BIOMEDICAL AND ENVIRONMENTAL SCIENCES **16**, 333-339 (2003)

Diet Rich in Saturated Fat Decreases the Ratio of Thromboxane/prostacyclin in Healthy Men[1]

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Objective To investigate the effect of dietary saturated fat (SFA) from animal sources on the urine excretion 11-dehydro thromboxane B_2 (TXB_2) and 6-keto prostaglandin F 1α (PGF 1α) in 27 healthy free-living male subjects aged 30 to 55 years. **Methods** It was a randomized crossover design. Each volunteer was randomly assigned to one of the two diets (high fat and low fat) for a period of 4 weeks, after which each subject resumed his usual diet for 2 weeks as a 'wash-out period', before being assigned to the other diet for an additional 4 weeks. **Results** Serum proportion of 20:4n-6 was 5% lower in the high fat (6.2% of total fatty acid) than in the low fat diet (6.5% of total fatty acid), which was associated with a significantly decreased ratio of the urinary excretion 11-dehydro TXB_2 to 6-keto PGF 1α ($P<0.05$). However, there was no significant fall in the absolute urinary excretion of 11-dehydro TXB_2. **Conclusions** Diet rich in SFA from animal sources may influence TXA_2 formation via effect on tissue proportion of 20:4n-6.

Key words: Thromboxane A_2; Prostacyclin I_2; Crossover; Saturated fat; Fatty acids

INTRODUCTION

Thrombus formation may be an integral part of atherosclerosis and the acute event, which leads to a myocardial infarction or sudden cardiac death. The thrombi formation is initialed by platelet aggregation. The ratio of thromboxane A_2/prostacyclin I_2 (TXA_2/PGI_2) plays a critical role in platelet aggregation[1]. TXA_2 and PGI_2 are biosynthesized from arachidonic acid (AA) through the cyclooxygenase pathway in the platelet membrane and arterial endothelial cells, respectively[2,3]. Any factors which affect the balance of TXA_2/PGI_2 in favour of PGI_2 should reduce the risk of thrombosis, whereas factors altering the balance in favour of TXA_2 should increase thrombosis tendency.

Evidences from dietary intervention studies have proved that the ratio of TXA_2/PGI_2 is decreased by marine omega-3 polyunsaturated fatty acid (n-3 PUFA) in humans and in animals[4,5] and by plant n-3 PUFA alpha-linolenic acid in both humans and animals[6,7].

[1] This project was supported by Department of Veterans Affairs, Australia.

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0895-3988/2003
CN 11-2816

Kelly *et al.* reported that diet enriched in 16:0 resulted in an increased *ex vivo* collagen and ADP induced whole blood platelet aggregation when compared with 18:0 enriched diet[8]. However, there are no data on the relation between dietary saturated fat from animal sources and the biosynthesis of TXA_2 and PGI_2 in the literature. The aim of the present study was to investigate the effect of dietary saturated fat from animal sources on urine stable metabolites of TXA_2 and PGI_2, 11-dehydro thromboxane B_2 (TXB_2) and 6-keto prostaglandin F 1α (PGF 1α)[9].

METHODS AND MATERIALS

Subjects and Study Design

The study protocol was approved by Deakin University Ethics Committee, and an informed written consent was obtained from each volunteer. Thirty-three healthy, free-living males aged 30 to 55 years were recruited through newspaper advertisements. Exclusion criteria for this study were: individuals with symptoms or prior diagnosis of cardiovascular and renal diseases, diabetes, or other chronic diseases, who were on long-term medications, athletes who trained regularly for competitive sports and whose alcohol consumption exceeded 10 percent of daily energy intake.

Prior to the commencement, participants were given detailed instructions on the diets to be consumed, and on how to accurately accomplish a weighed food record. Each subject was provided with a calibrated digital weighing scale (accurate to 1 gram), together with standard household measuring devices such as cups and spoons. Each volunteer was asked to complete a 4-day weighed diet record, including 2 weekend days, on their usual diets. The results of the initially weighed food record were used to calculate the daily energy allowance for each individual. The habitual diet and usual food choices of each individual were also considered in the planning of the diets to enhance compliance. The diets in this study were carefully calculated and planned by a dietitian.

