



浙江省第三届青年学术论坛

THIRD ZHEJIANG YOUTH ACADEMIC FORUM

WHO/FAO 高层专家论坛

“食品安全性、食品科技和食品产业发展国际学术研讨会”论文集

食品安全、营养与发展

EVOLUTION OF FOOD SAFETY AND NUTRITION

主编 励建荣 李铎

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主办

浙江省科学技术协会

WHO/FAO 食品营养专家组会议

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浙江省第三届青年学术论坛

——食品安全性与食品科技、产业发展国际学术研讨会

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浙江省第三届青年学术论坛论文集

浙江省青年学术论坛组委会编

单委会委员 一

浙江省第三届青年学术论坛论文集

——食品安全、营养与发展

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前 言

“浙江省青年学术论坛”是浙江省科学技术协会主办的高层次、大规模、综合性学术交流活 动。她面向广大青年科技工作者,依托所属 140 多个省级自然科学学会(协会、研究会),旨在营造贯彻“双百”方针、倡导创新文化、宏扬科学精神、符合时代潮流的学术环境和学术气氛,推动浙江省新世纪人才工程建设、文化大省建设和科教兴省。

本届学术论坛已是第三届。“食品安全性、食品科技和食品产业发展国际学术研讨会”作为分论坛暨 WHO/FAO 高层专家论坛,是继 2000 年首届学术论坛——“21 世纪食品与生物技术发展研讨会”后又一次与食品相关的高层学术会议。会议由浙江省科学技术协会、WHO/FAO 食品营养专家组主办,中国食品科学技术学会、浙江省食品学会、杭州商学院、浙江省农学会、浙江省预防医学会、浙江省生物工程学会、浙江省环境科学学会、浙江省营养学会、浙江省畜产品技术协会、浙江省植物保护学会、浙江省土壤肥料学会等 11 家单位联合承办。会议得到了各界人士的关注。FAO(联合国粮农组织)食品质量、安全与营养中心主任、国际营养科学联盟主席 Mark L Wahlqvist 教授、FAO/WHO 食品营养专家组成员 Gayle S Savige 博士、Naiyana Tikky Wattanapenpaiboon 博士、Robert Premier 博士、李铎(澳籍)博士等到会并作专题发言。会议还邀请了美国 Auburn 大学 Peggy Hsieh 教授和英国 Cranfield 大学 Anwar Haque 博士前来参会。本次会议共有来自世界和全国各地的专家、学者近 200 人参加。会议于 2002 年 10 月 23—24 日在杭州商学院举行。

本次会议多行业、多学科 的参与,正体现了会议的主题——食品安全、营养问题所涉及面之广,与各学科关系之密切。食品安全与营养是食品科学永恒的主题,它与环境质量、种养技术、生物技术、加工技术、检测技术以及卫生管理、疾病预防等密切相关。同时,食品安全与营养又是一个超越国界的全球性问题,它的意义不仅在于卫生和健康,还在于对经济的影响,特别是一些发达国家将其充当贸易保护的技术壁垒后,对已加入 WTO 的中国影响巨大。

本次会议共收到代表提交的论文近 100 篇。内容涉及食品科学与营养、食品安全、食品技术与产业发展等。特别是食品营养与人类健康、农业与食品安全、现代生物技术与食品安全、食品检测等方面的相关内容,反映了目前国际、国内在食品安全、营养等方面的研究进展和发展趋势。经过论文集编委会的认真审核与筛选,选择其中 87 篇入编论文集——《食品安全、营养与发展》;并根据来稿的内容和性质,分别编入“食品科学与营养”、“食品安全”、“食品技术与产业”三个篇章。

限于时间和水平,在编撰中难免有遗漏和错误,敬请各位作者和读者原谅!

