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Influence of Dietary Linoleic Acid Intake with Different Fat Intakes on Arachidonic Acid Concentrations in Plasma and Platelet Lipids and Eicosanoid Biosynthesis in Female Volunteers

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Key Words
Linoleic acid · Arachidonic acid · Prostaglandins · Desaturases · Diet

Abstract
Background/Aim: N-6 fatty acids are considered to promote diseases prevalent in industrialized countries and characterized by an increased eicosanoid biosynthesis from arachidonic acid (AA). We investigated the impact of the linoleic acid (LA) intake on AA levels in humans. ***Methods:*** Six healthy female volunteers (age range 23–34 years) were given liquid formula diets (LFD) devoid of AA for 6 weeks, providing a constant intake of zero energy% (LFD 0: protein 15%, carbohydrates 85%) or 20 energy% (LFD 20: protein 15%, carbohydrates 55%, fat 30%) LA, for 3 weeks each. Fatty acids of plasma cholesteryl esters and platelet lipids were determined each week, and the prostaglandin biosynthesis was measured in 24-hour urine samples. ***Results:*** LFD 0 increased (+31% of initial value) and LFD 20 lowered (–30% of initial value) the percentage of AA in plasma cholesteryl esters and platelet lipids. Moreover, absence of dietary AA lowered the percentages of AA in plasma (–31% week 0 vs. week 6) and platelet (–11%) lipids, indicating a low transformation of LA. LFD 0 reduced urinary metabolite levels of prostaglandins D, E, and F in 24-hour urine samples (–48%, $p < 0.001$) within 24 h, but did not significantly affect platelet aggregation (–10%) and thromboxane formation (–25%). LFD 20 significantly lowered platelet aggregation (–25%) and thromboxane formation (–43%). The prostaglandin metabolite levels increased during the first 10 days, declined thereafter, and were lower than the preexperimental values at the end of the 3-week period. ***Conclusions:*** The results show that dietary LA does not increase the AA levels in plasma or platelet lipids and does not persistently contribute to prostaglandin biosynthesis which is increased by AA intake with Western diets.

Introduction

Linoleic acid (LA) is the precursor of arachidonic acid (AA) in animals and in humans. The availability of AA is positively correlated with the biosynthesis of eicosanoids [1], especially thromboxane, which is elevated in atherosclerosis [2] and breast cancer [3]. Western diets are characterized by a prevalence of n-6 fatty acids, both LA and

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Table 1. Composition of conventional diet and LFDs

	Conventional diet		LFD 20		LFD 0	
	energy%	g/2,200 kcal	energy%	g/2,200 kcal	energy%	g/2,200 kcal
Fat	35	85.6	30	77.3	0	0
Carbohydrates	50	268.2	55	295	85	456.1
Protein	15	80.7	15	80.7	15	80.7
LA	7.6	16.5	20	43.5	0	0
AA	0.3	0.63	0	0	0	0
KCl		4		3		3
NaCl		8		5		5
Cholesterol		0.6		0.6		0.6

AA. Excessive intake of n-6 fatty acids with Western diets is incriminated as an important risk factor for diseases with an increasing prevalence in industrialized countries, like atherosclerosis, allergies, and immunological or certain neoplastic diseases [4]. To investigate the impact of LA on the AA prevalence, we studied healthy female volunteers being given liquid formula diets for 6 weeks, providing a defined intake of LA, but containing no AA.

Materials and Methods

Subjects

Six females (age range 23–34 years) were selected for the experiment on the basis of the following criteria: their body weight was within a body mass index of 20–24 kg/m^2, they were free from known metabolic abnormalities, and routine laboratory findings and clinical examination were unremarkable. All were medical students and continued their normal life and physical activities throughout the experiment. They came to the metabolic ward of the clinic every morning, and body weight and energy intake were determined and clinical evaluations performed. Most of the day they spent in the clinic, eating their formula diets under supervision.

