Butter-enriched Diets Reduce Arterial Prostacyclin Production in Rats

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Rats were fed diets containing 10%, 30% or 50% energy as fat derived predominantly from butter or lard. The protein content of the diets was maintained at 20%. After three weeks on the diets, the rats were killed and the following parameters measured: prostacyclin production in vitro from abdominal aorta and mesenteric artery; platelet aggregation to ADP and thrombin; fatty acid composition of the phospholipids in plasma, thoracic aorta and liver; smooth muscle reactivity and release of endothelial derived relaxing factor (EDRF) from aortic endothelium stimulated by acetylcholine. There was no significant effect of increasing fat content of the diets (neither lard nor butter) on platelet aggregation. In contrast, prostacyclin production in both the mesenteric artery and the abdominal aorta fell in a concentration-dependent manner in the butter-supplemented rats. However, no effect on prostacyclin production was detected in arteries from the lard-supplemented animals. The effects of the diets on prostacyclin (PGI_2) production correlated very well with the changes in plasma, aortic and liver phospholipid arachidonic acid (AA) and eicosapentaenoic acid (EPA) contents. AA decreased in a concentration-dependent manner in the rats fed the butter-enriched diets but did not change in those fed the lard-enriched diets, whereas EPA rose in a concentration-dependent manner in the butter-fed rats and was unchanged in the lard-fed animals. The clear-cut effects of the butter-enriched diets on aortic phospholipid fatty acid composition and aortic PGI_2 production were accompanied by a significant reduction in smooth muscle relaxation to EDRF. These results indicate that in the rat, enrichment of the diet with butter can reduce the concentration of AA and increase that of EPA in plasma and tissue phospholipids with a parallel reduction in arterial PGI_2 production and EDRF.

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It now is accepted widely that high levels of saturated fat in the diet increase the risk of coronary heart disease (1,2). The effects of dietary saturated fat appear to be mediated through two major pathways: elevated plasma cholesterol levels, resulting in increased risk of atherosclerosis (1), and increased thrombosis tendency (3). The mechanism by which thrombosis tendency is increased is not well understood. Renaud and coworkers have reported that dietary saturated fat is associated with an increase in the concentration of the fatty acid 20:3n-9 in plasma and platelet lipids in rats and humans (4,5), a phenomenon that usually occurs only in essential fatty acid deficiency (6). They attributed the increased thrombin-induced platelet aggregation associated with dietary saturated fat to the presence of this fatty acid (5). However, it also is possible that the increased levels of 20:3n-9 are a marker of other changes in plasma and tissue fatty acid composition that may affect directly the production of the arachidonic acid (AA)-derived prostanoids (prostacyclin [PGI_2] and thromboxane A_2) and thereby modulate thrombosis tendency. To examine this possibility, the aim of this study was to determine the effects of increasing the proportion of two different dietary fats (butter and lard) on vascular PGI_2 production and platelet aggregation, and plasma, aortic and liver phospholipid fatty acid composition in the rat. In addition, the effects of increased dietary fat (butter) on aortic smooth muscle reactivity and the release of endothelial derived relaxing factor (EDRF) were assessed.

METHODS

Animals. Male Sprague-Dawley rats weighing 120–170 g were used in all studies. They were housed in a temperature-controlled room with a 12-hr light-dark cycle. Food and water were provided ad libitum and food consumption and body weights recorded three times each week.

Diets. The nutrient composition of the experimental diets is shown in Table 1 and their fatty acid composition is given in Table 2. The diets were prepared from whole meal flour and skim milk powder (providing all the carbohydrate and protein) and lard or butter (providing most of the fat). The proportion of energy derived from protein was constant in all diets, while the proportions of fat and carbohydrate varied inversely. The P/S ratio fell as the proportion of fat rose on both the lard and butter diets, however the fall was more pronounced on the butter diet as the P/S ratio of butter is much lower than that of lard. Vitamins and minerals were added to reach the levels recommended by the American Institute of Nutrition (7).

Platelet aggregation. After three weeks on the diet, the rats were anesthetized with sodium pentobarbitone (50 mg/kg intraperitoneally), and blood was taken by cardiac puncture into 3.8% sodium citrate (9 vol blood: 1 vol citrate). The citrated blood was centrifuged

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Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; EC_{10}, EC_{50}, EC_{90}, the concentrations that will give 10%, 50% or 90% response (dilation or constriction), respectively; EDRF, endothelial-derived relaxing factor; EPA, eicosapentaenoic acid; KRB, Krebs Ringer bicarbonate buffer; NO, nitric oxide; PGI_2, prostacyclin I_2; PPP, platelet-poor plasma; PRP, platelet-rich plasma; P/S ratio, the ratio of polyunsaturated to saturated fatty acids; PUFA, polyunsaturated fatty acid; TXA_2, thromboxane A_2; TXB_2, thromboxane B_2; 6-keto-$PGF_{1\alpha}$, 6-keto-prostaglandin $F_{1\alpha}$; 20:3n-9, a 20 carbon fatty acid with three double bonds, the first one being nine carbons (n-9) from the methyl end.