编 者

2002 年 9 月于杭州

目 录

第一部分 食品科学与营养

□ Biosynthesis of 2-series Eicosanoids Influenced by Animal Fat Intake in Healthy Men	Duo Li(3)
□ Systematic Evaluation of the Functionality of New Lines of High Oleic Canola Oil in Deep Frying Applications	Xin-Qing Xu(9)
□ Physiological and Quality Responses of Postharvest Litchi Fruit to High O ₂ Atmospheres	Yueming Jiang 等(12)
□ 功能性食品的科学依据和全球观点	钱伯初 王 茵(17)
□ HPLC/MS 法分析柑桔皮中的柠檬苦素类似物配糖体	张 虹 张艳萍(22)
□ 青菜色泽及叶绿素含量无损伤检测的研究	王向阳 何 爽(26)
□ 中草药添加剂对猪肌肉相关酶活性、肌纤维特性及肉质的影响	韩剑众(33)
□ 共轭亚油酸产生菌株的筛选	赵广生等(37)
□ 光照对粉葛细胞的生长及次生代谢的影响	项雷文 赵 瑜(41)
□ 冷却肉气调保鲜的研究	蒋予箭 周小平(45)
□ 大麦麦叶中活性物质抗氧化能力的研究	许 钢(50)
□ 羊栖菜多糖的单糖组成及其含量的 GC 分析	张艳萍等(55)
□ 不同来源大豆(粕)的蛋白质特性及对小猪生长的影响	韩剑众等(59)
□ 壳聚糖——魔芋复合涂膜保鲜青虾的研究	沈月新等(63)
□ 各品种大麦叶生长过程中生物活性物质含量动态研究	许 钢等(67)
□ 羊栖菜抗氧化性质研究	张燕平等(71)
□ 紫苏清除自由基作用的研究	张燕平(76)
□ 论有机食品的发展	励建荣 张 晶(83)
□ 芥菜的营养保健作用与开发利用	唐晓珍等(88)
□ 生物合成虾青素	周 丽等(92)
□ 双歧杆菌耐驯化方法比较	陈荷凤等(96)
□ 茶多糖的提取工艺及其保健作用的研究进展	陈建国(99)
□ 鱼肉弹性测定方法的研究	陈立冬等(102)
□ 无花果保健茶的研制与功效	王宗道 东 阳(106)
□ 淀粉基生物降解塑料生物降解性研究	马 涛 刘长江(109)
□ 植酸的检测及其在食品中的应用	苗 颖 马 莺(118)
□ 发酵工程与功能食品	钱 方 姬德衡(122)
□ 栅栏技术及其在食品加工与保藏中的应用	钮昆亮(126)

第二部分 食品安全

- Current insight: Effect of Omega-3 Fatty Acid on Human Health Li Duo(133)
- The Principle of Salmonellae Contamination in Poultry Meat and Methods to Control in Processing Zhuang Rong-yu Huang Yao-wen(141)
- Rapid and Accurate Tests for the Assessment of Frying Oil Quality Xin-Qing Xu(147)
- 我国水产品安全质量问题的成因及其解决办法初探 顾振宇等(153)
- 农业环境质量与农产品安全 杨肖娥等(156)
- 食源性疾病与食品安全 蔡成岗 励建荣(164)
- 转基因食品安全性的分子解决方案:外源基因敲除技术 徐茂军(168)
- Estimation of the Degradation Rate of Insecticides on Tea Plants Huilong Xia(172)
- 酵母属生物制剂对果蔬采后病害形成的影响 孙 萍 郑晓冬(175)
- 杭州市区蔬菜基地蔬菜重金属含量研究 焦 荔等(180)
- 食品微生物快速检测技术及其自动化研究进展 张志洁 韩剑众(183)
- 果蔬抗氧化活性的研究 田迪英等(188)
- 禽肉软罐头生产线的微生物调查与控制点分析 赵 颖等(193)
- 转基因食品安全性评价和安全保障 王 扬等(198)
- 食品中细菌检验国标法急需改进 刘坚真等(202)
- 花卉食品的化学组成及花饮料的质量问题 陈洁迪(204)
- 开展绿色品牌建设全面提高“山水”果品安全质量和市场竞争力 汤虹美等(210)
- 浅析我国食品添加剂的安全性问题 朱学思(214)
- 100 家乡村饮食店卫生安全状况调查结果分析 沈心钿(217)
- 关于海通出口毛豆产品农残检测中发现的问题及探讨 陈亦辉等(219)
- 土壤、肥料与农产品安全生产 谢锦良(222)
- 散装冷荤食品卫生调查 姜松法等(226)
- 我国儿童食品的安全现状与管理对策 王 茵(231)

第三部分 食品技术与产业

- Isolation and Identification of Spoilage Fungi from Red Bay Berry Jian-Rong Li et al.(239)
- 魔芋涂膜保鲜草莓的工艺研究 陶宁萍等(246)
- 马铃薯渣提取膳食纤维的研究 赵 萍(251)
- 酶法水解玉米蛋白最佳条件的研究 林 莉 马 莺(255)
- 微胶囊技术在多维预混料中的应用 冯莉萍(259)
- 基因工程技术在食品品质改良中的应用 徐茂军(263)
- 苯甲醛高耐受性酵母菌的选育 梁新乐等(267)
- 保健蔬菜酸奶的加工工艺研究 栾金水 张 刚(270)
- Application of Plasma in Air Pollution Control Engineering Xie Ming(274)
- 高效毛细管电泳法短时间内同时测定咖啡因、山梨酸、苯甲酸、糖精的含量 宋广磊(280)
- 高效液相色谱在栀子成分测定中的近期研究进展 车双辉 杜琪珍(284)
- 青菜热处理保绿机理研究 袁海娜(287)
- 有机茶开发 陆海霞 励建荣(293)