A randomized crossover design was used to compare the effects of two diets. Each volunteer was randomly assigned to one of the two diets for a period of 4 weeks, after which each subject resumed his usual diet for 2 weeks as a 'wash-out period', before being assigned to the other diet for an additional 4 weeks. The two diets were designed to provide similar amounts of energy, protein, dietary fiber, and alcohol, differing only in the amount of fat. The high fat (HF) diet was designed to provide 10%-15% more energy from animal fat compared to the low fat (LF) diet. The HF diet provided approximately 42%-45% of energy from fat (22%-25% saturated fat) of full fat dairy products, and specially prepared biscuits containing lard. Butter, margarine, and lard-containing biscuits were provided free to each subject. The LF diet provided approximately 22%-25% of energy from fat (8% from saturated fat), and included low fat milk, cheese, yogurt, and monounsaturated margarine. The two diets were made isoenergetic by providing a greater amount of carbohydrate (55%-60% of total daily energy) during the LF diet period in the form of refined cereals, white bread, pasta, and sugar-containing beverages.

Both diets included 130 grams (raw weight) of very lean red meat each day, with a choice of beef or lamb. All meat consumed in the study was purchased from a single source (Top Cut Food Industries Pty., Ltd., Melbourne, Australia). The portion sizes of meat were pre-weighed and individually packed, and provided free of charge to each subject. Meals were prepared by the subjects and consumed at home. In addition to the dietary instructions, subjects were also asked to keep their physical activity pattern as similar as possible during

the two diets periods. The subjects were contacted weekly during the study to monitor compliance and to provide dietary counseling. On the last week of each diet, the subjects were instructed to accomplish a 7-day weighed diet record. All diet records were analyzed using FoodWorks version 1.2 (Xyris Software Pty. Ltd., Highgate Hill, Queensland, Australia), a dietary analysis software with nutrient composition data of Australian foods[10].

The height and weight of each subject were measured at the commencement of the study and after each diet period, and the body mass index (BMI) was calculated. Venous blood samples were collected into plain vacutainer tubes for the collection of serum, prior to the study and on two occasions three days apart at the end of each diet. Blood samples were collected after an overnight fast between 07:00 and 09:30. Blood samples were stored at −80℃ for later analysis.

Serum Fatty Acids

Serum lipids from 12 randomly selected subjects were extracted by chloroform: methanol (1:1, v/v) containing 10mg/L of butylated hydroxytoluene (Labco, VIC Australia), and 10 mg/L of C17:0 triacylglycerol (triheptadecanoin). Methyl esters of fatty acids of serum lipids were prepared by saponification using 0.68 mol/L KOH in methanol followed by transesterification with 14% BF_3 in methanol. Methyl esters of fatty acids were separated by gas chromatography as described by Sinclair *et al.*[11].

Urine Concentrations of 11-dehydro Thromboxane B_2 and 6-keto Prostaglandin F 1α

Twenty-seven subjects collected their 24-h urine on the last day of each of the diets. The samples were stored at -20℃ for later analysis. The concentrations of 11-dehydro TXB_2 and 6-keto PGF 1α in the urine were determined by using an enzyme immunoassay (EIA) method with commercially available EIA kits (Cayman Chemical Company, MI, USA) as described elsewhere[12].

Statistical Analyses

All data analyses were performed using the Statistical Package for the Social Sciences version 8.0 (SPSS Inc. Chicago, IL, USA). The general linear model (GLM) was used to compare the results at the end of the two diet periods, taking carry-over effects into consideration[13]. The values were expressed as $\bar{x}\pm s$ in all the results Tables. *P* values were two-sides, and <0.05 was considered as significant.

RESULTS

Thirty-three subjects were enrolled in the study, with a mean age of 41.2±7.8 years and a mean BMI of 26.5±3.0 kg/m^2 at baseline. However, only 27 subjects were included in the final results because six subjects did not collect urine at the end of both dietary periods. The mean daily intakes of total fat, saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) expressed as gram and percentage of total energy and cholesterol were significantly higher in the HF dietary period than in the LF dietary period ($P<0.01$). Compared with HF, mean daily intakes of carbohydrate and the ratio of PUFA to SFA were significantly higher in the LF dietary period (Table 1).

Serum proportion (% of total fatty acid) of total SFA, total n-6 PUFA, 14:0, 18:0, 20:0 and 18:1 were significantly higher, and 18:3n-3, 22:5n-3, total n-3 and the ratio of n-3 to n-6 were significantly lower in the HF dietary period than in the LF dietary period ($P<0.05$).

Serum proportion of 20:4n-6 was higher in the LF diet (6.5% of total fatty acid) than in the LF diet (6.2% of total fatty acid) (P=0.06) (Table 2).

