INFLUENCE OF *TRANS* FATTY ACIDS ON LINOLEIC ACID METABOLISM IN THE RAT

Cover illustration:

Some animals and plants contain trans fatty acids formed by a biohydrogenation process.

INFLUENCE OF *TRANS* FATTY ACIDS ON LINOLEIC ACID METABOLISM IN THE RAT

INVLOED VAN *TRANS* VETZUREN OP HET LINOLZUUR METABOLISME VAN DE RAT

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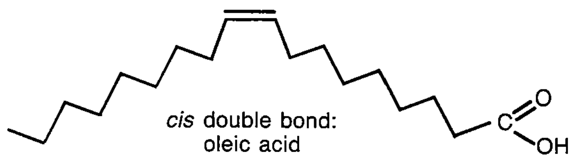

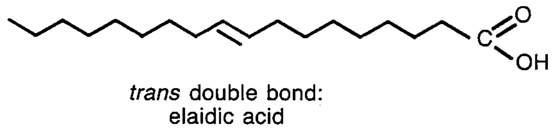

Structure of *cis* and *trans* fatty acids

Aan mijn Ouders en Suus.

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Chapter 1.

GENERAL INTRODUCTION AND OBJECTIVES OF THE STUDY

1. GENERAL INTRODUCTION AND OBJECTIVES OF THE STUDY

1.1. Introduction

Fats form an important constituent of our diet. They provide a considerable source of energy (about 40% of total energy in western, industrialized societies) and some essential nutrients, e.g. linoleic acid, α-linolenic acid and lipid-soluble vitamins. Dietary fats are mainly composed of triglycerides: a glycerol moiety with three fatty acids. There are many types of fatty acids, differing in chain length (number of carbon atoms) and in unsaturation (number, location and configuration of double bonds). The configuration of the double bonds can be either *cis* or *trans* (geometrical isomers). Most of the unsaturated fatty acids found in nature (e.g. oleic acid, linoleic acid and α-linolenic acid) have the *cis* configuration. *Trans* fatty acids can be formed during hydrogenation or hardening. This is a widely used process to shift the melting range of edible oils to higher temperatures with a view to obtain solid fats (hardstock) and to improve the stability of edible oils and fats for production of margarines and shortenings. These isomeric fatty acids, however, are also present in fats from ruminants (e.g. milkfat and butter) and in their meat. Because of the wide use of margarines, shortenings, ruminant- and milk-fat, butter and meat, *trans* fatty acids can be found in a wide variety of edible products. Since the discovery of the hydrogenation process by Sabatier in 1897 (Sabatier and Senderens, 1897) the availability of margarines and shortenings has clearly increased. This highly contributed to the gradual increase in the consumption of *trans* fatty acids in western, industrialized societies (Hunter and Applewhite, 1986). Depending on the method of calculation the average intake of *trans* fatty acids in the U.S.A. during the last decade is estimated to be between 6 and 12 g/capita/day (Enig et al., 1978; Emken, 1981; FASEB, 1985). A recent estimation based on detailed data on sales and composition of products containing *trans* fatty acids came to an average availability of about 8 g *trans* fatty acids per capita per

day (Hunter and Applewhite, 1986), which is about 6% of the total fat intake or 2.5% of total energy (en%). This figure is roughly similar to the estimates for intake in other western, industrialized societies (Heckers et al, 1979; Åkesson et al, 1981; Brisson, 1981; Gurr, 1983).

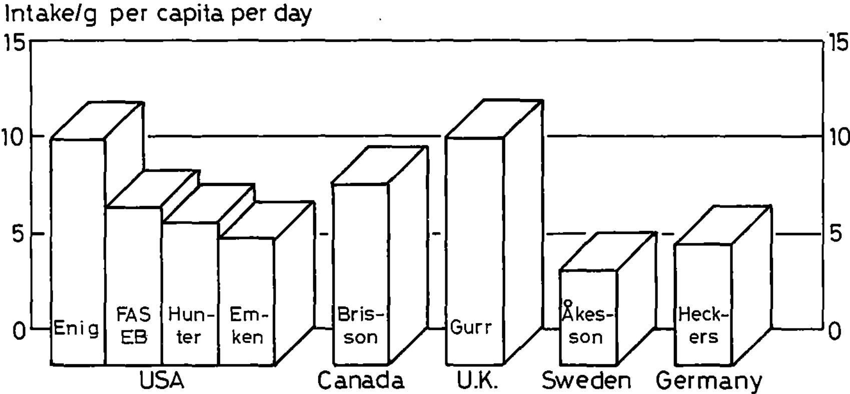


Fig. 1 Estimated average daily intake of *trans* fatty acids for some western, industrialized societies as calculated by the authors indicated.

Because *cis* isomers are the predominant unsaturated fatty acids in natural oils and fats, the safety of *trans* isomers has since long been the subject of many investigations. The first extensive experiment to evaluate the safety of *trans* fatty acids was reported in 1945 (Deuel et al, 1945). This and subsequent experiments of this group consisted of multi-generation studies with rats fed hydrogenated vegetable oils (see Chapter 7 and FASEB, 1985 for reviews). Also in our laboratory long-term studies with hydrogenated soybean oils were performed (Vles and Gottenbos, 1972a and b; Vles et al, 1977). In these long-term feeding experiments with various animal species, no adverse effects of *trans* fatty acids as present in (partially) hydrogenated vegetable oils were found, provided an adequate amount of linoleic acid was present in the diet (for recent reviews see: Beare-Rogers, 1983; Emken, 1983; Gottenbos, 1983; Gurr, 1983). Also the long-standing experience with *trans* fatty acids in human nutrition confirmed the safety of these fatty acids as a dietary constituent. Short-term animal experiments, however, indicate that *trans* fatty acids can influence

the metabolism of linoleic acid and its conversion products (other long-chain polyunsaturated fatty acids) in vivo (Anderson et al, 1975; Hill et al, 1979 and 1982; Holman, 1981; Kinsella et al, 1981). These findings have led to a considerable interest in the metabolism of *trans* fatty acids and the mechanism by which they are assumed to influence linoleic acid metabolism.

1.2. Aim of thesis

At the start of the work described in this thesis, most reviewers on *trans* fatty acids agreed that these isomeric fatty acids did not induce undesirable effects, provided sufficient linoleic acid was present in the diet (Beare-Rogers, 1983; Emken, 1983; Gottenbos, 1983; Gurr, 1983). However, *trans* fatty acids were reported to aggravate symptoms of essential fatty acid deficiency in experimental animals (poor growth, deteriorated skin, impairment of spermatogenesis) and cause alterations in tissue fatty acid composition and decrease prostaglandin synthesis (Aaes-Jørgensen and Hølmer, 1969; Hill et al, 1979; Kinsella et al, 1981). It was our aim to define the minimum requirement for linoleic acid necessary to prevent specific or adverse effects (like aggravation of linoleic acid deficiency and decrease in prostaglandin synthesis) of *trans* fatty acids from partially hydrogenated vegetable oils (mainly C18 *trans* fatty acids). Moreover, we wanted to get a better insight into the effects of *trans* fatty acids on linoleic acid metabolism, because it was suggested that changes in metabolism of linoleic acid and polyunsaturated fatty acids derived thereof could be the underlying cause of the alleged adverse effects of *trans* fatty acids (Kinsella et al, 1981; Hill et al, 1982).