□双歧杆菌豆酸奶的研制	陈荷凤等(299)
□磨菇气调包装系统的研究	雷 桥 徐文达(303)
□姬松茸原生质体制备条件的研究	陈 敏等(307)
□黑豆蛋白肽果汁复合饮料的研制	刘恩岐等(311)
□转基因食品的标识	张 刚(314)
□HACCP 体系在虾仁重组生产中的应用	俞 红等(318)
□山茱萸中总皂甙的提取工艺研究	夏道宗等(321)
□高速逆流色谱分离红曲黄色素	夏 明 杜琪珍(326)
□浅谈我国芦荟产业现状及综合开发的途径	杨远晶 毛杭莉(330)
□方便米饭的种类及 α -化米饭的加工	孟凌华等(334)
□生姜调味酱(姜膏)的研制	田迪英 杨荣华(339)
□浙江省发展有机食品的可行性探讨	楼菊青 马香娟(342)
□肉及肉制品保鲜技术的探讨	徐 波(346)
□浅谈榨菜的加工及包装保存技术	杨 伟(352)
□瓶装饮用纯净水水质净化及运行管理	田迪英(357)
□柑桔深加工技术研究和开发的必要性	陈福青(360)
□山药的综合利用途径浅探	吴增军等(365)
□加快培育和发展食品加工业集聚形态的思考——山核桃加工业发展状况调查	徐 星(368)
□论绿色食品生产的三效合一	林海萍 张立钦(373)
□碎小虾仁重组大虾仁工艺的研究	洪咏平等(377)
□食品企业 CIMS 初探	俞 红(382)
□An Increase in Stability of Ternary Platinum(II) Complexes by the Intramolecular Aromatic-Ring Staking Interaction	Sun Hongliang(384)
□Urban Air Pollution Control and Public Health	Hai-xia Lu Jian-rong Li(389)

Biosynthesis of 2-series Eicosanoids Influenced by Animal Fat Intake in Healthy Men

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第一部分

食品科学与营养

动物脂肪影响人体 2-系列二十烷类的生物合成

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摘要: 在健康男性中, 动物脂肪摄入量对 2-系列二十烷类生物合成 (2-series eicosanoids) 的影响进行了研究。在为期 4 周的实验中, 受试者被分为两组: 高脂肪组 (HF) 和低脂肪组 (LF)。在 HF 组中, 动物脂肪摄入量显著增加, 导致 2-系列二十烷类生物合成率显著增加。相反, 在 LF 组中, 动物脂肪摄入量显著减少, 导致 2-系列二十烷类生物合成率显著减少。这些结果表明, 动物脂肪摄入量对 2-系列二十烷类生物合成具有重要影响。此外, 我们还观察到, 动物脂肪摄入量对 2-系列二十烷类生物合成的影响与血清胆固醇水平密切相关。这些发现对于理解饮食因素对炎症反应和心血管疾病风险的影响具有重要意义。

1. Introduction

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... synthesis of eicosanoids in healthy men. The...
... synthesis is inhibited by plasma aggregation...
... role of cholesterol...
... (Savinick and Sinclair 1993, Sinclair and Savolainen 1993)

Biosynthesis of 2-series Eicosanoids Influenced by Animal Fat Intake in Healthy Men

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Abstract In the present study we investigated the effect of dietary saturated fat (SFA) from animal sources on the urine excretion 11-dehydro thromboxane B₂(TXB₂) and 6-keto prostaglandin F 1 α (PGF 1 α) in 27 healthy aged 30 to 55 years free-living male subjects. Each volunteer was randomly assigned to one of the two diets (high fat (HF) and low fat (LF)) 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 a further 4 weeks. Serum proportion of 20:4n-6 was 5% lower in the HF (6.2% of total fatty acid) than in the LF diet (6.5% of total fatty acid), which was associated with a significantly decreased ratio of the urinary excretion 11-dehydro TXB₂ to 6-keto PGF 1 α ($p < 0.05$). However, there was no significant fall in the absolute urinary excretion of 11-dehydro TXB₂. Diet rich in SFA from animal sources may influence TXA₂ formation via effect on tissue proportion of 20:4n-6.

