in serum levels of total-Chol among the four groups. The levels of higher low-density lipoprotein (LDL)-Chol and lower high-density lipoprotein (HDL)-Chol were observed in the butter groups but not in the safflower oil groups ($p < 0.005$, respectively).

The effects of dietary protein, casein and soy protein, on the serum levels of Chol were compared (Table 2). The levels of total-, LDL-, and HDL-Chol were almost the same in the casein groups compared with the soy protein groups. There was no interaction between dietary fat and protein on the levels of total-, LDL-, and HDL-Chol.

Table 1 Fatty acid composition of the butter and safflower oil used

Fatty acid	Butter	Safflower oil
	(% of total fatty acids)	
8:0	1.46	-
10:0	4.23	-
12:0	4.51	-
14:0	13.86	0.12
16:0	35.24	7.36
18:0	10.51	2.34
18:1 n-9	21.34	14.34
18:2 n-6 (LA)	2.01	70.63
18:3 n-3 (LN)	0.41	0.36
20:4 n-6 (AA)	0.11	-
n-3/n-6 PUFA ratio	0.193	0.005

n-3/n-6 ratio indicates the total n-3 fatty acid/n-6 fatty acid ratio.

Effects of dietary fat and protein on the levels of triglycerides and phospholipids in serum

The effects of dietary fats and protein on the levels of triglycerides (TG) and phospholipids (PL) were compared (Table 2). The contents of TG and PL were significantly higher in the butter groups than those in the safflower oil groups ($p < 0.005$, respectively). Significantly high levels of TG in the casein groups were found compared to those in the soy protein groups ($p < 0.01$). The levels of PL were almost the same in the casein groups compared with the soy protein groups. There was no interaction between dietary fat and protein on the levels of TG and PL.

Effects of dietary fat and protein on the levels of tocopherol in serum

The effects of dietary fats and protein, on the levels of α -tocopherol (Toc) are shown in Table 2. The Toc contents were significantly higher in the butter groups (6.34 and 5.09 μ g/ml in BuCa and BuSo, respectively) than in the safflower oil groups (5.18 and 4.76 μ g/ml in SaCa and SaSo, respectively) ($p < 0.05$). In comparison with dietary proteins, significantly higher levels of Toc were found in the casein groups (6.34 and 5.18 μ g/ml in BuCa and SaCa, respectively) than in the soy protein groups (5.09 and 4.76 μ g/ml in BuSo and SaSo, respectively) ($p < 0.01$). There was no interaction between dietary fat and protein on the levels of Toc.

Effects of dietary fats on fatty acid composition in serum

The serum fatty acid amounts in the four dietary groups are shown in Table 3. The amounts of saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) were higher in the butter groups than the safflower oil groups ($p < 0.005$). Polyunsaturated fatty acids (PUFA) in the butter groups were lower than those in the safflower oil groups ($p < 0.005$). When comparing each fatty acid amounts, for linolenic acid (LN, C18:3n-3), the butter groups showed significantly higher levels of 37 and 134 μ g/100ml for BuCa and BuSo, respectively, than those of the safflower oil groups (under 1 μ g/100ml for SaCa and SaSo, respectively) ($p < 0.05$, respectively). The levels of eicosapentaenoic acid (EPA, C20:5n-3) in the safflower oil groups (SaCa and SaSo) were very low, under 0.01mg/100ml, compared with those in the butter groups (BuCa and BuSo) of 1.89 and 1.73mg/100ml, respectively ($p < 0.005$). The levels of docosahexaenoic acid (DHA, C22:6n-3) in the butter groups were also higher than those in the safflower oil groups (6.23 and 4.91 mg/100 ml in BuCa and BuSo, under 0.01 and 0.16 mg/100 ml in SaCa and SaSo, $p < 0.005$, respectively). The serum levels of linoleic acid (LA, C18:2n-6) and arachidonic acid (AA, C20:4n-6) in the butter groups were lower than those for the safflower oil groups ($p < 0.005$). Serum levels of n-3 PUFA and n-6 PUFA are shown in Fig. 1. The total amounts of n-3 PUFA in the butter groups, 8.15 and 6.78mg/100ml in BuCa and BuSo, respectively, were significantly higher than those in the safflower

Table 2 Effects of dietary fat and protein on body weight gain and serum lipids in rats