TABLE 1

Composition of the Experimental Diets

	Butter			Lard		
Constituents (g/100 g)	10%	30%	50%	10%	30%	50%
Whole meal flour	73.1	42.5	4.6	73.5	43.8	4.8
Skim milk powder	20.0	38.7	61.3	20.1	39.8	64.9
Butter	2.5	14.4	29.7	—	—	—
Lard	—	—	—	2.0	12.0	25.9
Choline bitartrate	0.1	0.1	0.1	0.1	0.1	0.1
Mineral mix	3.3	3.3	3.3	3.3	3.3	3.3
Vitamin mix	1.0	1.0	1.0	1.0	1.0	1.0
Composition (% energy)						
Protein	20	20	20	20	20	20
Carbohydrate	70	50	30	70	50	30
Fat	10	30	50	10	30	50
P/S ratio	0.56	0.10	0.05	0.54	0.24	0.19

TABLE 2

Fatty Acid Composition of the Diets

	Butter			Lard		
Fatty acid	10%	30%	50%	10%	30%	50%
8:0	1.2	1.4	1.9	—	—	—
10:0	2.1	2.77	4.2	—	—	—
12:0	2.4	2.8	3.4	—	—	—
14:0	6.5	10.5	11.3	1.8	1.8	1.7
14:1	0.5	0.7	0.8	—	—	—
15:0	0.8	1.2	1.2	—	—	—
16:0	24.7	30.7	29.8	25.1	30.9	27.0
16:1	1.4	1.6	2.9	1.2	0.8	2.1
17:0	0.8	1.0	0.6	0.9	0.6	0.6
18:0	9.4	14.9	14.0	14.4	16.5	17.9
18:1	23.0	25.1	25.9	32.5	36.3	40.6
18:2n-6	24.6	5.7	2.4	21.3	11.1	8.2
18:3n-3	2.2	1.1	1.2	1.6	1.0	0.9
20:0	0.2	0.3	0.2	0.3	0.3	0.3
20:1	0.2	0.3	0.2	0.9	0.7	0.7
Saturates	48.1	65.5	66.6	42.5	50.1	47.5
Monoenes	25.1	27.7	29.8	34.6	37.8	43.4
Polyenes	26.8	6.8	3.6	22.9	12.1	9.1
n-6/n-3 ratio	11.2	5.2	2.0	13.3	11.1	9.1

at 150 × g for 10 min at room temperature to prepare platelet-rich plasma (PRP). After removal of the PRP, the remaining blood was centrifuged for 15 min at 3000 × g at room temperature to obtain platelet-poor plasma (PPP). The platelet count of the PRP was determined using a Coulter Counter (Model SII) and adjusted with PPP to give a count of 300,000 platelets/μl. Platelet aggregation measurements were performed with a Payton dual-channel aggregometer: 450 μl PRP was stirred at 1000 rpm at 37 C and 50 μl of the appropriate aggregating agent added: ADP (2 and 5 μM), thrombin (1 and 2 U/ml) or AA (6.4 mM). Platelet aggregation was monitored continuously for four min. To quantitate platelet aggregation, the maximum change in light transmittance through PRP was expressed as a percentage of the light transmittance through PPP. Five min after the addition of the aggregating agent, a 200 μl aliquot was mixed with 200 μl of ethanol, centrifuged and the supernatant stored at -20 C for subsequent radioimmunoassay of thromboxane B_2 (TXB_2), the stable metabolite of thromboxane A_2 (TXA_2) (8).

Vascular PGI_2 production. Immediately after blood collection, the rat was perfused with Krebs Ringer Bicarbonate buffer (KRB), pH 7.4, containing 5.5 mM glucose, to flush out the remaining blood, leaving the artery preparations free of adhering blood clots. The mesenteric artery and abdominal aorta were removed carefully, cleared of connective tissue and cut into rings. These rings were incubated in 4 ml of KRB containing 5.5 mM glucose at 37 C for three hr in a shaking water bath and 100 μl aliquots taken at zero time and 15, 30, 60, 120 and 180 min later. These samples were stored at -20 C and the time course of PGI_2 production determined subsequently by radioimmunoassay of its stable metabolite, 6-keto $PGF_{1\alpha}$ (9). At the end of the incubation, the rings were removed, blotted on filter paper and weighed to obtain the "wet weight." In order to control for the presence of any cross-reacting material interfering with the RIA of 6-keto-$PGF_{1\alpha}$ produced during the incubation of arteries, we incubated aortic rings from butter-fed rats (10% and 50%) in the presence and absence of indomethacin (10 μg/ml). Indomethacin completely prevented the production of any immuno-reactive material from these aortea, confirming the specificity of the assay for 6-keto-$PGF_{1\alpha}$.