Liquid Formula Diets

The compositions of the liquid formula diets (LFDs) and of the conventional diet are shown in table 1. For each volunteer, the necessary caloric intake was calculated to maintain the body weight. The LFD 20 provided 20 energy% LA (43.5 g/2,200 kcal) included in the total amount of 30 energy% of fat (appropriate mixtures of safflower oil and olive oil). The protein intake with both LFDs containing 92% whey protein was 15 energy% (Hyperprotidine; Laboratoires Guigoz, Marne-la-Vallée, France). With the LFD 20, carbohydrates (Maltodextrine; Maizena, Krefeld, Germany) containing 94% oligopolymers of glucose provided 55% and with the LFD 0 (containing no fat) 85% of the total energy intake. To ensure a constant composition of dietary fat, appropriate mixtures of safflower oil and olive oil were prepared before the experiment, stored under nitrogen, and deep-frozen (–20°C) in brown bottles until use. The fatty acid composition was checked before and every week during the experiment. LFDs were prepared each day for every volunteer in amounts ensuring an adequate caloric intake; 3 g KCl, 5 g NaCl, and 0.6 g cholesterol were added per 2,200 kcal LFD, 1 capsule of vitamins (Protovita; Roche, Grenzach-Whylen, Germany) was given daily, and 100 mg of $FeCl_2$ was given every other day. Water intake was allowed ad libitum.

Experimental Design

Eight days before the experiment, our volunteers started recording their diet to calculate their usual caloric intake. Then the volunteers were randomized and allocated to LFD 0 or LFD 20. Three of them (volunteers A, B, and C) were started on LFD 20, the remaining 3 (volunteers D, E, and F) on LFD 0, changing to the other LFD after 3 weeks. The experiments were approved by the ethical committee of the institution, and written informed consent was given by the volunteers.

24-hour urine samples were collected 8 days before and during the experimental period by each volunteer, the volumes of which were immediately determined and the samples deep-frozen (–20°C) in appropriate amounts until analysis of prostaglandin (PG) and uric acid levels. The LFDs were free from purines, and adherence to LFDs was monitored by measuring the uric acid excretion. Fasting venous blood samples were collected in EDTA-coated tubes every 2nd day (4 times) during the preexperimental period and every 7th day during each experimental period. For platelet aggregation studies, venous blood samples were collected in trisodium citrate (final concentration 12.9 m*M*). At the same time points nonanticoagulated blood samples (2 × 1 ml) were taken for determinations of serum thromboxane levels expressed as thromboxane B_2 (TXB_2).

Measurements

Cholesteryl esters (CE) were isolated by thin-layer chromatography on precoated silica gel plates (Schleicher & Schuell, Dassel, Germany) and identified by standards (Sigma, Freiburg, Germany) on each plate as described elsewhere [5]. Total cholesterol and free cholesterol concentrations were determined by a commercial test set (Boehringer Mannheim, Germany), and cholesterol esters were calculated as difference of these values. Platelets were isolated from EDTA-plasma by fractional centrifugation, deep-frozen at –20°C, and lipids were extracted as previously described [6]. Methyl esters were prepared by transmethylation with 5% HCl in CH_3OH [5], and capillary gas-liquid chromatography was performed on a Fractovap series 4160 apparatus equipped with a flame ionization detector and a Spectra Physics SP 4100 computing integrator (Carlo Erba, Milan

Italy). The capillary fused silica column was coated with M20 (Chrompack, Berlin, Germany). Most samples were run in duplicate, questionable samples were reexamined on a Hewlett-Packard model 5971a gas-liquid chromatography-mass spectrometry system. The known molar weight, the amount of CE, and the percentages of LA, dihomo-γ-LA, and AA in CE allowed for calculating the quantities (mg%) of these fatty acids.

Determinations of uric acid were done using a Technicon autoanalyzer II system (Technicon Instruments, Tarrytown, N.Y., USA) by a routine method (Boehringer Mannheim, Germany). The urinary PG metabolites were determined by measuring tetranorprostanedioic acid (TNPDA), the joint metabolite of PGD, PGE, and PGF, on two packed columns with liquid phases of different polarity (M20 and SP2340; Chrompack), as previously described [7]. The serum TXB_2 concentration was measured in duplicate in 1 ml of nonanticoagulated blood samples which were allowed to clot in a tube at 37°C for 1 h. TXB_2 was measured by a commercial radioimmunoassay (Code NEK-024; Du Pont de Nemours, Bad Homburg, Germany).