TABLE 1

Nutrient Intake During the Low Fat and High Fat Diets (n=27)

	Low Fat Diet	High Fat Diet
Energy (MJ)	9.1 ± 1.6	9.3 ± 1.7
Protein (g)	87.4 ± 9.9	99.0 ± 12.3
Fat (g)	59.9 ± 9.1	103.3 ± 17.8
SFA (g)	19.3 ± 3.2	50.2 ± 8.7[a]
MUFA (g)	23.6 ± 3.3	30.8 ± 10.6[b]
PUFA (g)	12.5 ± 3.4	11.9 ± 3.0
P: S Ratio	0.7 ± 0.2	0.2 ± 0.03[b]
Carbohydrate (g)	307.7 ± 84.9	226.9 ± 47.7[b]
Cholesterol (mg)	138.0 ± 18.5	341.6 ± 75.0[b]
Fibre (g)	28.6 ± 6.4	27.9 ± 5.7
Alcohol (g)	5.1 ± 6.6	5.4 ± 7.4
Protein (% energy)	16.7 ± 1.9	16.5 ± 1.2
Carbohydrate (% energy)	55.0 ± 3.3	38.9 ± 2.9[b]
Fat (% energy)	24.5 ± 2.2	41.3 ± 2.7[b]
SFA (% energy)	8.5 ± 1.1	21.6 ± 1.6[b]
MUFA (% energy)	10.4 ± 1.2	14.5 ± 1.2[b]
PUFA (% energy)	5.5 ± 1.1	5.1 ± 0.8
Alcohol (% energy)	1.6 ± 1.9	1.5 ± 2.0

Note. Values are $\bar{x}\pm s$. Significantly differences: [a]: $P<0.05$, [b]: $P<0.01$ compared with low fat diet. SFA, saturated fat; MUFA, monounsaturated fat; PUFA, polyunsaturated fat; P:S ratio, polyunsaturated to saturated fat ratio.

TABLE 2

Serum Fatty Acid Composition (Percentage of Total Fatty Acid) During the Low Fat and High Fat Diets (n=12)

Fatty Acid	Low Fat Diet	High Fat Diet
14:0	1.5 ± 0.3	1.8 ± 0.5[a]
16:0	22.4 ± 2.2	22.9 ± 2.1
18:0	6.2 ± 0.5	6.7 ± 0.5[a]
20:0	0.4 ± 0.1	0.5 ± 0.1[b]
Total SFA	30.4 ± 2.3	31.9 ± 2.5[b]
16:1	2.3 ± 0.6	2.5 ± 0.9
18:1	23.4 ± 2.0	22.5 ± 2.1[b]
Total MUFA	25.7 ± 2.4	25.0 ± 2.8
18:2n-6	25.2 ± 3.3	25.9 ± 4.0
20:3n-6	1.6 ± 0.2	1.6 ± 0.2
20:4n-6	6.5 ± 1.6	6.2 ± 1.4
22:4n-6	0.3 ± 0.1	0.2 ± 0.1
22:5n-6	0.1 ± 0.1	0.1 ± 0.0
Total n-6	33.8 ± 4.5	34.0 ± 4.8
18:3n-3	0.9 ± 0.3	0.6 ± 0.1[b]
20:5n-3	0.9 ± 0.2	0.9 ± 0.2
22:5n-3	0.8 ± 0.2	0.7 ± 0.1[a]
22:6n-3	1.5 ± 0.3	1.4 ± 0.2
Total n-3	4.2 ± 0.6	3.5 ± 0.4[b]
n-3/n-6	0.12 ± 0.02	0.10 ± 0.01[b]
Total PUFA	37.9 ± 4.6	37.6 ± 5.0

Note. Values are $\bar{x}\pm s$. Significantly differences: [a]: $P<0.05$, [b]: $P<0.01$ compared with low fat diet. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

Serum concentrations of total and LDL cholesterol were significantly higher in the HF diet compared with in the LF diet. There was no significant difference in serum HDL cholesterol and triacylglycerol concentrations between two diets.

The concentrations of daily urine excretion of 11-dehydro TXB_2 and 6-keto PGF 1α are shown in Figs. 1 and 2. Mean daily urine excretion of 11-dehydro TXB_2 was 903±65 ng/day and 1007±63 ng/day, 6-keto PGF 1α was 377±37 ng/day and 360±32 ng/day for the HF diet and LF diet, respectively. Ratio of 11-dehydro TAB_2 to 6-keto PGF 1α in urine was significantly lower in the HF dietary period (2.7±0.2) than in the LF dietary period (3.1±0.3) (Fig. 2).