	Butter		Safflower oil		ANOVA		
	Casein	Soy protein	Casein	Soy protein	Fat	Protein	Fat $\times$ Protein
Food intake (g/day)	12.1 $\pm$ 0.6	12.9 $\pm$ 0.6	10.4 $\pm$ 0.5	11.0 $\pm$ 0.5	$p < 0.005$	$p < 0.05$	ns
Body weight gain (g/47weeks)	209 $\pm$ 26	196 $\pm$ 9	196 $\pm$ 19	163 $\pm$ 20	$p < 0.05$	$p < 0.05$	ns
Heart weight (mg)	937 $\pm$ 97	923 $\pm$ 45	937 $\pm$ 83	920 $\pm$ 67	ns	ns	ns
Total cholesterol (mg/100ml)	73.8 $\pm$ 9.8	66.7 $\pm$ 4.2	70.3 $\pm$ 3.1	71.7 $\pm$ 6.0	ns	ns	ns
LDL-cholesterol (mg/100ml)	35.5 $\pm$ 6.0	28.4 $\pm$ 3.0	21.9 $\pm$ 2.9	21.7 $\pm$ 4.5	$p < 0.005$	ns	ns
HDL-cholesterol (mg/100ml)	38.3 $\pm$ 4.3	38.3 $\pm$ 1.2	48.5 $\pm$ 2.9	50.0 $\pm$ 2.3	$p < 0.005$	ns	ns
Triglycerides (mg/100ml)	146.5 $\pm$ 36.0	97.0 $\pm$ 19.3	87.8 $\pm$ 29.5	65.4 $\pm$ 21.2	$p < 0.005$	$p < 0.01$	ns
Phospholipids (mg/100ml)	140.4 $\pm$ 18.7	126.8 $\pm$ 6.7	115.5 $\pm$ 4.9	114.3 $\pm$ 12.1	$p < 0.005$	ns	ns
α -Tocopherol (μ g/ml)	6.34 $\pm$ 0.78	5.09 $\pm$ 0.38	5.18 $\pm$ 0.43	4.76 $\pm$ 0.68	$p < 0.05$	$p < 0.01$	ns

Table 3 Effects of dietary fat and protein on fatty acid composition in serum and hearts

Fatty acid	Butter		Safflower oil		ANOVA		
	Casein	Soy protein	Casein	Soy protein	Fat	Protein	Fat $\times$ Protein
Serum fatty acid (mg/100ml)							
Saturated	88.4 $\pm$ 14.4	69.4 $\pm$ 9.7	57.7 $\pm$ 6.2	55.7 $\pm$ 7.5	$p < 0.005$	$p < 0.05$	ns
Monounsaturated	100.9 $\pm$ 22.2	62.5 $\pm$ 7.1	20.0 $\pm$ 4.1	16.9 $\pm$ 3.1	$p < 0.005$	$p < 0.01$	$p < 0.01$
Polyunsaturated	39.7 $\pm$ 5.0	44.5 $\pm$ 2.1	106.9 $\pm$ 14.1	100.0 $\pm$ 13.1	$p < 0.005$	ns	ns
18:3 n-3	0.037 $\pm$ 0.083	0.134 $\pm$ 0.136	<0.001	<0.001	$p < 0.05$	ns	ns
20:5 n-3	1.89 $\pm$ 0.23	1.73 $\pm$ 0.25	<0.001	<0.001	$p < 0.005$	ns	ns
22:6 n-3	6.23 $\pm$ 1.02	4.91 $\pm$ 0.40	<0.001	0.16 $\pm$ 0.23	$p < 0.005$	$p < 0.05$	$p < 0.01$
18:2 n-6	8.64 $\pm$ 1.35	10.4 $\pm$ 0.8	54.4 $\pm$ 11.1	45.5 $\pm$ 7.3	$p < 0.005$	ns	ns
20:3 n-6	1.56 $\pm$ 0.27	1.77 $\pm$ 0.32	0.53 $\pm$ 0.12	0.58 $\pm$ 0.15	$p < 0.005$	ns	ns
20:4 n-6	21.4 $\pm$ 3.2	25.6 $\pm$ 1.0	52.0 $\pm$ 3.7	53.8 $\pm$ 6.6	$p < 0.005$	ns	ns
Heart fatty acid (mg/g)							
Saturated	7.08 $\pm$ 0.47	7.50 $\pm$ 0.36	6.90 $\pm$ 0.22	7.04 $\pm$ 0.56	ns	ns	ns
Monounsaturated	3.19 $\pm$ 0.42	2.95 $\pm$ 0.25	0.98 $\pm$ 0.12	1.00 $\pm$ 0.22	$p < 0.005$	ns	ns
Polyunsaturated	8.39 $\pm$ 0.26	9.13 $\pm$ 0.50	10.65 $\pm$ 0.34	10.29 $\pm$ 0.81	$p < 0.005$	ns	$p < 0.05$
18:3 n-3	0.0089 $\pm$ 0.0044	0.0127 $\pm$ 0.0016	<0.0001	<0.0001	$p < 0.005$	ns	ns
20:5 n-3	0.0653 $\pm$ 0.0077	0.0538 $\pm$ 0.0043	<0.0001	<0.0001	$p < 0.005$	$p < 0.05$	$p < 0.05$
22:6 n-3	2.203 $\pm$ 0.097	2.347 $\pm$ 0.157	0.208 $\pm$ 0.015	0.272 $\pm$ 0.039	$p < 0.005$	$p < 0.05$	ns
18:2 n-6	1.73 $\pm$ 0.13	2.25 $\pm$ 0.11	5.50 $\pm$ 0.21	5.47 $\pm$ 0.46	$p < 0.005$	ns	$p < 0.05$
20:3 n-6	0.334 $\pm$ 0.019	0.319 $\pm$ 0.008	0.077 $\pm$ 0.006	0.071 $\pm$ 0.005	$p < 0.005$	ns	ns
20:4 n-6	4.05 $\pm$ 0.20	4.34 $\pm$ 0.28	4.87 $\pm$ 0.14	4.48 $\pm$ 0.35	$p < 0.005$	ns	$p < 0.01$