The platelet aggregation was measured with a Born-type aggregometer (Fresenius, Oberursel, Germany). Following a 10-min incubation period at 37°C in a shaking water bath, platelet aggregation to collagen was performed in aliquots (0.5 ml) from each sample in triplicate, as previously described [6].

Statistics

Statistical analyses were performed using the nonparametric two-way analysis of variance (Friedman test); statistical differences were determined by using the Wilcoxon signed-rank test [8]. The resulting two-by-two frequency table was evaluated descriptively by the chi-square test (exact Fisher test) [8].

Results

All volunteers completed the study without noticeable side effects. The adherence to the LFDs was good, as assessed by determination of the uric acid excretion. No difference between both LFDs was found for the uric acid excretion which returned to the basal levels of 258 ± 16 mg/dl in the study participants on purine-free LFDs. The low amount of sodium in LFDs caused an average loss of body water of 1.1 kg during the first 3 days on LFD. The volunteers' switching to the other LFD after the crossing-over had no effect on the body weight which remained constant within ± 0.5 kg after the first 3 days during the rest of the experimental period.

With the LFD 0, the LA decreased in CE of plasma from 49 ± 4.8% to 36.5 ± 3.3% (fig. 1). With the LFD 20, an enrichment of LA was observed which already was completed after the 2nd week on LFD 20 (fig. 1).

AA and DHGLA percentages in plasma cholesteryl esters were inversely correlated to LA (table 2). With the LFD 0, the AA concentration in CE increased from 11.3 ± 2.3% to 15.2 ± 2.1% of total fatty acids as compared with the values measured in the volunteers on a conventional diet ($p < 0.01$). Most of the increase was reached already after the 2nd week on the LFD 0 (fig. 1). The increase of cholesteryl linoleate with the LFD 20 lowered the AA concentration in CE from 10.3 ± 2.9% to 8.1 ± 1.2% after 3 weeks on this diet ($p < 0.01$). We found a distinct influence of the precedent diet on cholesteryl arachidonate. In experimental subjects A, B, and C, coming from a highLA intake with the LFD 20, the increase in the AA concentration with the LFD 0 was less pronounced (30% over preexperimental value than in subjects D, E, and F, starting with the LFD 0 (51%; fig. 2). The LFD 20 reduced the AA concentration by 31% in subjects A, B, and C and by 51% in subjects D, E, and F ($p < 0.01$; fig. 2).

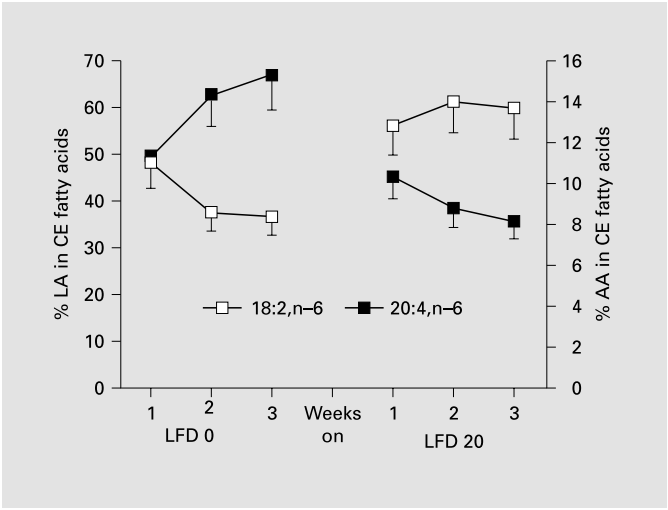

Fig. 1. Percentage of LA (□) and AA (■) in CE of plasma measured after 1, 2, and 3 weeks in the volunteers on LFD 0 and LFD 20.