In our feeding experiments we used a special, partially hydrogenated soybean oil. This oil contained a high amount of various monoenoic *trans* isomers, but also a relatively high amount of dienoic *trans* fatty acids: isomers of linoleic acid. The diets were composed

in such a way as to contain 16 en% *trans* monoenes and maximally about 3.2 en% *trans* dienes (about 0.2 en% 9*t*,12*t*-18:2 and 0.2 en% other 9,12-18:2 isomers and 3.1 en% non-9,12-18:2 isomers (*cis* and *trans*)), thus by far exceeding the *trans* content of human diets. The advantage of this approach over feeding isolated fatty acids is that both monoenoic and polyenoic *trans* fatty acids are present in the experimental diet and that the distribution of positional isomers is similar to that in the human diet. So, conclusions can be reached with regard to the nutritive value of *trans* fatty acids-containing food products, consumed in realistic amounts.

We chose the rat as an animal model for our studies for a number of reasons. The rat has been the most frequently used animal to study effects of diet on fatty acid metabolism. The biological consequences of *trans* fatty acids have been studied mainly in rats. As a consequence a firm basis for a study of specific effects of *trans* fatty acids on linoleic acid metabolism in rats already existed. Although it is well established that the linoleic acid metabolism in the rat differs quantitatively from that of man, results of rat feeding studies are often used to indicate possible consequences of dietary components for human health. Ethical and technical reasons prevent studies on the effects of *trans* fatty acids on linoleic acid metabolism in man. The rat seems to be the most suitable animal model for studies of that type at present.

1.3. Brief outline of linoleic acid metabolism in the rat

Linoleic acid is an essential fatty acid (EFA): it cannot be synthesized in the body. As soon as linoleic acid is taken up from the diet, a number of pathways can be followed. It can be stored in triglycerides in adipose tissue and be transported in the blood mainly as cholesterol-ester. It can also be incorporated into structural lipids (phospho-lipids); esterified in this way it is part of all membranes in the body. Part of the linoleic acid is converted into other polyunsaturated fatty

acids and via this route it supplies precursors for prostaglandins, leukotrienes and hydroxy fatty acids. These are biologically very active compounds, having a number of special effects. And finally, linoleic acid can be catabolized (mainly via β-oxidation) to yield energy.

Insufficient supply of linoleic acid or other polyunsaturated fatty acids of the n-6 family in the diet leads to a number of biochemical and clinical symptoms, like changes in the polyunsaturated fatty acid composition of tissue lipids, decreased eicosanoid biosynthesis, diminished growth, impaired skin function, disturbed reproduction. A number of internal and external factors are reported to interfere with linoleic acid metabolism, and to potentially aggravate some of the symptoms mentioned above. Among these factors are protein deficiency, diabetes, high n-3 fatty acid intake and consumption of large amounts of *trans* fatty acids (Brenner, 1981; De Schrijver and Privett, 1982a; De Gomez Dumm et al, 1983; Kirstein et al, 1983; Mahfouz et al, 1984).

In this part of this chapter the metabolism of linoleic acid and its derivatives in the rat is discussed as a basis for the review of possible effects of *trans* fatty acids on polyunsaturated fatty acid-metabolizing pathways in the next section.

In Fig. 2 a reminder is given of the various types of fatty acids and their nomenclature.

Activation

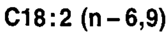
Prior to their participation in most of the fatty-acid-metabolizing reactions, fatty acids have to be activated to acyl-CoA. This process is catalyzed by acyl-CoA synthetase (acid:CoA ligase, EC 6.2.1.3) and requires ATP. This enzyme is found in various locations within most cells, including microsomes, mitochondria and peroxisomes. The presence of a double bond in the fatty acid increases the rate of activation (Normann et al, 1981). The apparent Km-values of most of the common unsaturated fatty acids (oleic, linoleic, α-linolenic and arachidonic acid) are in the same range (Normann and Flatmark, 1980; Normann et al, 1981). These fatty acids are competitive inhibitors of the activation of each other (Marcel and Suzue, 1972). However, in vitro experiments

The fatty acids differ in their carbon chain in:

- the number of carbon atoms,
- the number of the double bonds,
- the position of the double bonds.

Fatty acids are often represented by a **SYMBOL** indicating the above three properties.

For example the symbol of LINOLEIC ACID is:



This means:

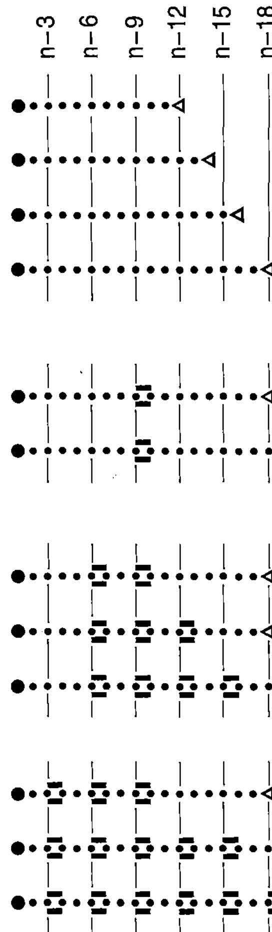
C18 the chain has 18 (C)arbon atoms

:2 and 2 double bonds (=)

n – 6 one = between the **6th** and 7th carbon atom from the methyl group (CH_3)
(or at the 18 - 6 = 12th carbon atom from the carboxyl group, COOH)

n – 9 and one = between the **9th** and 10th carbon atom from the methyl group (CH_3)
(or at the 18 - 9 = 9th carbon atom from the carboxyl group, COOH)

FATTY ACID	STRUCTURE	SYMBOL
saturated		
– lauric		C12:0
– myristic		C14:0
– palmitic		C16:0
– stearic		C18:0
mono-unsaturated		
– oleic		C18:1 (n – 9)
– erucic		C22:1 (n – 9)
poly-unsaturated		
linoleic acid (n – 6) family – linoleic		C18:2 (n – 6,9)
– gamma-linolenic		C18:3 (n – 6,9,12)
– arachidonic		C20:4 (n – 6,9,12,15)
linolenic acid (n – 3) family – alpha-linolenic		C18:3 (n – 3,6,9)
– eicosa-pentaenoic		C20:5 (n – 3,6,9,12,15)
– docosa-hexaenoic		C22:6 (n – 3,6,9,12,15,18)



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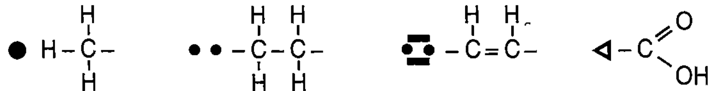

Fig. 2 Types of fatty acids and their nomenclature.

generally indicate that acyl-CoA synthetase is not the rate-limiting enzyme in the metabolism of long-chain fatty acids (Brenner, 1981). Once activated, fatty acids can undergo a number of reactions, i.e. incorporation in phospholipids, triglycerides etc., desaturation/ elongation and oxidation.