oil groups, under 0.01 and 0.16 mg/100 ml in SaCa and SaSo, respectively ($p < 0.005$). On the other hand, the total amounts of n-6 PUFA in the butter groups (31.6 and 37.7 mg/100 ml in BuCa and BuSo, respectively) were lower than those in the safflower oil groups (106.9 and 99.9 mg/100 ml in SaCa and SaSo, respectively) ($p < 0.005$). The ratios of n-3/n-6 PUFA in serum were significantly higher in the butter groups, 0.259 and 0.180 in BuCa and BuSo, respectively, than those in the safflower oil groups, under 0.001 and 0.002 in SaCa and SaSo, respectively ($p < 0.005$) (Fig. 2).

Effects of dietary protein on fatty acid composition in serum

The effects of dietary protein on fatty acid composition in serum were assessed (Table 3). Dietary casein (BuCa and SaCa) induced increases in SFA and MUFA than those on the soy protein diet (BuSo and SaSo) ($p < 0.05$ and $p < 0.01$, respectively). As shown in Fig. 1, with the casein diet, the levels of n-3 PUFA in serum were affected by dietary protein ($p < 0.05$). No significant differences were found in the levels of n-6 PUFA between the casein and soy protein diets. The ratios of n-3/n-6 PUFA were higher in the casein diet than those of the soy protein diet in the case of butter as the dietary fat (0.259 and 0.180 in BuCa and BuSo, respectively) ($p < 0.005$) (Fig. 2). For all four dietary groups, the interaction between dietary fat and protein were found, for the serum levels of n-3 PUFA and the ratios of n-3/n-6 PUFA ($p < 0.05$ and $p < 0.005$, respectively).

Effects of dietary fats on fatty acid composition in the heart

The amounts of fatty acids in the heart in the four dietary groups are shown in Table 3. There were no significant differences in the amounts of SFA among the four groups. MUFA amounts were higher in the butter groups than the safflower oil groups ($p < 0.005$). PUFA were lower than those in safflower oil groups ($p < 0.005$). For LN (C18:3n-3), the butter groups showed significantly higher concentrations of 8.9 and 12.7 $\mu\text{g/g}$ for BuCa and BuSo, respectively, than those of the safflower oil groups (under 0.1 $\mu\text{g/g}$ for SaCa and SaSo, respectively) ($p < 0.005$). The levels of EPA (C20:5n-3) in the butter groups of 65.3 and 53.8 $\mu\text{g/g}$ for BuCa and BuSo, respectively, were higher compared with those in the safflower oil groups of under 0.1 $\mu\text{g/g}$ (SaCa and SaSo, respectively) ($p < 0.005$). The levels of DHA (C22:6n-3) in the butter groups, 2.203 and 2.347 mg/g for BuCa and BuSo, respectively, were also higher than those in the safflower oil groups (0.208 and 0.272 mg/g in SaCa and SaSo, respectively) ($p < 0.005$). In the butter groups, LA (C18:2n-6) and AA (C20:4n-6) were lower than those for the safflower oil groups ($p < 0.005$).