Table 2. Plasma concentrations (mg/100 ml) of LA, γ-linolenic acid, and AA in plasma CE

	LFD 0	LFD20
LA	16.2 ± 1.4	30.0 ± 3.1*
γ-Linolenic acid	2.1 ± 0.1	1.1 ± 0.1
AA	7.2 ± 0.6	4.8 ± 0.4*

* $p < 0.05$ (Wilcoxon signed-rank test).

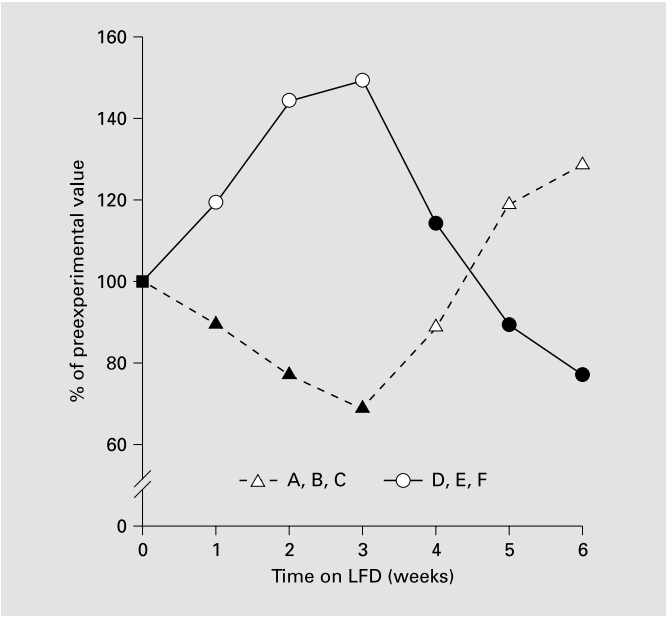

Fig. 2. AA levels measured in plasma CE of the volunteers A, B, and C, starting on the LFD 20 (▲) and thereafter put on the LFD 0 (△), and volunteers D, E, and F, starting on the LFD 0 (○) and thereafter put on the LFD 20 (●). The values are given as mean percentages of the preexperimental value (conventional diet).

Table 3. LA and AA concentrations (mean ± SD) in platelet phospholipids of 6 healthy females measured before the experiment, with the volunteers on a conventional diet and at the end of LFD 0 and LFD 20 given to the volunteers for 3 weeks each

	Platelet phospholipids		
	conventional diet	LFD 0	LFD 20
LA, % of total fatty acids	5.8 ± 1.2	4.5 ± 0.6	8.3 ± 1.1[a,b]
AA, % of total fatty acids	41.4 ± 1.4	37.1 ± 1.1[a]	35.4 ± 1.1[a]

[a] $p < 0.01$ versus preexperimental value.
[b] $p < 0.01$ versus LFD 0 (Wilcoxon signed-rank test).

Table 4. TXB_2 and platelet aggregation measured in 6 healthy volunteers during LFD 0 and LFD 20 compared to the values measured during conventional diet (0.3 g/day AA)

	% reduction compared to preexperimental value	
	LFD 0	LFD 20
TXB_2	–25	–43*
Platelet aggregation	–10	–25*

* $p < 0.01$ versus the preexperimental value (Wilcoxon signed-rank test).

At the end of the 6 weeks on LFD, the mean AA values in CE were approximately 20% lower than the preexperimental values, indicating a decrease of AA in CE during LFDs devoid of AA.

Total cholesterol and CE decreased during LA intake with the LFD 20. Consequently, the concentrations of LA and DHGLA in plasma decreased more than their relative percentage values (table 2). In platelet lipids, the percentage of LA decreased by –22% during the LFD 0, and at the end of the LFD 20, an increase of 43% was observed. In contrast to the findings in CE, the AA concentration was lowered with both LFDs in platelet lipids (table 3).