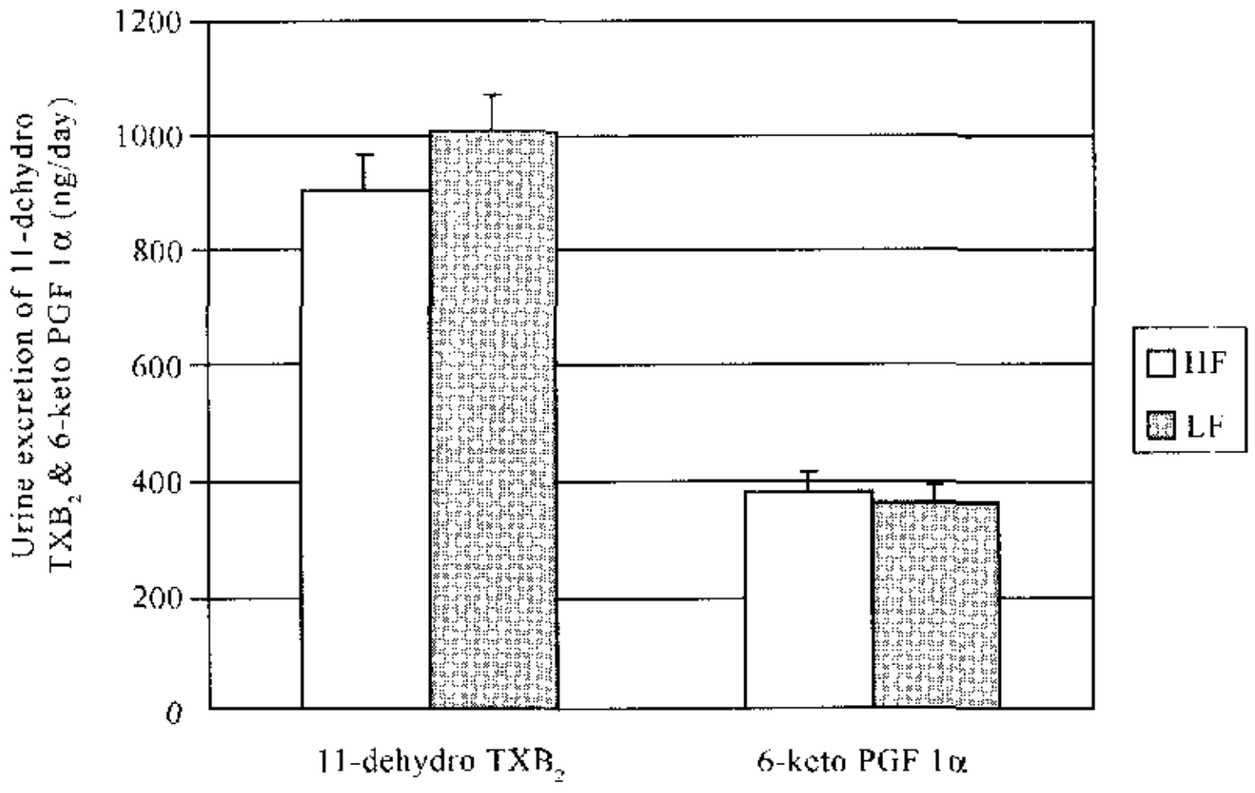

FIG. 1. The concentrations of daily urine excretion of 11-dehydro thromboxane B_2 and 6-keto prostaglandin F 1α (ng/day).