Incorporation

Fatty acids are generally distributed non-randomly in phospholipids, the membrane structural lipids. This asymetric distribution is either accomplished during the de novo synthesis of these lipids or by replacement of certain acyl chains of the completed lipids by different ones through deacylation followed by reacylation. Reacylation of lyso-phospholipid by activated fatty acids requires the action of acyltransferases (EC 2.3.1.23). There are at least two different acyltransferases. Both types are widely but not equally distributed within the cells. The one specifically attaches a saturated acyl group to the 1-position (e.g. acyl-CoA:2-acyl-PC-acyltransferase); the other, acting at the 2-position (e.g. acyl-CoA:1-acyl-PC-acyltransferase), prefers unsaturated fatty acids. These enzymes are thought to play a major role in the general pattern of fatty acid composition of phospholipids in most animal tissues. As a consequence of this specificity, the 1-position of these lipids is predominantly occupied by a saturated fatty acid, while the 2-position commonly contains an unsaturated fatty acid.

Most experimental evidence leads us to believe that deacylation is usually the reaction limiting the rate of acylgroup turnover, while reacylation is the reaction conferring specificity to the retailoring process. Because fatty acid turnover is often quite fast, even a modest shift in acyltransferase specificity can quickly be reflected in phospholipid fatty acid composition (Thompson and Martin, 1976).
The specificity of the 1-acyl-PC-acyltransferase in rat liver microsomes is decreasing in this order: 20:4 n-6, 20:5 n-3, 20:3 n-6 > 18:2 n-6 > 18:1 n-9 > 18:3 n-3 > 18:3 n-6 > 22:6 n-3 > saturated fatty acids (Hill and Lands, 1968; Lands et al, 1982). Competition experiments indicated that 18:3 n-6, 18:3 n-3 and 20:5 n-3 were the most effective competitors with arachidonic acid for incorporation at the 2-position of PC. Saturated fatty acids, 18:2 n-6 and 22:6 n-3 did not or only slightly compete with arachidonic acid (Lands et al, 1982). The 2-acyl-PC-acyltransferase showed the strongest preference for stearate and the

lowest for polyunsaturated fatty acids (18:0 > 16:0 > 14:0 = 18:1 > polyunsaturated fatty acids) (Van Den Bosch et al, 1968; Lands et al, 1966). Rat erythrocytes showed a similar pattern of specificities of 1-acyl-PC-acyltransferase with regards to polyunsaturated fatty acids. The transfer rate of 9*c*-18:1, however, was much smaller than that of polyunsaturated fatty acids (Waku and Lands, 1968). Significant differences have been detected in the specificity for acylation of the various lyso-phospholipids. α-Linolenic acid and arachidonic acid, for example, have a greater affinity for lyso-PE than for lyso-PC while linoleic acid contributes non-selectively to both lyso-phospholipids (De Tomás and Brenner, 1970). This could at least partly explain compositional differences in phospholipid species.
Neither the nutritional state of rats (fed, starved, starved-refed) (Pugh and Kates, 1984; Kawashima et al, 1985) nor the type of dietary fat (corn oil versus coconut oil) (Pugh and Kates, 1984) had a significant effect on acylCoA:1-acyl-PC-acyl-transferase activity in rat liver microsomes, while desaturation activities were influenced. This points to an independent regulation of desaturases and acyltransferases. The good agreement of the distribution of various fatty acids in phospholipids in vivo with the pattern predicted from acyltransferases information makes it probable that these enzymes have a significant function in vivo in influencing lipid composition (Lands, 1979).

Desaturation and elongation

The biosynthesis of polyunsaturated fatty acids is performed by aerobic desaturations and elongations of fatty acids previously formed or provided by the food. The mitochondrial and microsomal elongation system elongates acyl-CoA with a C2-unit at the carboxyl end. This process is similar to the repeated elongation during fatty acid synthesis, but the enzymes involved are membrane-bound. Desaturation is the introduction of a *cis* double bond into the acyl chain. This process takes place in the endoplasmatic reticulum (microsomes) and is catalyzed by a complex of membrane-bound enzymes: a reductase, cytochrome b5 and the actual desaturase. Acyl-CoA form the substrates. Although desaturation working

on polyunsaturated fatty acids incorporated in phospholipid has been described, the physiological significance of this pathway remains to be established (Pugh and Kates, 1979). Four different desaturases have been identified in most mammalian systems: the Δ9-, Δ6-, Δ5- and Δ4-desaturase. The desaturation of saturated fatty acids to form monoenoic acids is catalyzed by the Δ9-desaturase, introducing a double bond at the 9-position. Thus, oleic acid is produced from stearic acid and palmitoleate from palmitate. The other three desaturases, in conjunction with elongase, serve to synthesize a whole range of polyunsaturated fatty acids (for reviews see: Brenner, 1971; Sprecher, 1981). A general characteristic of these enzymes is their inability to introduce a double bond between the methyl end of the acyl chain and the first double bond from that side. An important consequence is that polyunsaturated fatty acids can be grouped in families, e.g. the n-6, n-3, n-9 and n-7 families, with the first double bond on the 6th, 3rd, 9th and 7th carbon from the methyl end, respectively. Irrespective of further desaturations or elongations, unsaturated fatty acids will remain within their family. The general scheme for the synthesis of polyunsaturated fatty acids from their precursors is shown in Figure 3.

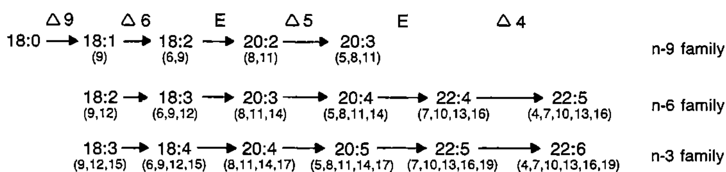

Fig. 3 Main desaturation and elongation pathways in the biosynthesis of polyunsaturated fatty acids. The location of the double bonds is indicated between brackets. Desaturation reactions: Δ9, Δ6, Δ5 and Δ4; elongation reactions: E.

Because of its high polyunsaturated fatty acids-synthetizing capacity and high mass, the liver is probably the dominant site for polyunsaturated fatty acid synthesis. Although many other organs investigated

are reported to possess desaturation and elongation systems (adrenals, testes (Brenner, 1971), brain (Cook, 1978), intestine (Garg et al, 1988)), there are indications that at least part of the polyunsaturated fatty acids in their lipids are derived from the liver and transported via the blood.