动物脂肪影响人体 2 - 系列二十烷类的生物合成

李 锋

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摘 要 在本课题中,我们对食物中动物饱和脂肪酸对尿液里 11-dehydro thromboxane B₂(TXB₂) 和 6-keto prostaglandin F 1 α (PGF 1 α)在 27 位年龄在 30 至 55 之间健康男子的排泄进行了研究。我们将志愿者任意分成高脂肪和低脂肪饮食两组共四周,然后志愿者恢复他们的习惯饮食两周作为“洗脱期”,再吃四周的另一种饮食。血清花生四烯酸的比例在高脂肪饮食中比在低脂肪饮食中低 5%,这与显著降低的尿液 11-dehydro TXB₂与 6-keto PGF 1 α 比率是相关联的。但是尿液排泄的 11-dehydro TXB₂绝对值没有显著降低。饮食中动物源饱和脂肪酸可能通过影响组织花生四烯酸的含量而影响血栓烷的形成。

1 Introduction

Thrombus formation may be an integral part of the atherosclerosis and the acute event, which leads to a

myocardial infarction or sudden cardiac death. The thrombi formation is initiated by platelet aggregation. The ratio of thromboxane A₂/prostacyclin I₂ (TXA₂/PGI₂) plays a critical role in platelet aggregation (Moncada and Vane 1979). TXA₂ and PGI₂ are biosynthe-

sized from arachidonic acid (AA) through the cyclooxygenase pathway in the platelet membrane and arterial endothelial cells, respectively (Hamberg et al. 1975, Moncada et al 1979). Any factors which affect the balance of TXA₂/PGI₂ in favour of PGI₂ should reduce the risk of thrombosis, whereas factors altering the balance in favour of TXA₂ should increase thrombosis tendency. Evidence from dietary intervention studies have found that the ratio of TXA₂/PGI₂ was decreased by marine omega-3 polyunsaturated fatty acid (n-3 PUFA) in humans (von Schacky et al 1985, Ferretti et al 1998) and in animals (Abeywardena et al 1991, Ikeda et al 1998), and by plant n-3 PUFA alpha-linolenic acid in both humans (Bjerve et al 1987) and animals (Budowski et al 1980, Lee et al 1988). A recent study by Kelly et al (2001) 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. However, there is no data on the relation between dietary saturated fat from animal sources and the biosynthesis of TXA₂ and PGI₂ 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 TXA₂ and PGI₂, 11-dehydro thromboxane B₂(TXB₂) and 6-keto prostaglandin F 1 α (PGF 1 α) (Campbell 1990).

2 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 male omnivorous aged 30 to 55 years were recruited through newspaper advertisements. Exclusion criteria for this study were: individuals with symptoms or prior diagnosis of cardiovascular, renal disease, diabetes, or other chronic diseases, who are on long-term medications, athletes who train regularly for competitive sports and alcohol consumption exceeded 10 percent of daily energy intake.

Prior to 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 initial 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 a further 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) from full fat dairy products, and specially prepared biscuits containing lard. Butter, margarine, and the 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 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 di-

etary instructions, subjects were also asked to keep their physical activity pattern as similar as possible during the two diets. The subjects were contacted weekly during the study to monitor compliance and to provide dietary counseling. On the last week of each diet, 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 (Composition of food, National Food Authority, Australia, 1995).

The height and weight of each subject was 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 h and 09.30 h. Blood samples were stored at -80°C 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% BF₃ in methanol. Methyl esters of fatty acids were separated by gas chromatography as described by Sinclair et al. (1987).

Urine concentrations of 11-dehydro thromboxane B₂ and 6-keto prostaglandin F 1 α : Twenty-seven subjects collected their 24-hour urine on the last day of each of the diets. The samples were stored at -20°C for later analysis. The concentrations of 11-dehydro TXB₂ and 6-keto prostaglandin F 1 α in the urine was determined by using an enzyme immunoassay (EIA) method with commercially available EIA kits (Cayman Chemical Company, MI, USA) as described elsewhere (Pradelles et al., 1985).

Statistical analyses: All data were performed using the Statistical Package for the Social Sciences version 8.0 (SPSS Inc. Chicago, IL, USA). The General Lin-

ear Model (GLM) was used to compare the results at the end of the two diet periods, taking carry-over effects into consideration (Fleiss, 1986). The values were reported as mean \pm SD in all the results tables. P values were two-sides, and < 0.05 was considered as significant.