The eicosanoid biosynthesis, measured as TNPDA, decreased within 24 h with the experimental subjects on the LFD 0 (fig. 3). With the LFD 20 an increase of eicosanoids transformable to TNPDA was observed till the 10th day, then the TNPDA levels gradually decreased, and we found lower amounts in the 24-hour urine samples than observed with the conventional diet. Platelet aggregation and TXB_2 formation were decreased during all LFD, and the augmented LA intake with LFD 20 additionally lowered both parameters (table 4).

Discussion

Diets high in LA may have adverse effects, if they result in augmented availability of AA, the precursor of eicosanoids. Exaggerated eicosanoid formation promotes platelet aggregation and mediates immunological, proliferative, and allergic responses of cells [9]. Transformation of LA to AA is an enzymatic process, dependent on the activity of δ-6-desaturase, an enzyme inhibited by all polyunsaturated fatty acids, including LA [10, 11]. With the LFD 20, we observed an impressive decrease in the percentage of AA in CE of plasma which can be attributed either to inhibition of desaturases or replacement of AA on the binding sites by LA or displacement of AA in cell lipids. In platelet lipids we found no significant differ-

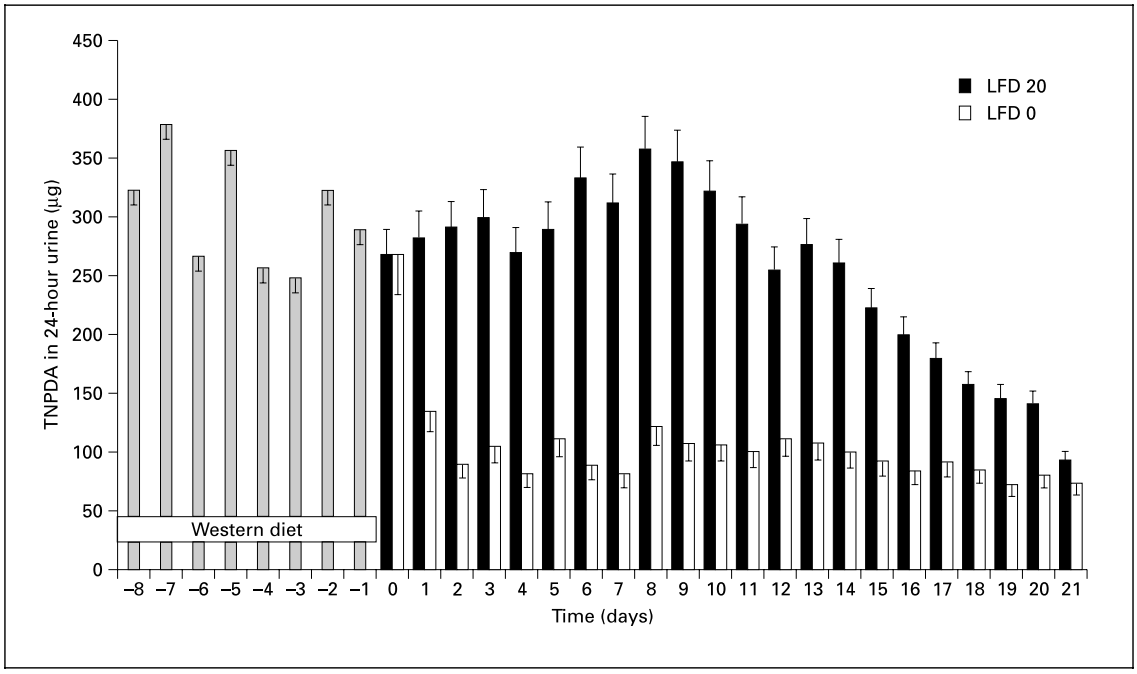

Fig. 3. Mean values (± SEM) of TNPDA, the joint metabolite of PGE, PGF, and PGD, measured in 24-hour urine samples in the volunteers A–F on a Western diet (preexperimental) and on the LFD 20 (dark columns) or the LFD 0 (open columns).

ences in AA percentages with LFD 0 or LFD 20. So we were unable to find a displacement of AA in cell lipids. In cell cultures, LA competes poorly with AA for incorporation [12]. Inhibition of LA desaturation, seems relevant in humans, as in our experiments the DHGLA and AA levels were decreased with the LFD 20. The biosynthesis of AA from LA is sluggish [11], but increases with fasting [13] or diets devoid of polyunsaturated fatty acids [9]. This was confirmed by the increase of the AA concentrations with the LFD 0 in our experiments.