The relative rates of conversion of fatty acids of the n-6 family by desaturases and elongase from rat liver microsomes have been measured by Bernert and Sprecher (1975). In their system the Δ6-desaturation of linoleic acid was slightly faster than the Δ5-desaturation of dihomo-γ-linolenic acid. The elongation from γ-linolenic acid to dihomo-γ-linolenic acid was much faster. Elongation of arachidonic acid, however, occurred at a similar rate as the Δ5- and Δ6-desaturations of their respective substrates. The Δ4-desaturation seemed to proceed at a very slow rate. Although the Δ6-desaturation certainly has a key-position in the synthesis of polyunsaturated fatty acids because it is the first enzyme involved, these in vitro data do not support the hypothesis that the Δ6-desaturase is the rate-limiting enzyme in the biosynthesis of arachidonic acid in the rat. Also the Δ5-desaturase may have a strong regulatory function (Brenner, 1981). The relative rates of the above mentioned reactions involving n-6 fatty acids, suggest that accumulation of other n-6 fatty acids than linoleic acid and arachidonic acid is prevented.
As evident from the scheme in Fig. 3. the desaturase enzymes show a strong specificity towards specific acyl-CoA. For the Δ9-desaturase stearic acid is the preferred substrate (Jeffcoat et al, 1977). Only a few unsaturated fatty acids are reported to be desaturated at the 9-position and then only in the absence of saturated fatty acids. The preferred substrates for the Δ6-desaturase in the rat are α-linolenic acid > linoleic acid > oleic acid (Brenner and Peluffo, 1966). In vitro experiments with rat liver microsomes clearly indicated that linoleic acid, α-linolenic acid and oleic acid compete for the Δ6-desaturation (Brenner and Peluffo, 1969). The conversion of linoleic acid into γ-linolenic acid was competitively inhibited by other unsaturated fatty

acids, in the following order: α-linolenic acid > docosahexaenoic acid > oleic acid. Conversely, the Δ6-desaturation of α-linolenic acid was inhibited by linoleic acid (Brenner and Peluffo, 1966).
Chain elongation of preformed fatty acids is generally faster than desaturation (Bernert and Sprecher, 1975). The rate of elongation is determined by the chain length and the number and positions(s) of the double bond(s). Maximal rates were obtained with C18 acyl chains. No clear relationship between the number and position of the double bonds and the rate of elongation was observed (Ludwig and Sprecher, 1979).

The desaturation and elongation process can be influenced by a number of external (e.g. dietary) or internal (e.g. hormonal) factors (see for a recent review: Brenner, 1981). A diurnal variation in Δ6-desaturase activity and decrease with aging have also been reported (Jeffcoat and James, 1977; Horrobin, 1981; De Gomez Dumm et al, 1984). Dietary protein and EFA-deficiency activate the Δ6-desaturation, while fasting, carbohydrates and diets high in polyunsaturated fatty acids generally inhibit the enzyme-activity (Inkpen et al, 1969; De Gomez Dumm et al, 1972; Kurata and Privett, 1980; De Schrijver and Privett, 1983; De Gomez Dumm et al, 1984). Especially n-3 fatty acids have a strong influence on the linoleic acid metabolism (Mohrhauer and Holman, 1963; De Schrijver and Privett, 1982a; Holman et al, 1983). In contrast, the Δ5-desaturase activity is increased by dietary polyunsaturated fatty acids and decreased by fasting (Jeffcoat and James, 1977; De Gomez Dumm et al, 1984). Dietary polyunsaturated fatty acids strongly depress the activity of the Δ9-desaturase (Inkpen et al, 1969; Jeffcoat and James, 1977; De Schrijver and Privett, 1983). In rats fed diets with a sufficient essential fatty acid content (3 - 4 % of total energy) the conversion of linoleic acid to arachidonic acid is reported to be slow (Pascaud and Strouvé, 1968; Sinclair, 1975) and is a minor route for linoleic acid metabolism.

Eicosanoid synthesis

Eicosanoids is the generic name for hormone-like substances derived from C20 polyunsaturated fatty acids. Arachidonic acid is the most common

precursor of this class of compounds which encompasses prostaglandins, thromboxanes and leukotrienes (for reviews see: Higgs et al, 1986; Samuelsson, 1986). Stimulation of a tissue results in the release of free polyunsaturated fatty acids (i.e. arachidonic acid) from phospholipids. Subsequently these free fatty acids are rapidly converted into hydro peroxides by cyclooxygenase or lipoxygenases, located in the endoplasmatic reticulum. Cyclooxygenase forms endoperoxides, precursors for a whole range of prostaglandins (e.g. $PGF_{2\alpha}$, PGE_2, PGD_2, PGI_2 (prostacyclin)) and thromboxane A_2 (TXA_2). A number of lipoxygenases convert polyunsaturated fatty acids into hydroperoxy-acids, which are rapidly transformed to hydroxy fatty acids (e.g. hydroxy-eicosatetra-enoic acid (HETE)) and to leukotrienes. A schematic overview of the arachidonic acid metabolism is given in Fig. 4.

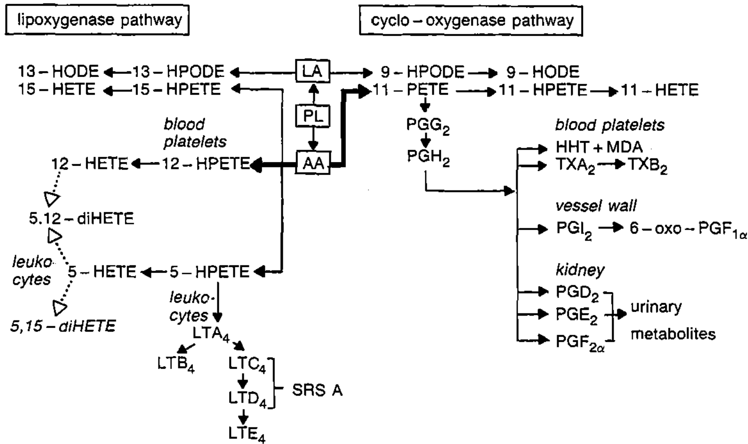

Fig. 4 Scheme of transformations of arachidonic acid by the cyclo-oxygenase and lipoxygenase pathways. Adapted from: J.J. Gottenbos (1985). The role of linoleic acid, in: The role of fats in human nutrition (Padley, F.B. and Podmore, J., eds.). Ellis Horwood Ltd., Chichester, England.