Heart levels of n-3 PUFA and n-6 PUFA are shown in Fig. 1. The total amounts of n-3 PUFA in the butter groups, 2.28 and 2.42 mg/g in BuCa and BuSo, respectively, were over 9 times higher than those in the safflower oil groups, 0.21 and 0.27 mg/g in SaCa and SaSo, respectively ($p < 0.005$). On the other hand, the total amounts of n-6 PUFA in the butter groups (6.11 and 6.71 mg/g in BuCa and BuSo, respectively), were lower than those in the safflower oil groups (10.4 and 10.0 mg/g in SaCa and SaSo, respectively) ($p < 0.005$). The ratios of n-3/n-6 PUFA were over 13 times higher in the butter groups, 0.373 and 0.362 in BuCa and BuSo, respectively, than in those in the safflower oil groups, 0.020 and 0.027 in SaCa and SaSo, respectively, in the heart ($p < 0.005$) (Fig. 2).

Effects of dietary protein on fatty acid composition in the heart

There were no significant effects of dietary casein and soy protein on the amounts of SFA and PUFA in the heart (Table 3). The levels of n-3 PUFA in the casein diet groups (2.28 and 0.21 mg/g in BuCa and SaCa, respectively), were lower than those of the soy protein diet groups (2.42 and 0.27 mg/g in BuSo and SaSo, respectively) ($p < 0.05$) (Fig. 1). No significant differences were found in the levels of n-6 PUFA (Fig. 1) and the ratios of n-3/n-6 PUFA between the casein and soy protein diets groups (Fig. 2). A significant interaction between dietary fat and protein were found on the levels of n-6 PUFA ($p < 0.05$) (Fig. 1).

Fig. 1 Concentrations of n-3 PUFA and n-6 PUFA in serum and the hearts of rats kept for 47 weeks on BuCa, butter-casein diet; BuSo, butter-soy protein diet; SaCa, safflower oil-casein diet; or SaSo, safflower oil-soy protein diet. Data (mean $\pm$ SD) represent the serum and heart values from 5 rats each, using ANOVA.

Fig. 2 Ratio of n-3 /n-6 PUFA in serum and the hearts of rats kept for 47 weeks on BuCa, butter-casein diet; BuSo, butter-soy protein diet; SaCa, safflower oil-casein diet; or SaSo, safflower oil-soy protein diet. Data (mean $\pm$ SD) represent the serum and heart values from 5 rats each, using ANOVA.

Discussion

The fat of SFA and animal protein increased plasma total Chol while the fat of PUFA and plant protein decreased it ²⁻⁷. However, in the experiment conducted by Ishihara et al., safflower oil was not hypocholesterolemic as compared with lard ²⁷. In this study, no significant differences were found in the mean levels of serum total-Chol among the four dietary groups. This suggests that butter SFA and casein animal protein have no effect on increasing total-Chol in normocholesterolemia.

Although total-Chol levels were the same, lower levels of HDL-Chol and higher levels of LDL-Chol were found in the butter groups in comparison with the safflower oil groups (Table 1). In general, n-3 PUFA increases the levels of HDL-Chol ^{15, 28}, but butter with a higher content of n-3 PUFA than safflower oil induced lower HDL-Chol. This difference indicates that n-3 PUFA amounts in butter were not enough to increase HDL-Chol. It is reported that SFA increase LDL-Chol by decreasing the LDL receptor ²⁹ and increase plasma total-Chol mainly due to an increase in LDL-Chol ³⁰. In this study, total-Chol levels were the same in both diets of SFA fat, butter, and PUFA fat, safflower oil. These findings suggest that the n-3 PUFA has a role in increasing the LDL-Chol receptor and can offer protection against an increase of LDL-Chol, which should increase due to the higher content of SFA in butter.