Aortic smooth muscle reactivity and the release of EDRF. Rats were stunned and the thoracic aorta carefully dissected free of surrounding tissue. The aorta was pinned down at either end on a silastic rubber-coated dish filled with cold, oxygenated Krebs' solution composition (in mM) Na^+ 144, K^+ 5.9, Ca^{++} 2.5, Mg^{++} 1.2, Cl^- 128.7, HCO_3^- 25, SO_4^{2-} 1.2, glucose 11, EDTA 0.27 aerated with a gas mixture of 95% O_2 and 5% CO_2. Two three-ring segments each 3 mm long were cut transversally from the aorta. Each ring was suspended on two surgical stainless steel Z-shaped support wires 500 μm diameter in a heated organ bath. One wire was attached to an isometric force transducer (Grass FTO3C), the second wire to an acrylic leg mounted on a micrometer screw gauge. After 30 min

equilibration, the micrometer was advanced to stretch the artery in steps every minute. The passive force-diameter relationship was determined from the measurements as described (10). The internal diameter (D) and circumference (L) of the vessel was calculated for an equivalent transmural pressure of 100 mm Hg. The artery was released slightly to a circumference of 0.9 L and left at this passive stretch for the remainder of the experiment. These initial steps were taken to ensure that each artery segment was under similar resting passive tone before drug addition. From each rat, one ring was contracted to a steady level of active force by phenylephrine hydrochloride 1 μM, then relaxed by the cumulative addition of acetylcholine bromide at 0.5 log unit increments (from 10–1000 nM) to test the EDRF activity. Relaxation responses were averaged at fixed concentrations of each.

Fatty acid composition of plasma aortic, and liver phospholipids. Lipid extracts were prepared from plasma, thoracic aorta and liver by chloroform/methanol extraction (11). Phospholipids were separated by thin layer chromatography and the concentration of the component fatty acids determined by capillary gas liquid chromatography using heptadecanoic acid as an internal standard as described (12). The identification of 20:3n-9 was based on identical retention times with 20:3n-9 isolated from essential fatty acid deficient rat liver when separated on a 50 m x 0.32 mm bonded phase capillary column coated with CP Sil 88. On this column, 20:3n-9 was separated clearly from 20:2n-6, 20:3n-6 and 22:0.

Statistical analyses. Differences between groups were established using analysis of covariance or Student's unpaired t-test. Results are expressed as mean ± SEM.

MATERIALS

Pentobarbitone sodium was purchased from Civa Chemicals (Hornsby, N.S.W., Australia), ADP, TXB_2 standard, 6-keto $PGF_{1\alpha}$ standard, acetylcholine bromide and indomethacin were from Sigma Chemical Co. (St. Louis, MO); thrombin (Fibrindex, human) from Orthodiagnostic Systems Inc. (Raritan, NJ); arachidonic acid and heptadecanoic acid were from NuChek Prep (Elysian, MN), TXB_2 antibody was a gift from Laurence Levine, Brandeis University (Waltham, MA); 6-keto-$PGF_{1\alpha}$ antibody was a gift from L.C. Best, University of Sheffield (Sheffield, U.K.); tritiated 6-keto-$PGF_{1\alpha}$ and TXB_2 were from Amersham International (Amersham, U.K.); bonded phase capillary column from Chrompak (Middelburg, The Netherlands); and phenylephrine hydrochloride from Stirling Pharmaceuticals (Sydney, Australia).

RESULTS

Whether the rats were fed butter or lard diets did not affect their weight gain. At 10%, 30% and 50% butter diets, the rats gained 7.3 ± 0.1, 7.0 ± 0.3 and 6.3 ± 0.3 g/day, while with the 10%, 30% and 50% lard diets they gained 7.5 ± 0.1, 7.1 ± 0.2 and 6.1 ± 0.3 g/rat/day. However, in both dietary studies (butter and lard), there was a reduction in weight gain as the fat content of the diet increased. This probably was due in large part to the major changes in salt and water balance that occur on low carbohydrate diets (13,14).

There was no effect of either type of dietary fat on ADP-induced platelet aggregation (Table 3). Although there was a trend towards increased platelet aggregation in response to the higher concentration of thombin (2 U/ml) with increasing fat intake (both lard and butter), it was not statistically significant. Thromboxane production by platelets in vitro was not influenced by either type of dietary fat (Table 3).