In platelets arachidonic acid is metabolized primarily to TXA_2, 12-hydroxy-heptadecatetraenoic acid (HHT: a co-product of the TXA_2-synthesis) and 12-HETE (Wolfe, 1982; Sprecher, 1986). Thromboxane A_2 is a potent stimulant of platelet aggregation and a vasoconstrictor (see Granström et al, 1982, for a review). In contrast, prostacyclin, the main arachidonic acid metabolite in vascular tissue is a potent inhibitor of platelet aggregation and relaxes arterial smooth muscle (see Dusting et al, 1982, for a review). So, both the anti-aggregatory

PGI_2 and the pro-aggregatory TXA_2 are formed from the same precursor arachidonic acid, via the same intermediate. It has been proposed that the balance between TXA_2 and PGI_2 determines arterial thrombosis tendency (Moncada and Vane, 1978). This ratio certainly plays an important role in this process; however, the exact regulation of thrombosis is not yet fully understood. Apart from platelets and vascular tissue, kidney (PGE_2, $PGF_{2\alpha}$), intestine (PGD_2), stomach fundus (PGI_2), liver, spleen and lung are capable of producing high amounts of eicosanoids (Nugteren et al, 1966; Pace-Asciak and Rangaraj, 1977; Marshall et al, 1987; Kivits and Nugteren, 1988).

Many naturally occuring polyunsaturated fatty acids can be converted by cyclooxygenase of sheep vesicular glands in vitro (Van Dorp, 1964; Beerthuis et al, 1971; Nugteren and Christ-Hazelhof, 1987). Except for arachidonic acid, especially n-6 polyunsaturated fatty acids like dihomo-γ-linolenic acid (20:3), dihomo-linoleic acid (20:2), columbinic acid and γ-linolenic acid (18:3) are oxygenated in good yield. But also α-linolenic acid (18:3) and eicosapentaenoic acid (20:5), both n-3 fatty acids are oxygenated. These fatty acids could therefore compete with arachidonic acid for oxygenation by cyclooxygenase. However, inhibition by other common polyunsaturated fatty acids of the n-6 and n-3 family is only significant at high concentrations compared to that by arachidonic acid (Pace-Asciak and Wolfe, 1968), as cyclooxygenase has a strong preference for arachidonic acid. It should be kept in mind that only dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid can be converted to prostaglandins to a significant degree.

Dietary polyunsaturated fatty acids can significantly alter the eicosanoid biosynthesis in rats. Linoleate or linoleic acid-rich oils often increase prostaglandin synthesis (Hwang et al, 1975; Nugteren et al, 1980; Ten Hoor et al, 1980; Mathias and Dupont, 1985; Blankenship et al, 1986) while essential fatty acid deficiency consistently depresses eicosanoid production. Also dietary fatty acids of the n-3 series depress the synthesis of eicosanoids (Hwang and Carroll, 1980; Ten Hoor et al, 1980; Hornstra et al, 1983; Blankenship et al, 1986). Precursor fatty acid availability (i.e. arachidonic acid) seems to be an important determinant of eicosanoid biosynthesis (Hwang and Kinsella, 1978; Hwang and Carroll, 1980; Hornstra et al, 1983).

The turnover of arachidonic acid via eicosanoids in rats on normal diets is low: the total excretion of the main prostaglandins or their metabolites via urine is estimated at 15 - 25 μg/day (Nugteren et al, 1980; Zijlstra and Vincent, 1985). Ramesha et al. (1985) reported a somewhat higher production (50 - 100 μg/day). This is only a fraction of the total intake of n-6 fatty acids, the main prostaglandin precursors in chow-fed rats (about 0.5 g/day). Dietary linoleic acid and especially arachidonic acid increased the urinary excretion of prostaglandin metabolites severalfold (Nugteren et al, 1980; Ramesha et al, 1985).

Oxidation

Oxidation of fatty acids serves to supply the body with relatively large amounts of energy and with smaller components for use in the synthesis of other compounds (i.e. acetyl-CoA). β-Oxidation in mitochondria is probably by far the most important mechanism by which fatty acids are degraded to acetyl-CoA and water and finally to CO_2 by the citric acid cycle. In the liver and to some extent in the kidneys, the acetyl-CoA can also be converted into ketone bodies, which are fuel for other tissues, e.g. brain. Polyunsaturated fatty acids require three auxiliary enzymes in addition to the enzymes necessary for the complete β-oxidation of saturated fatty acids in mitochondria (for a recent review see: Bremer and Osmundsen, 1984). Recently a revised pathway was proposed in which 2,4-dienoylCoA reductase functions as an auxiliary enzyme. Both mitochondria and peroxisomes are capable of oxidizing polyunsaturated fatty acids via this pathway (Hiltunen et al, 1983; Hiltunen et al, 1986; Schulz and Kunau, 1987). The peroxisomal β-oxidation oxidizes fatty acids only partly (2 - 5 cycles), resulting in the release of shortened fatty acids. These fatty acids are further metabolized by the mitochondria. Peroxisomes may be involved in the retroconversion of very-long-chain polyunsaturated fatty acids, like 22:4 n-6 into arachidonic acid (Christensen et al, 1986). In hepatocytes from fasted rats, arachidonic acid was mainly oxidized by mitochondria (Christensen et al, 1986), although a distinct role for peroxisomal arachidonic acid oxidation was observed. Eicosapentaenoic acid is

oxidized by peroxisomes to a higher extent than arachidonic acid. In the perfused rat liver, it was found that except for arachidonic acid, the rate of peroxisomal β-oxidation of a number of unsaturated fatty acids was almost proportional to the mitochondrial rate of oxidation (Hiltunen et al, 1986). The relative contribution of peroxisomes to the polyunsaturated fatty acid β-oxidation in the intact animal under normal metabolic conditions is still under debate (Masters and Crane, 1984; Osmundsen, 1985).

Peroxisomal β-oxidation can be stimulated by high-fat diets, particularly diets containing a high amount of fatty acids that are poorly oxidized by mitochondria (i.e. very-long chain monounsaturated fatty acids). The mitochondrial oxidation is affected to a much lesser extent (Bremer and Osmundsen, 1984).

Of various unsaturated fatty acids investigated (18:1 and the n-6 fatty acids 18:2, γ-18:3, 20:4 and 22:6) the rate of oxidation of γ-linolenic acid was by far the fastest and that of arachidonic acid slowest in peroxisomes. In mitochondria arachidonic acid is oxidized at a similar rate as other polyunsaturated fatty acids (Hiltunen et al, 1986). The oxidation rates of oleic acid and linoleic acid in both mitochondria and peroxisomes are quite similar. γ-Linolenic acid and docosahexanoic acid were slowly oxidized by mitochondria compared to the rate of oxidation in peroxisomes and are also powerful potential inhibitors of mitochondrial oxidation (Hiltunen et al, 1986). Peroxisomal β-oxidation is particularly well suited for the catabolism of fatty acids which are poor substrates for mitochondrial β-oxidation (Bremer, 1977).

In agreement with the data obtained in cellular fractions, the rate of oxidation of oleic acid and linoleic acid to CO_2 and acid-soluble products in hepatocytes of fasted rats was very similar (Christensen et al, 1986). Arachidonic acid and eicosapentaenoic acid were slightly less rapidly catabolized.