The LFD 20 transiently increased the PGD, PGE, and PGF levels transformable to TNPDA (fig. 3). It seems feasible that excessive amounts of LA, as provided by the LFD 20, result in a replacement of AA to maintain an appropriate cell fluidity. This process may continue, until normal cell fluidity is regained, thus leading to a temporarily increased release of AA and stimulated PG biosynthesis [7]. This process is terminated after 2 weeks, when we observed a reduction of TNPDA despite the continued high LA intake. It cannot be excluded that formation of isoprostanes additionally contributes to the temporarily increased TNPDA formation [14].

With the LFD 0, the AA concentrations increased in the CE of our volunteers. It is very unlikely that release of AA from fat stores contributes appreciable to the increase in AA levels, as both LFD 0 and LFD 20 provided the same amount of energy, and our volunteers did not loose weight during the LFD 0. Moreover, it is known that release of AA from fat stores is very limited, as AA mostly is stored in the sn-3 position of triglycerides which is not accessible for lipases [9]. It is interesting that exclusion of LA from the diet augments AA percentages in CE of plasma, but lowers PG biosynthesis. AA has been supposed to contribute essentially to cell membrane fluidity [15], and eventually this compartment is not accessible for the activity of phospholipase A_2, relevant to release of AA and subsequent PG biosynthesis [16]. Our findings would fit excellently into the concept that desaturation of fatty acids regulates cell fluidity and does not favor the AA cascade for PG formation. This assumption is affirmed by the findings in rats given a fat-free diet [17]. After 18 weeks on the diet, the overall degree of unsaturation in phosphatidyl choline fatty acids was not decreased and always higher in the outer than in the inner leaflets of liver microsomal membranes.

LFD devoid of AA decreased the percentage of this fatty acid during the 6-week experimental period, indicating that endogenous AA formation was not sufficient to

maintain the high levels of AA provoked by the intake of AA with meat and other products of animal origin in our volunteers during the preexperimental period on a Western diet. This finding is consistent with results of other experiments obtained with LFDs devoid of AA [7]. Also with conventional diets high in LA, only minor changes in AA percentages are observed [18]. Investigations performed in vegetarians, living on a diet without animal products, revealed that the AA percentages in blood, cells, and tissues are lower than in omnivores [19]. These findings underline the importance of dietary AA provided with products of animal origin.

The importance of dietary AA is further strengthened by the finding of inhibited platelet aggregation and reduced TXB_2 formation with both LFDs (table 4). The difference in fatty acid composition of plasma lipids and platelets is consistent with results reported in the literature and may indicate a special metabolic behavior of platelets [20]. Despite unaltered percentages of AA, augmentation of LA has been shown to diminish TXB_2 release and platelet response to aggregating agents [21], similar to the findings in our experiments.

In conclusion, we found that the transformation of LA to AA in our volunteers was very low, leading to an inverse relationship of dietary LA intake and percentages of AA in CE during LFDs devoid of AA. The reduction of AA by LA depends on a diet devoid of AA, e. g., a vegetarian diet, as experiments with a conventional diet fail show this effect [18]. In view of common diseases in Western societies, such as atherosclerosis or immunologic or allergic diseases, this points to the importance of dietary AA in maintaining unwanted high levels of this precursor of eicosanoids.