The effect of increasing the level of butter in the diet on the time-course of PGI_2 production by the abdominal aorta is shown in Figure 1. PGI_2 production over the three hr was reduced consistently as the proportion of dietary fat rose from 10% to 50% of energy (analysis of covariance, $p < 0.001$). Subsequent results are presented as the PGI_2 production in the first hour

TABLE 3

The Effects of the Diets on Platelet Aggregation and Thromboxane A_2 Production in Platelets (See Methods)

	Butter			Lard		
Platelet aggregation (%)	10% n = 10	30% n = 10	50% n = 10	10% n = 5	30% n = 4	50% n = 3
ADP 2 μM	24.1 ± 2.2	26.3 ± 3.0	22.8 ± 4.0	23.3 ± 1.9	26.0 ± 4.9	24.5 ± 6.2
5 μM	31.9 ± 3.8	36.8 ± 2.1	33.3 ± 3.8	36.0 ± 3.6	36.8 ± 5.9	23.7 ± 4.5
Thrombin 1 U/ml	12.9 ± 4.9	12.9 ± 4.5	13.5 ± 5.3	12.3 ± 4.8	14.1 ± 4.8	27.0 ± 12.9
2 U/ml	28.4 ± 4.0	35.6 ± 2.9	40.4 ± 4.4	26.4 ± 2.6	33.6 ± 6.4	37.8 ± 5.0
Thromboxane B_2 production (ng/ml PRP)						
ADP 2 μM	3.2 ± 0.2	3.0 ± 0.4	2.8 ± 0.2	2.6 ± 0.3	3.4 ± 1.1	2.6 ± 0.3
5 μM	3.8 ± 0.3	3.2 ± 0.2	3.7 ± 0.2	3.1 ± 0.5	3.6 ± 0.7	2.7 ± 0.5
Thrombin 1 U/ml	2.8 ± 0.2	2.6 ± 0.2	2.4 ± 0.1	2.3 ± 0.2	4.1 ± 2.1	2.8 ± 0.3
2 U/ml	6.6 ± 1.2	12.5 ± 5.1	6.4 ± 1.3	8.4 ± 1.4	11.9 ± 2.2	7.0 ± 1.8
Arachidonic acid 6.4 mM	1440 ± 141	1406 ± 159	1671 ± 204	1413 ± 85	1202 ± 67	1365
Platelet-poor plasma	2.9 ± 0.3	2.2 ± 0.2	2.4 ± 0.1	1.9 ± 0.1	2.7 ± 0.7	1.8 ± 0.2

Mean ± SEM.

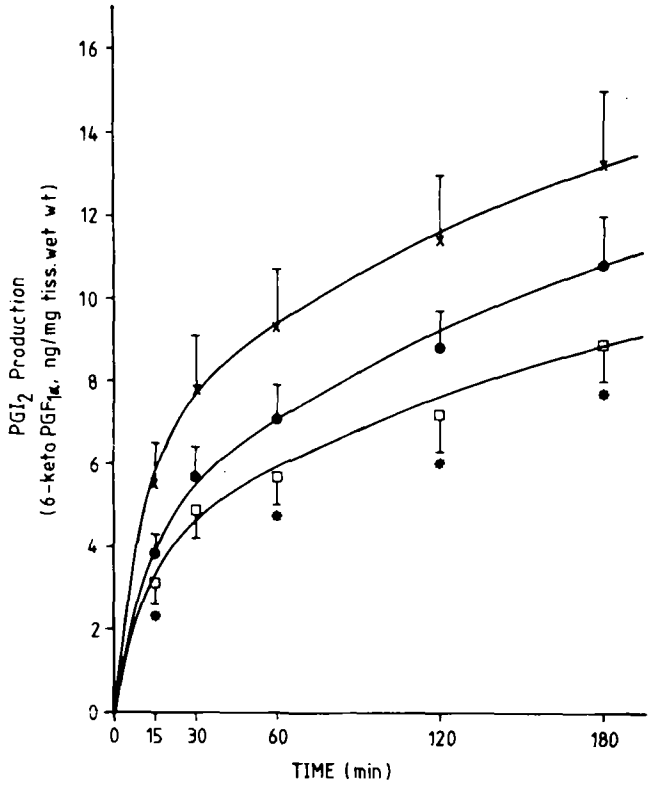

FIG. 1. Time course of prostacyclin production by rings of abdominal aorta from rats fed diets containing 10% (x), 30% (•) or 50% (□) energy from butter (ng 6-keto $PGF_{1\alpha}$/mg tissue wet weight, mean ± SEM).