The expiration of CO_2 as a measure of total oxidation of a number of fatty acids was determined in weanling rats. Of the n-6 fatty acids, linoleic acid was oxidized at a higher rate than γ-linolenic acid, which

in turn was oxidized faster than arachidonic acid and dihomo-γ-linolenic acid (Leyton et al, 1987). Also in adult rats, the catabolism of arachidonic acid was significantly lower than that for other fatty acids. This seems to be connected with its high incorporation into tissue phospholipids (Coots, 1964a; Coots, 1965). Generally, there is a strong inverse relationship between the incorporation of fatty acids into structural lipids (phospholipids) and oxidation (McGarry et al, 1973; Stakkestad and Lund, 1984).
The rates of fatty acid oxidation and of ketogenesis are mainly regulated by the concentration of free fatty acids offered to the tissues. However, especially in the liver, the nutritional and hormonal state of the animal has a strong influence on fatty acid oxidation (for a review see: Bremer and Osmundsen, 1984).

1.4. Review of effects of *trans* fatty acids on linoleic acid metabolism in the rat

Trans fatty acids are often viewed as one single group with identical properties, but this is a gross oversimplification. A distinction should be made between monoenoic and polyenoic *trans* fatty acids (Houtsmuller, 1978). The most common *trans* fatty acids in the diet are by far the monoenoic isomers. These *trans* isomers in partially hydrogenated oils and in ruminant fats form a heterogeneous group of positional isomers with a double bond on positions 6 to 16 (Dutton, 1979; Ohlrogge, 1983). In industrially hydrogenated fats the δ8- to δ11-*trans* isomers are the most abundant (Dutton, 1979), while bio-hydrogenated fats (e.g. butter) contain predominantly the δ11-isomer (Parodi, 1976). The metabolism of each isomer may differ significantly (for recent reviews see: Emken, 1983, 1984), and the biological effects of the isomers could vary accordingly.

A number of *trans* isomers of linoleic acid has also been identified in dietary lipids, especially in partially hydrogenated soybean oil, as a consequence of the partial hydrogenation of linolenic acid and

linoleic acid. Due to the extremely difficult analysis of the various *trans* dienes, our knowledge on the consumption of these *trans* fatty acids is limited. It is estimated that *trans* linoleic acid isomers do not constitute more than 10% of the total *trans* fatty acid intake (Adlof and Emken, 1986). The best known representative of this group of isomeric fatty acids is linolelaidic acid (9*t*,12*t*-18:2), which can be synthesized fairly easily and has been used in many in vitro and animal studies (Privett et al, 1967; Kinsella et al, 1981; Hwang et al, 1982). However, as pointed out in some recent reviews, the amount of this fatty acid in commercial partially hydrogenated oils is very low (Beare-Rogers, 1983; Emken, 1983). As a consequence, the total dietary intake of 9*t*,12*t*-18:2 by humans is very low (Brussaard, 1986; Van Den Reek et al, 1986).

1.4.1. In vitro studies

In the following section, in vitro studies on the effects of (mainly C18) *trans* fatty acids on some polyunsaturated-fatty-acid-metabolizing enzyme systems are discussed. The metabolism of isomeric fatty acids in in vitro systems is also included. The second part of this paragraph reviews some rat feeding studies with *trans* fatty acids. Especially interferences of *trans* fatty acids with the polyunsaturated fatty acid metabolism are discussed.

Activation

The activation rate of 9*t*-18:1, 11*t*-18:1 and 9*t*-18:2 by acyl-CoA synthetase in both microsomes and mitochondria of rat liver is reported to be higher than that of the corresponding *cis*-isomers (Lippel, 1973). In this system, without added albumin *trans* isomers are more like saturated fatty acids in this respect than like *cis*-isomers. The activation of *trans* octadecenoic acids with double bonds at positions 8 to 12 was slower than that of the other positional *trans* isomers (Lippel et al, 1973). Neither the melting points of the fatty acids nor their solubility correlated with the activation

profile. However, when albumin was added to the system no differences in activation of *cis*- and *trans*-isomers could be found (Lippel, 1973). This is in accordance with the data of Normann et al. (1981). They found no effect of the configuration of the double bond in a number of monoenoic acids (9-16:1, 6-18:1, 9-18:1, 11-18:1 and 13-22:1) and a dienoic acid (9,12-18:2). Also the position of the double bond was of minor importance, except for 22:1, which revealed a 2-fold lower activity of the 13-isomer than the 11-isomer. As in vivo albumin, or other fatty acid binding proteins, are always present, it is not likely that biologically significant differences in the activation rate between *trans* and *cis* isomers exist in vivo. Differences in the metabolism of *trans* and *cis* fatty acids are most likely due to specificity differences in enzymatic reactions following the activation with CoA (Normann et al, 1981).

Incorporation

Both the supply of a fatty acid and its suitability as a substrate for acyltransferases affect the extent to which it appears in cellular phospholipids. All evidence on the selectivity for acyltransferase acting at the 1-position indicates a strong influence of conformation upon the rate of incorporation. The rate of incorporation at the 1-position of PC by acyltransferase in rat liver microsomes was higher for the most common *trans* isomers than for 9*c*-18:1. The acyltransferase activity for the δ10-*trans* isomer, however, was remarkably low (Okuyama et al, 1972). Reitz et al. (1969) reported that the common *cis*-18:1 isomers, 9 and 11, are poor substrates for transfer to the 1-position of PC. On the other hand, the 1-acyl-PC-acyltransferase seems to have a higher affinity for fatty acids with double bonds at the 5-, 9- and 12-position, irrespective of the geometrical configuration. Oleic acid was the preferred substrate of all 18:1-isomers at the 2-position of PC. In vitro experiments with rat liver microsomal protein indicated that 9*t*-18:1 was preferentially incorporated at the 1-position of PC, whereas the 9*c*-isomer was predominantly attached to the 2-position (Lands et al, 1966). Linolelaidic acid, 9*t*,12*t*-18:2, was

incorporated at a much higher rate in the 1-position than in the 2-position. Just as the 9*c*,12*c*-isomer (and 18:3 and 20:4), 9*c*,12*t*-18:2 displayed a higher preference for the 2-position than for the 1-position. There was no preference for either position of the 9*t*,12*c*-isomer. The rates of incorporation of all investigated *trans* fatty acids at the 2-position exceeded those of saturated fatty acids. The two investigated *trans* fatty acids without *cis*-double bonds (9*t*-18:1 and 9*t*,12*t*-18:2) showed an even higher rate of transfer into the 1-position of PC than saturated fatty acids (Lands et al, 1966). The patterns of acyltransferase specificities for octadecenoic acids for incorporation into PE were very similar to those for PC (Okuyama et al, 1972). For acyl-transfer activities by acyltransferase from rat liver mitochondria, similar preferences as for microsomal preparations have been found (Lands, 1966).