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References

1 Sautebin L, Caruso D, Galli G, Paoletti R: Preferential utilization of endogenous arachidonate by cyclo-oxygenase in incubations of human platelets. FEBS Lett 1983;157:173–178.
2 van Kooten F, Ciabattoni G, Koudstaal PJ, Grobbee DE, Kluft C, Patrono C: Increased thromboxane biosynthesis is associated with poststroke dementia. Stroke 1999;30:1542–1547.
3 Nigam S, Zakrzewicz A, Eskafi S, Roscher A: Clinical significance of prostacyclin and thromboxane in cancer of the female breast and genital tract. Cancer Metastasis Rev 1992;11:411–421.
4 Okuyama H, Kobayashi T, Watanabe S: Dietary fatty acids – the n-6/n-3 balance and chronic elderly diseases: Excess linoleic acid and relative n-3 deficiency syndrome seen in Japan. Prog Lipid Res 1997;35:409–457.
5 Zöllner N: Allgemeine Untersuchungsmethoden; in Zöllner N, Eberhagen D (eds): Untersuchung und Bestimmung der Lipoide im Blut. Berlin, Springer, 1965, pp 35–206.
6 Adam O, Wolfram G, Zöllner N: Prostaglandin formation and platelet aggregation during fasting and linoleic acid intake. Res Exp Med (Berl) 1985;185:169–172.
7 Adam O, Wolfram G, Zöllner N: Prostaglandin formation in man during intake of different amounts of linoleic acid in formula diets. Ann Nutr Metab 1982;26:315–323.
8 Sachs L: Statistische Auswertmethoden; in Sachs L (ed): Angewandte Statistik. Heidelberg, Springer, 1991, pp 224–296.
9 Adam O: Immediate and long range effects of the uptake of increased amounts of arachidonic acid. Clin Investig 1992;70:721–727.
10 Brenner RR: Endocrine control of fatty acid desaturation. Biochem Soc Trans 1990;18:773–775.
11 Jeffcoat R: The biosynthesis of unsaturated fatty acids and its control in mammalian liver. Essays Biochem 1979;15:1–36.
12 Ziboh VA, Miller CC, Cho Y: Metabolism of polyunsaturated fatty acids by skin epidermal enzymes: Generation of anti-inflammatory and antiproliferative metabolites. Am J Clin Nutr 2000;71(suppl 1):361–366.
13 Zhou L, Nilsson A: Fasting increases tissue uptake and interconversion of plasma unesterified linoleic acid in guinea pigs. Biochim Biophys Acta 1999;1436:499–508.
14 Liu T, Stern A, Roberts LJ, Morrow JD: The isoprostanes: Novel prostaglandin-like products of the free radical-catalyzed peroxidation of arachidonic acid. J Biomed Sci 1999;6:226–235.
15 Ghebremeskel K, Harbige LS, Williams G, Crawford M, Hawkey C: The effect of dietary change on in vitro erythrocyte haemolysis, skin lesions and alopecia in common marmosets *(Callithrix jacchus)* Comp Biochem Physiol A 1991;100:891–896.
16 Baciulis V, Lüthy C, Hofer G, Toplak H, Wiesman UN, Oetliker OH: Specific and non-specific stimulation of prostaglandin release by human skin fibroblasts in culture: Are changes of membrane fluidity involved? Prostaglandins 1992;43:293–304.
17 Iritani N, Mizoguchi H, Kitaya M: Asymmetric distribution of arachidonic acid and n-3 polyunsaturated fatty acids in rat liver microsomal membranes under a fat-free diet. Lipids 1987;22:45–50.
18 Kinsella JE, Broughton KS, Whelan JW: Dietary unsaturated fatty acids: Interactions and possible needs in relation to eicosanoid synthesis. J Nutr Biochem 1990;1:123–141.
19 Phinney SD: Metabolism of exogenous and endogenous arachidonic acid in cancer. Adv Exp Med Biol 1996;399:87–94.
20 Lemaitre-Delaunay D, Pachiaudi C, Laville M, Pousin J, Armstrong M, Lagarde M: Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [$^{(13)}$C]DHA in phosphatidylcholine. J Lipid Res 1999;40:1867–1874.
21 Hajarine M, Lagarde M: Liberation and oxygenation of polyenoic acids in stimulated platelets. Biochimie 1988;70:1749–1758.