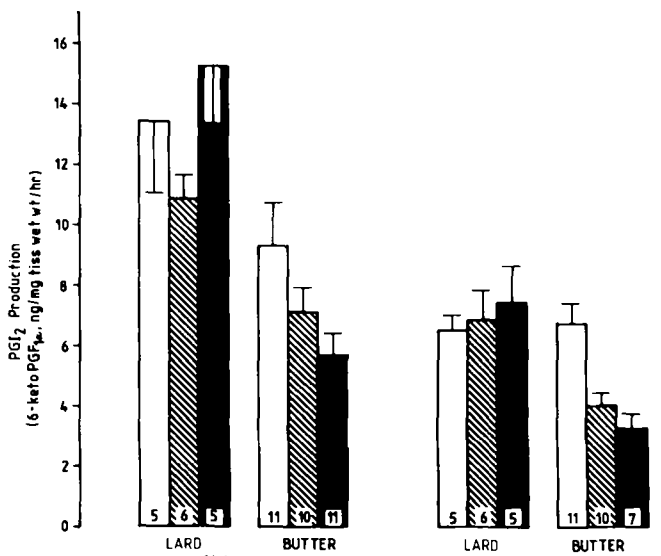

FIG. 2. Prostacyclin production by rings of abdominal aorta and mesenteric artery from rats fed diets containing 10% (open bars), 30% (hatched bars) or 50% (black bars) energy from butter or lard.

Statistics:	Abdominal aorta	Mesenteric artery
10% vs 30%	butter n.s., lard n.s.	butter $p < 0.005$, lard n.s.
10% vs 50%	butter $p < 0.05$, lard n.s.	butter $p < 0.001$, lard n.s.
30% vs 50%	butter n.s., lard n.s.	butter n.s., lard n.s.

of incubation in order to summarize the data in a single figure (Fig. 2). In both the mesenteric artery and the abdominal aorta, the PGI_2 production decreased in a dose-dependent manner as the proportion of butter in the diet rose from 10% to 50% energy. In contrast, lard supplementation had no detectable effect on PGI_2 production in either artery.

The effects of butter-enrichment on the fatty acid composition of plasma phospholipids are shown in Table 4. Increasing the butter content of the diet was associated with striking concentration-dependent changes in the proportions of the eicosanoid fatty acids: AA fell ($p < 0.001$), EPA rose ($p < 0.001$) and eicosatrienoic acid (20:3 n-9) rose ($p < 0.001$). In contrast, lard-enrichment had no effect on the plasma phospholipid fatty acid composition. None of the lard diets differed from the 10% butter diet or from each other (Table 5).

The effect of increasing the butter content of the diet on the fatty acid composition of aortic phospholipids is presented in Table 6. The major changes were in the proportions of the eicosanoid fatty acids that were similar but less pronounced than those in the plasma phospholipids. The proportion of AA fell but that of EPA and 20:3 n-9 rose as the butter content of the diet increased. Enrichment of the diet with lard had no effect on the fatty acid composition of aortic phospholipids.

The concentration of phospholipid in plasma fell dose-dependently on the butter-enriched diets: 10% = 54.8 ± 3.0, 20% = 50.0 ± 2.1, 50% = 41.6 ± 2.6 mg phospholipid fatty acid / 100 ml plasma (50% < 10%, $p<0.01$; 50% < 30%, $p<0.05$). The concentration of phospholipids in aorta and liver was not affected by increasing the butter content of the diet. Increasing the proportion of dietary fat as lard did not affect the phospholipid concentration in plasma, aorta or liver. The data are not shown but in all three lard-enriched diets, it was essentially the same as that in the 10% butter diet.

In view of the changes in aortic lipid composition and the marked reduction in aortic prostacyclin production on the butter-enriched diets, we considered the possibility that the synthesis and/or release of EDRF from the endothelium also could have been influenced. To test for this possibility, aortic smooth muscle reactivity and the release of EDRF were determined. There was a small but significant difference in the contraction response to submaximal concentrations of phenylephrine (1 μM) between the 10% butter diet (2.73 ± 0.08 g, n=6) and the 50% butter diet (3.09 ± 0.07 g, n=6, $p<0.02$) (Fig. 3). In the 50% butter diet, the maximum relaxation to acetylcholine was significantly less (1.08 g) than that on the 10% butter diet (1.62 g). In percentage terms, these relaxations were 34.9% and 59.3%, respectively. The sensitivity EC_{50} of the two diet groups to acetylcholine was similar (EC_{50}: 10% butter diet = 0.21 μM, 50% butter diet = 0.33 μM).