From these in vitro data it can be predicted that most *trans* monoenoic fatty acids and 9*t*,12*t*-18:2 will effectively compete with saturated fatty acids for incorporation at the 1-position of phospholipids. In the presence of linoleic acid and/or other polyunsaturated fatty acids, these isomeric fatty acids will predominantly be incorporated at the 1-position and will not interfere directly with the incorporation of polyunsaturated acids at the 2-position. However, the pattern of incorporation of individual *trans* monounsaturated fatty acids into phospholipids, as observed in vivo (Wood and Chumbler, 1978; Reichwald-Hacker et al, 1979) does not correlate with in vitro specificities of acyl-CoA synthetases and transferases towards the individual octadecenoyl-CoA (Reichwald-Hacker et al, 1979). The *cis*,*trans*-dienes have some affinity for the 2-position and therefore compete with polyunsaturated fatty acids.

Desaturation and elongation

Effects of isomeric fatty acids on desaturation and elongation processes have been investigated in vitro and in vivo. For in vitro studies rat liver microsomes have been used most frequently. Essential-fatty-acid-

deficient (EFAD) rats were used in the majority of the studies in order to have an active desaturation system, as both the Δ9- and the Δ6-desaturation systems are activated by EFAD. Under non-saturating conditions, low concentrations of fatty acids (substrate and inhibitor) and a relatively high amount of microsomal fraction, the competition between the fatty-acid-metabolizing enzymes for the available fatty acids is studied. In this system, with inhibitor/substrate ratios of much more than 1, 9*t*-, 9*c*-, 6*c*- and 7*c*-18:1 and 9*t*, 12*t*-18:2 showed only a very mild inhibition on the Δ6-desaturation of linoleic acid (Brenner and Peluffo, 1969), whereas α-linolenic acid was strongly inhibitory. Saturating conditions, high fatty acid levels and a low microsome level, giving a direct competition between fatty acids for the available enzymes, e.g. desaturases, are more generally used to investigate effects of isomeric fatty acids on desaturation and elongation processes. Brenner and Peluffo (1969) showed that elaidic acid (9*t*-18:1) had hardly any effect on the Δ6-desaturation, 9*t*, 12*t*-18:2 inhibited slightly, but not significantly more than oleic acid (9*c*-18:1). Again, only a polyunsaturated fatty acid, like γ-linolenic acid could inhibit the linoleic acid desaturation more than 50%.
The effects of the position of the double bonds in *trans*- and *cis*-octadecenoic acids on the Δ9-, Δ6- and Δ5-desaturation were extensively investigated. With inhibitor-to-substrate ratios of 3 under saturating conditions, the maximal inhibition of the Δ9-desaturation displayed by *trans* octadecenoic acids was about 50% compared to that of stearic acid (Mahfouz et al, 1980a). The δ3, δ5, δ7, δ10, δ12, δ13 and the δ16 isomers inhibited most. Also the Δ6-desaturation of linoleic acid could be inhibited by 50% maximally by some *trans* fatty acids compared to stearic acid, of which δ3, δ4, δ7 and δ15 were the most active (inhibitor/substrate ratio's of 3). The Δ5-desaturation of dihomo-γ-linolenic acid, at inhibitor/substrate ratio of 6, was inhibited mainly by the δ3-, δ9-, δ13- and the δ15-*trans* monoenoic acids. Lineweaver-Burk plots of the kinetic data, although not strictly applicable, suggest that the inhibitions found are competitive. In a similar study with *cis*-monoenoic acids it was shown that the sensitivity of the

desaturation enzymes to double bond configuration is different for *cis* fatty acids than for *trans* fatty acids (Mahfouz et al, 1981). Some *cis* monoenoic acids inhibited the Δ9-, Δ6- and Δ5-desaturases also to about 50% compared to stearic acid. In a direct comparison, three *trans* monoenoic acids (6*t*, 9*t* and 11*t*) were equally or less inhibitory on the Δ9-desaturation of stearic acid than their *cis* isomers (Chang et al, 1973). In fresh liver microsomes from chow-fed rats 9*t*,12*t*-18:2 inhibited the Δ6-desaturation of linoleic acid 23% compared to oleic acid (Shimp et al, 1982). No information is available on the effect of *trans* polyenoic isomers other than linolelaidic acid, on desaturation enzymes.

Apart from influencing the metabolism of other fatty acids, *trans* fatty acids can be desaturated and elongated too. Pollard et al. (1980a,b) investigated the desaturation of a number of *cis* and *trans* monoenoic and dienoic fatty acids, by rat liver microsomes, under non-saturating conditions to obtain high yields of products for analyses. Most *trans*-18:1 isomers were desaturated by the Δ9-desaturation to a similar degree as stearic acid, except of course the δ8-, δ9- and δ10-isomers where Δ9-desaturation is not possible. *Cis* isomers were clearly desaturated less well. When the Δ9-desaturase was inhibited, some *trans* isomers were also converted by the Δ6- or Δ5-desaturation systems: 8*t*-18:1 and 10*t*-18:1 giving 6*c*,8*t*- and 6*c*,10*t*-18:2 and 9*t*-, 14*t*- and 15*t*-18:1 giving 5*c*,9*t*-, 5*c*,14*t*- and 5*c*,15*t*-18:2. Conjugated dienes could thus be formed in vitro. Some *trans* dienoic fatty acids were extensively Δ6-desaturated (compared to linoleic acid): 9*c*,11-13*t*-18:2 for more than 25% in this system, while the 9*c*,14-15*t*-isomers were hardly converted. Linolelaidic acid (9*t*,12*t*-18:2) was not desaturated via the Δ6-desaturation pathway, but small quantities of 5*c*,9*t*,12*t*-18:3 were found. The microsomal systems used in these studies contain all desaturation and elongation enzymes; so each fatty acid added may be a substrate for the Δ9-, Δ6-, Δ5- or Δ4-desaturation and elongation.

Trans monoenoic acids were also reported to undergo an isomerisation under conditions optimized for the desaturation of oleic acid. The

Table 1. Unsaturated fatty acids formed from *trans* fatty acids in rat liver microsomes in vitro, under various conditions.