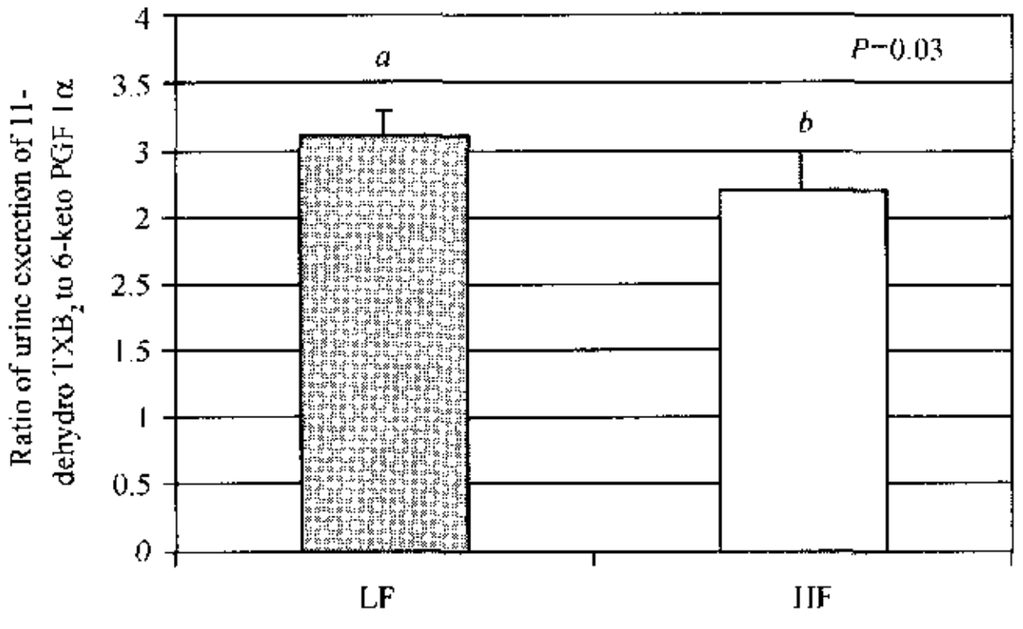

FIG. 2. Ratio of urine excretion of 11-dehydro TAB_2 to 6-keto PGF 1α.

DISCUSSION

Effect of n-3 and n-6 PUFA on TXA_2 and PGI_2 has been well documented in both

human and animal studies. However, there are no data on the effects of the diet rich in saturated fat from animal sources in the ratio of urine stable metabolites of TXA_2/PGI_2 in literature. We have measured fatty acid composition in serum as a marker of dietary individual fatty acid intake, since a comprehensive database on the individual fatty acid content of foods is not available in Australia.

In the present study, HF diet resulted in a 10% decreased 1-dehydro TXB_2 (P=0.09) and 13% decreased ratio of urine excretion of 11-dehydro TXB_2 to 6-keto PGF 1α, which was associated with a decreased proportion of 20:4n-6 by 5% in serum (P=0.06) compared with the LF diet (P=0.03). Decreased ratio of urine excretion of 11-dehydro TXB_2 to 6-keto PGF 1α may also be caused by increased 18:0 intake in HF diet since high 18:0 diet has been proposed to influence TXA_2 biosynthesis in platelet[8].

TXA_2 is formed in platelets and it is a potent cellular regulatory agent with a strong platelet-aggregating activity[2] and is also a potent vasoconstrictor[14]. TXA_2 can be broken down nonenzymatically ($t_{1/2}$= 3 minutes) into TXB_2, a stable metabolite[9]. PGI_2 is formed in vascular endothelial cells[3]. PGI_2 is released by endothelium and it only affects the local environment. It is a powerful vasodilator on the abluminal side of vessels and inhibits platelet aggregation on the luminal side[15]. PGI_2 is hydrolyzed nonenzymatically ($t_{1/2}$ = 3 minutes) to 6-keto-PGF1α[9].

Biosynthesis of TXA_2 can be interfered with by long chain n-3 PUFA. Dietary n-3 PUFA can be incorporated into platelets, where they compete with AA for the 2-acyl position of membrane phospholipids[16]. Numerous dietary intervention studies have found that TXA_2 production is more sensitive to alteration to the diet compared with PGI_2[4,17,18]. The ratio of urine excretion 11-dehydro TAB_2 to 6-keto PGF 1α was decreased by 20% when 34 healthy men aged 24 to 57 had supplementary fish oil at 15 g/d for 10 weeks compared with placebo (48% of lard, 40% beef tallow and 12% of corn oil)[19]. Daily urine excretion of 11-dehydro TAB_2 was reduced by 14%, while 6-keto PGF 1α was decreased only by 2% when 25 healthy subjects (male 12, female 13) aged 22 to 52 years consumed an average 133 g raw Atlantic salmon per day for two weeks compared with after one-week vegetarian diet[17]. When 8 healthy male volunteers aged 20 to 40 years consumed a high-DHA diet containing 6 g/d of DHA for 120 days, 11-dehydro TAB_2 was decreased by 35%, while 6-keto PGF 1α was decreased only by 8% compared with the control diet with trace amounts of DHA (n=4)[18].

In conclusion, the present results indicate that high SFA intake from animal fat results in a decreased serum proportion of 20:4n-6 and increased 18:0, which is associated with a significantly decreased ratio of the urinary excretion of 11-dehydro TXB_2 to 6-keto PGF 1α. In addition, high SFA intake is associated with an increase in other usual factors such as serum total and LDL-C. Based on the present results, we do not suggest that diet rich in SFA from animal sources has a favourable effect on thrombosis tendency, nevertheless, it can be suggested that the population reduce their SFA intake to improve their lipid profile.

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(*Received December* 24, 2002 *Accepted May* 20, 2003)