DISCUSSION

The major finding in this study was that increasing the level of butter in the diet resulted in a progressive reduction in PGI_2 production in aorta and mesenteric

TABLE 4

The Effects of Butter-enrichment on the Fatty Acid Composition of Plasma Phospholipids

Fatty acid	Percentage distribution of fatty acids (Mean ± SEM) Diet 10% n=11	30% n=9	50% n=10	Statistical comparisons 10% vs 30%	10% vs 50%	30% vs 50%
16:1	24.6 ± 0.6	23.1 ± 1.0	22.6 ± 0.7	—	—	—
16:1	1.4 ± 0.2	1.1 ± 0.2	0.9 ± 0.2	—	—	—
18:0	20.3 ± 0.6	23.2 ± 0.9	23.2 ± 0.7	0.05	0.01	—
18:1	9.4 ± 0.2	10.8 ± 0.7	13.5 ± 0.9	—	0.001	0.05
18:2n-6	13.3 ± 0.3	13.6 ± 0.4	11.9 ± 0.4	—	0.05	0.05
20:3n-9	0.7 ± 0.06	1.2 ± 0.09	1.9 ± 0.1	0.001	0.001	0.001
20:3n-6	0.9 ± 0.08	1.2 ± 0.09	1.6 ± 0.05	0.05	0.001	0.01
20:4n-6	23.4 ± 0.4	17.3 ± 0.9	12.3 ± 0.7	0.001	0.001	0.001
20:5n-3	0.2 ± 0.03	0.9 ± 0.08	2.6 ± 0.3	0.001	0.001	0.001
22:5n-3	0.6 ± 0.06	0.9 ± 0.1	1.5 ± 0.09	—	0.001	0.01
22:6n-3	5.2 ± 0.3	6.7 ± 0.4	8.0 ± 0.4	0.01	0.001	0.05

TABLE 5

The Effects of Lard-enrichment on the Fatty Acid Composition of Plasma Phospholipids

Fatty acid	Percentage distribution of fatty acids (mean ± sem) Diet 10% n = 4	30% n = 4	50% n = 4
16:0	24.1 ± 1.9	21.3 ± 2.0	23.5 ± 2.2
16:1	1.0 ± 0.2	0.5 ± 0.08	0.4 ± 0.2
18:0	18.4 ± 1.5	21.2 ± 2.2	20.0 ± 0.6
18:1	8.2 ± 0.5	5.9 ± 1.0	9.9 ± 0.8
18:2n-6	12.8 ± 0.7	11.4 ± 0.7	13.7 ± 1.4
20:3n-9	0.5 ± 0.07	0.5 ± 0.03	0.5 ± 0.05
20:3n-6	0.5 ± 0.08	0.7 ± 0.03	0.9 ± 0.09
20:4n-6	29.6 ± 1.9	31.9 ± 2.8	25.7 ± 1.8
20:5n-3	0.1 ± 0.02	0.3 ± 0.09	0.6 ± 0.2
22:5n-3	0.5 ± 0.07	0.7 ± 0.1	0.7 ± 0.2
22:6n-3	4.4 ± 0.7	5.6 ± 0.8	4.1 ± 0.7

artery in vitro. This effect on arterial PGI_2 production was accompanied by profound changes in the fatty acid composition of plasma phospholipids which were also evident but less marked in aortic phospholipids. The proportion of AA fell markedly as the level of butter in the diet increased, whereas the proportion of EPA and eicosatrienoic acid (20:3n-9) rose. The reductions in arterial PGI_2 production can be explained in terms of these fatty acid changes: the fall in the concentration of its precursor (AA) in arterial wall phospholipids, the rise in the concentration of EPA (an inhibitor of cyclooxygenase [15]), or a combination of both.

Substitution of lard for butter as the major source of fat in the diet did not result in any changes in fatty acid composition of plasma, aortic or liver phospholipids, nor was it associated with any reduction in arterial PGI_2 production. This latter result provides good evidence that the progressive reduction in arterial PGI_2 production in the butter–fed rats was not due to the increasing proportion of dietary fat per se. It also raises a number of questions.

Were the different responses to enrichment of the diet with butter or lard due to the different P/S ratios of the diets? The P/S ratios of the 10% fat diets were quite similar regardless of whether lard or butter were used (0.54 and 0.56, respectively). However, as the fat content of the diet was increased to 30% and 50% the P/S ratio fell much more with butter (0.10 and 0.05) than it did with lard (0.24 and 0.19). It is possible that both the concentrations and the relative proportions of particular polyunsaturated fatty acids (PUFA) are critical factors in determining the changes in phospholipid fatty acid composition and the consequent physiological responses.