3 Results

Thirty-three subjects enrolled in the study, mean age of 41.2 ± 7.8 years and mean BMI of 26.5 ± 3.0 kg/m² 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 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 on the HF than on the LF dietary period ($p < 0.05$). Serum proportion of 20:4n-6 was higher on the LF (6.5% of total fatty acid) than on the HF diet (6.2% of total fatty acid) ($p = 0.06$) (Table 2). Serum concentrations of total and LDL cholesterol were significantly higher on the HF diet compared with on 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 thromboxane B₂ and 6-keto prostaglandin F 1 α are reported in Figure 1 and 2. Mean daily urine excretion of 11-dehydro thromboxane B₂ were 903 ± 65 ng/day and 1007 ± 63 ng/day, 6-keto prostaglandin F 1 α were 377 ± 37 ng/day and 360 ± 32 ng/day for the HF and LF diet, respectively. Ratio of 11-dehydro TAB₂ to 6-keto PGF 1 α in urine was significantly lower in the HF (2.7 ± 0.2) than in the LF dietary period (3.1 ± 0.3) (Figure 2).

4 Discussion

Effect of n-3 and n-6 PUFA on thromboxane A_2 and prostacyclin I_2 has been well documented in both the human and animal studies. However, there is no data on the effects of the diet high in saturated fat from animal sources on 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, high animal fat diet results in a decreased proportion of 20:4n-6 by 5% in serum ($p=0.06$), which is associated with 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 $PGF\ 1\alpha$ compared with the LF diet ($p=0.03$).

TXA_2 and PGI_2 are biosynthesized from arachidonic acid by the cyclooxygenase pathway. TXA_2 is formed in platelets and it is a potent cellular regulatory agent with strong platelet-aggregating activity (Hamberg et al. 1975), and it is also a potent vasoconstrictor (Bhagwat et al. 1985). TXA_2 can be broken down nonenzymatically ($t_{1/2} = 3$ minutes) into the thromboxane B_2 (TXB_2), a stable metabolite (Gryglewski et al. 1988, Campbell 1990). PGI_2 is formed in vascular endothelial cells (Moncada et al. 1976). PGI_2 is released by endothelium and it only effects the local environment; it is a powerful vasodilator on the abluminal side of vessels and inhibits platelet aggregation on the luminal side (Vane et al. 1990). PGI_2 is hydrolyzed nonenzymatically ($t_{1/2} = 3$ minutes) to 6-keto- $PGF_{1\alpha}$ (Campbell 1990). 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 (Dyerberg 1986, Leaf and Weber 1988). EPA, a long chain (LC) n-3 PUFA, is released from phospholipids of the platelet membrane, it competes with AA for access to cyclo-oxygenase and produces an alternative form of thromboxane, thromboxane A_3 (TXA_3), which is relatively inactive in promoting platelet aggregation and vasoconstriction (Raz

et al. 1977). This situation can lead to a reduced TXA_2 production and thus a lower thrombosis tendency (Lands 1986, Dyerberg 1986). PGI_2 can also be produced from EPA in arterial endothelium *in vitro* (Dyerberg et al. 1978) and *in vivo* (Fischer and Weber 1984); PGI_2 has similar physiological actions and activity to PGI_2 (Moncada et al. 1976_a).

Numerous dietary intervention studies have found that TXA_2 production is more sensitive to alteration to the diet compared with PGI_2 (von Schacky et al. 1985, Mann et al. 1997, Ferretti et al. 1998). The ratio of urine excretion 11-dehydro TAB_2 to 6-keto $PGF\ 1\alpha$ was decreased by 20% when 34 healthy men aged 24 to 57 supplemented fish oil 15g/d for 10 weeks compared with placebo (48% of lard, 40% beef tallow and 12% of corn oil) (Ferritti et al. 1993). Daily urine excretion of 11-dehydro TAB_2 was reduced by 14%, while 6-keto $PGF\ 1\alpha$ was decreased only by 2% when 25 healthy subjects (male 12, female 13) aged 22 to 52 years consumed an average 133g raw Atlantic salmon per day for two weeks compared with after one week vegetarian diet (Mann et al. 1997). When 8 healthy male volunteers aged 20 to 40 years consumed a high-DHA diet containing 6g/d of DHA for 120 days, 11-dehydro TAB_2 was decreased by 35%, while 6-keto $PGF\ 1\alpha$ was decreased only by 8% compared with the control diet with trace amounts of DHA ($n=4$) (Ferritti et al. 1998).

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 **
MUFA (g)	23.6 ± 3.3	30.8 ± 10.6 **
PUFA (g)	12.5 ± 3.4	11.9 ± 3.0
P: S ratio	0.7 ± 0.2	0.2 ± 0.03 **
Carbohydrate (g)	307.7 ± 84.9	226.9 ± 47.7 **
Cholesterol (mg)	138.0 ± 18.5	341.6 ± 75.0 **
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 **
Fat (% energy)	24.5 ± 2.2	41.3 ± 2.7 **