Toc, an antioxidant, prevents oxidation of PUFA, with a resulting decrease in the Toc level ³¹. Thus, a high intake of Toc is required when the intake of PUFA is high ³². It seems to be reasonable that the safflower oil diets containing large amounts of PUFA induced a low serum level of Toc in comparison to the butter diet containing low amounts of PUFA.

LDL oxidation is involved in the development of atherosclerosis ²⁶. An inverse correlation between the plasma levels of Toc and mortality from CVD is shown ²³. Those findings indicate that Toc as an antioxidant can inhibit the oxidation of LDL and then decrease the risk of CVD. The present results suggest that higher levels of Toc in the butter groups than in the safflower oil groups may mean that there is enough protection of LDL from oxidation in butter diets in comparison to safflower oil diets. Hence, it is likely that in spite of higher levels of LDL in butter groups than safflower oil groups, protection against the development of atherosclerosis is offered by the effect of the higher levels of Toc.

Higher levels of TG were found in BuCa than in SaSo, respectively, and TG levels in BuSo were almost the same in SaCa. SFA has been reported to increase TG and PUFA and n-3 PUFA to decrease TG ^{7, 14, 15}. As shown in Table 2, soy protein decreased TG levels as compared with casein. It seems that the n-3 PUFA in butter and soy protein decrease of TG in BuSo to the levels of TG in SaCa.

The low incidence of CVD among Greenland Inuits has

been attributed to their greater consumption of fish enriched with n-3 PUFA ³³. Dyerberg found that Greenland Inuits have high EPA levels and low AA levels in the blood and that a high n-3/n-6 PUFA ratio could reduce the development of thrombosis and atherosclerosis ³³. From the standpoint of the effects of n-3 PUFA and n-6 PUFA on anti-thrombotic and anti-atherosclerotic activities, the ratio of n-3 PUFA to n-6 PUFA is one of the indicators of those activities and the risk of CVD ¹⁴. When our butter groups were compared with the safflower oil groups, they showed higher n-3 PUFA contents and lower n-6 PUFA contents in serum and heart ($p < 0.005$, respectively) (Fig. 1). The ratios of n-3/n-6 PUFA in the butter groups in serum and heart were higher than those of the safflower oil groups (Fig. 2). From these findings, it seemed that our results of lower levels of n-3 PUFA and higher levels of n-6 PUFA in serum and heart in safflower oil groups could offer an explanation for the higher CVD incidence in a high n-6 PUFA diet ^{9, 10}.

The present results demonstrate that the fatty acid composition in serum and heart differs with the type of oil consumed. Although neither both butter nor safflower oil contain EPA or DHA, both EPA and DHA are synthesized from LN. In rat fed both fats, the n-3 PUFA percentages for total fatty acids and n-3/n-6 PUFA ratio amounts in heart were higher than those in serum. In particular, in the heart, the n-3 PUFA and n-3/n-6 PUFA ratios were over 8 times those in the butter groups as compared with the safflower oil groups. Our data indicate that the fatty acid composition in the heart was more responsive to dietary fatty acid composition than in serum and the degree of storage in n-3 PUFA was highly preserved for a functional organ, in this case, the heart.

The results of this study on the fatty acid composition of serum and heart of rats in response to dietary fat emphasizes the importance of considering the dietary fatty acid composition. Our results revealed that diets with greater levels of n-3 PUFA and n-3/n-6 PUFA ratio resulted in a higher degree of n-3 PUFA and n-3/n-6 PUFA ratio in the organs. Thus, the organ fatty acid composition depends not only on the dietary fatty acids but also on the partitioning of those fats for organ function.

In general, the fatty acid composition in serum and tissue reflects the composition of the food consumed. Also, an inverse relationship has been reported between fish consumption and mortality from coronary heart disease ^{11, 12}. Our present study indicates that dietary levels of n-3 PUFA and n-3/n-6 PUFA ratio influence the levels of n-3 PUFA and n-3/n-6 PUFA ratio in serum and in the heart. Thus, consuming more n-3 PUFA and n-3/n-6 PUFA ratio should lower the risk for CHD.

In conclusion, the results from this study indicate that higher amounts of n-3 PUFA and the higher ratio of n-3/n-6 PUFA as an indicator of metabolically important activities in the heart in the butter groups may be expected to play a role in the prevention of CVD in comparison with the safflower oil groups.

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