What was the source of the increased concentration of EPA in plasma and tissue phospholipids of the butter–fed rats? It is unlikely to have come from the butter itself because the only PUFA we could detect in butter were linoleic and linolenic acids. A more likely explanation could be that the ratio of linoleic acid/linolenic acids was much lower in the butter than the lard diets. In the 50% fat diets, this ratio was 9:1 with lard and only 2:1 with butter. Since linolenic acid is a more effective competitor for the desaturase enzymes than linoleic acid in the rat (16), the lower linoleate/linolenate ratio in the butter diets may have facilitated the synthesis of EPA at the expense of AA.

Studies on the effects of different types of dietary fat on arterial PGI_2 production in the rat and rabbit have yielded conflicting results (17–20). Not unexpectedly, essential fatty acid deficiency reduced aortic PGI_2 production (17). Diets enriched with linoleic acid (the precursor of AA) have not been associated with enhanced PGI_2 production but rather a reduction (18,19). Lard supplementation has been reported not to change (20) or to increase (19) basal aortic prostacy-

TABLE 6

The Effects of Butter-enrichment on the Fatty Acid Composition of Aortic Phospholipids

Percentage distribution of fatty acids (Mean ± sem)				Statistical comparisons		
Fatty acid	Diet					
	10% n=9	30% n=8	50% n=9	10% vs 30%	10% vs 50%	30% vs 50%
16:0	23.1 ± 0.8	22.7 ± 0.9	23.1 ± 1.1	—	—	—
16:1	2.9 ± 0.4	2.5 ± 0.5	3.0 ± 0.7	—	—	—
18:0	17.4 ± 0.9	18.5 ± 1.1	16.7 ± 1.4	—	—	—
18:1	15.0 ± 0.5	16.1 ± 0.3	16.7 ± 0.3	—	0.05	—
18:2n-6	6.0 ± 0.3	5.6 ± 0.2	5.2 ± 0.3	—	—	—
20:3n-9	1.5 ± 0.09	1.4 ± 0.2	2.0 ± 0.1	—	0.01	0.01
20:3n-6	1.8 ± 0.07	1.9 ± 0.09	1.6 ± 0.07	—	—	—
20:4n-6	23.9 ± 0.5	22.4 ± 0.8	21.1 ± 0.8	—	0.01	—
20:5n-3	0.5 ± 0.05	0.9 ± 0.2	2.2 ± 0.1	0.01	0.001	0.001
22:4n-6	3.0 ± 0.2	2.7 ± 0.2	1.9 ± 0.1	—	0.001	0.001
22:5n-6	0.8 ± 0.04	0.8 ± 0.09	0.5 ± 0.03	—	0.001	0.001
22:5n-3	1.0 ± 0.07	1.4 ± 0.1	2.2 ± 0.1	0.01	0.001	0.001
22:6n-3	3.1 ± 0.1	3.1 ± 0.2	3.8 ± 0.3	—	—	—

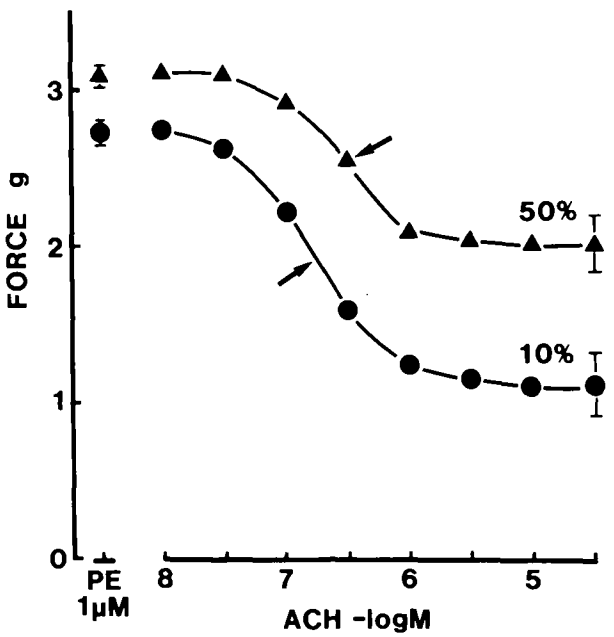

FIG. 3. Average acetylcholine-relaxation curves in six rings precontracted by phenylephrine (1 μM). The isometric force (g) is calculated as the change in the active force after the initial stretch-force procedure. The aortas were removed from rats on the 10% butter diet (•) or 50% butter diet (▲). Error bars shown are ± 1 SEM.
PE, phenylephrine 1 μM. ACH, acetylcholine. EC_{50} values are indicated by arrow.

clin production and to stimulate PGI_2 synthesis from exogenous AA (20). In contrast to the results of the present study, Galli and coworkers reported increased aortic PGI_2 production in rabbits red diets containing 25% energy as butter (19). It is possible that the variable effects of dietary saturated fat on aortic prostacyclin production in the rat that have been reported in the literature (18–21) also may be due to variations in the fatty acid composition of the rat chows used for the control diets. We have observed wide variations in plasma phospholipid fatty acid composition between rats fed different batches of chow (purchased from the same supplier), which is the reason we now prepare all diets in the laboratory. In the few instances in which effects of the diets on the fatty acid composition of aortic phospholipids have been measured, any changes in PGI_2 production have been mirrored by changes in aortic phospholipid AA content. When AA content increased (19,20) so did PGI_2 production and vice versa (17,22). This highlights the importance of measuring effects of diets on phospholipid fatty acid composition. In this study, changes in plasma phospholipid fatty acid composition were similar but more pronounced than those in the aorta.

Sinzinger and coworkers have suggested that the relative resistance of rats to the dietary induction of atherosclerosis may be related, at least in part, to their particularly high capacity for arterial PGI_2 synthesis relative to man, rabbit and a series of other animals that were tested (23). This characteristic, in turn, may be related to the high levels of AA in rat arterial phospholipids (20). It also is possible that the negligible thromboxane production by platelets after stimulation with ADP and thrombin also could contribute to this resistance to vascular disease.

Although the butter-enriched diets had clear-cut effects on the fatty acid composition of plasma and tissue phospholipids that correlated well with reductions in arterial PGI_2 production in vitro, there were no significant effects on ADP or thrombin-induced platelet aggregation. It is possible that the diet period (three weeks) was not sufficiently long for any such effects to be evident (24). Longer feeding periods

should clarify this question. Similarly, TXA_2 production by platelets in vitro was not affected by increasing levels of either type of fat in the diet. Indeed, TXA_2 production by platelets after stimulation with ADP and thrombin generally was insignificant, i.e. not significantly greater than that generated by the PPP "blank."

These results also may shed light on the observation of Morita and coworkers that dietary supplementation with EPA inhibited platelet aggregation in rats only when the background diet had been enriched with butter (25). The mechanism by which butter–enrichment unmasked this effect of EPA may be related to effects on the fatty acid composition of platelets. Presumably, the butter, being particularly low in PUFA and with an unusually low linoleate/linolenate ratio. would have been associated with reduced AA synthesis and thereby have facilitated the incorporation of the EPA dietary supplement into platelet phospholipids, resulting in reduced platelet aggregation. The data from the present study suggest that there is enrichment of tissue phospholipids with EPA even in the absence of supplementation.

In view of the changes in aortic phospholipid fatty acid composition on the butter–enriched diet, the possibility that alterations in membrane lipids could affect the synthesis and/or the release of EDRF from the endothelial cell or even the membrane receptor for the EDRF releasing agonist such as acetylcholine was considered. A very recent paper (26) has identified EDRF as nitric oxide (NO), at least in bovine endothelial cells stimulated by bradykinin. EDRF is a highly labile (27) nonprostanoid factor released from endothelial cells that has a powerful vasodilatory action on the underlying smooth muscle in response to some vasoactive substances such as acetylcholine, substance P, bradykinin and ATP (28). Because in the rat aorta (as in other species) the relaxation response to acetylcholine completely is endothelium-dependent (29), the reactivity to acetylcholine can be used to assess the entire process from EDRF release to smooth muscle relaxation. The 50% butter diet attenuated the magnitude of the EDRF-mediated response range suggesting either a reduction in the EDRF synthesis or release, or in smooth muscle responsiveness. Our experiments cannot distinguish between these possibilities. The sensitivity (ED50) was altered little, suggesting that the acetylcholine receptor coupling probably was unaffected by the dietary change. These changes to the EDRF activity may have been related to the marked changes in the PUFA composition of aortic phospholipids. However, the marked reduction in prostacyclin release measured in the separate series of experiments could not have been responsible for these changes in EDRF response since indomethacin was present throughout these latter experiments.

In conclusion, these results provide strong evidence that in the rat (i) dietary saturated fat in the form of butter can reduce the concentration of AA in plasma and tissue phospholipids, (ii) vascular PGI_2 production appears to be modulated directly by the level of AA in arterial wall phospholipids and/or inversely by the level of EPA, (iii) changes in plasma phospholipid fatty acid composition appear to be an accurate reflection of changes in arterial wall phospholipid fatty acid composition, (iv) these changes in aortic phospholipid fatty acid composition and PGI_2 production were accompanied by a reduction in smooth muscle relaxation to EDRF. Finally, butter-supplementation may provide an ideal model in which to examine the effects of particular PUFA supplements on vascular PGI_2 production in the rat.

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