substrate	product	reaction[a]	ref[b].
5*t*-16:1	5*t*,9*c*-16:2	Δ9	1.
7*t*-16:1	7*t*,9*c*-18:2	Δ9	1.
8*t*-16:1	6*c*,8*t*-16:2	Δ6	1.
5*t*-17:1	5*t*,9*c*-17:2	Δ9	1.
9*t*-17:1	5*c*,9*t*-17:2	Δ5•	2.
12*t*-17:1	9*c*,12*t*-17:2	Δ9	1.
4*t*-18:1	4*t*,9*c*-18:2	Δ9	3.
,,	4*c*,9*c*-18:2	Δ9,I	3.
5*t*-18:1	5*t*,9*c*-18:2	Δ9	1.
,,	5*c*,9*c*-18:2	Δ9,I	3.
6*t*-18:1	6*t*,9*c*-18:2	Δ9	3.
,,	6*c*,9*c*-18:2	Δ9,I	3.
7*t*-18:1	7*t*,9*c*-18:2	Δ9	3.
,,	9*t*-20:1	E	4.
8*t*-18:1	6*c*,8*t*-18:2	Δ6	1.
,,	10*t*-20:1	E	4.
9*t*-18:1	5*c*,9*t*-18:2	Δ5	1.
,,	11*t*-20:1	E	4.
10*t*-18:1	6*c*,10*t*-18:2	Δ6	1.
,,	5*c*,10*t*-18:2	Δ5	1.
,,	12*t*-20:1	E	4.
11*t*-18:1	9*c*,11*t*-18:2	Δ9	1.,3.
,,	9*c*,11*c*-18:2	Δ9,I	3.
,,	13*t*-20:1	E	4.
12*t*-18:1	9*c*,12*t*-18:2	Δ9	3.
,,	9*c*,12*c*-18:2	Δ9,I	3.
,,	14*t*-20:1	E	4.
13*t*-18:1	9*c*,13*t*-18:2	Δ9	1.,3.
,,	9*c*,13*c*-18:2	Δ9,I	3.
14*t*-18:1	9*c*,14*t*-18:2	Δ9	1.,3.
,,	9*c*,14*c*-18:2	Δ9,I	3.
15*t*-18:1	9*c*,15*t*-18:2	Δ9	1.,3.
9*t*-20:1	5*c*,9*t*-20:1	Δ5	2.
9*c*,12*t*-17:2	6*c*,9*c*,12*t*-17:3	Δ6	1.
9*c*,11*t*-18:2	6*c*,9*c*,11*t*-18:3	Δ6	1.
9*c*,12*t*-18:2	6*c*,9*c*,12*t*-18:3	Δ6	1.
9*c*,13*t*-18:2	6*c*,9*c*,13*t*-18:3	Δ6	1.
9*c*,14*t*-18:2	6*c*,9*c*,14*t*-18:3	Δ6	1.
9*c*,15*t*-18:2	6*c*,9*c*,15*t*-18:3	Δ6	1.
9*t*,12*t*-18:2	5*c*,9*t*,12*t*-18:3	Δ5	2.

a) Δ9, Δ6 and Δ5 are respective desaturations; I stands for isomerization and E for elongation.

b) References: 1) Pollard et al, 1980a; 2) Pollard et al, 1980b; 3) Mahfouz et al, 1980b; 4) Kameda et al, 1980.

9*c*,x*t*-18:2 fatty acids resulting from desaturation of x*t*-18:1, were isomerised to 9*c*,x*c*-18:2 (Mahfouz et al, 1980b). Especially the 5*t*-isomer gave relatively high yields of 5*c*,9*c*-18:2.

Finally, the elongation of *trans* monoenoic acids was investigated in a microsomal system optimised for the elongation of oleic acid (Kameda et al, 1980). The 4*t*-, 5*t*-, 6*t*-, 13*t*-, 14*t*- and 15*t*-isomers were not elongated at rates above experimental error. The *trans* isomers with a double bond between positions 6 and 12 were elongated at a rate of 40% or less than that of 9*c*-18:1.

All fatty acids which were found to be formed from *trans* fatty acids by rat liver microsomes in vitro, under various conditions, are given in Table 1.

These in vitro studies show possible pathways of metabolism of *trans* fatty acids. However, it must be emphasized that the reported findings are obtained in artificial systems in which the microsomes were supplied with high amounts of *trans* fatty acids. Under normal conditions in vivo, the cells will always have the availability of many types of fatty acids, including the common substrates for the desaturation and elongation enzymes, and *trans* fatty acids will generally be available in much lower amounts. Moreover, the desaturation enzymes display a high affinity for their "normal" substrates, like the Δ9-desaturation for palmitic and stearic acid, the Δ6-desaturation for linoleic and linolenic acid and the Δ5-desaturation for dihomo-γ-linolenic and 8,11,14,17-eicosa-tetraenoic acid. A good example of these "natural" preferences is that 5,8,11-20:3 ("Mead's acid") can only be formed by Δ6- and Δ5-desaturation and elongation from oleic acid in vivo when the linoleic acid and linolenic acid availability is very low (e.g. in EFAD).

Therefore, although some inhibition of desaturation systems by *trans* fatty acids can be measured in vitro, this is no proof that *trans* fatty acids will interfere with the conversion of fatty acids in vivo under normal conditions, i.e. when the diet is well balanced in its fatty acid composition.

Prostaglandin synthesis

Only two studies on the effect of *trans* isomers on prostaglandin synthesis in vitro have been reported. In those studies, microsomal preparations of vesicular glands of sheep have been used. Nugteren (1970) found that only *trans* fatty acids with a *trans,cis*-mono-conjugated system inhibited the conversion of dihomo-γ-linolenic acid into PGE_1 very strongly. Other *trans* isomers of the substrate or of arachidonic acid did not inhibit this synthesis more than *cis* polyunsaturated fatty acids. This was recently re-confirmed by Nugteren and Christ-Hazelhof (1987).

Oxidation

Rat heart mitochondria oxidized CoA-esters of elaidic acid and 7*t*-16:1 at a significantly lower rate than esters of the corresponding *cis* isomers and stearic or palmitic acid (Lawson and Kummerow, 1979). Most *trans* octadecenoic isomers displayed a lower rate of oxidation than the *cis* isomers in liver and heart mitochondria (Lawson and Holman, 1981). This was especially marked for the δ9- and δ11-isomers. But the δ8-, δ10-, and δ14-*trans* isomers were catabolized faster than the *cis* isomers. Most *trans* isomers were also oxidized more slowly than stearic acid. In another type of study, radio-labelled 18:1- and 18:2-isomers were incubated with rat liver mitochondria. With this technique the slower oxidation of elaidic acid compared to oleic acid was confirmed (Anderson, 1967). The mono-*trans*-isomers (mixture of 9*t*,12*c*- and 9*c*,12*t*-18:2) and 9*t*,12*t*-18:2 were catabolized to $^{14}CO_2$ more rapidly than linoleic acid (Anderson, 1968). No shorter chain fatty acids were detected, so all isomers were completely oxidized via β-oxidation by mitochondria. In contrast to mitochondria, isolated peroxisomes oxidized *trans* monoenoic CoA-esters at similar rates as saturated fatty acid esters and faster than the corresponding *cis* isomers (Osmundsen et al, 1979; Neat et al, 1981). Rat heart homogenates preferentially oxidized oleic acid compared to elaidic acid: the former was oxidized 35 - 40% faster (oxidation measured as the sum of the $^{14}CO_2$-production and amount of ^{14}C-acid

soluble products) (Lanser et al, 1986). In 1966, Willebrands and Van Der Veen described the formation of shortened fatty acids from *t*-octadecenoic acids in perfused rat heart. The product 5*t*-14:1 was identified after perfusion with 9*t*-18:1. This was believed to be a product from partial β-oxidation in mitochondria at that time. We now know that peroxisomal β-oxidation of *t*-18:1 isomers could yield *t*-12:1 and *t*-14:1 isomers. Finally, contrary to the rate of oxidation in isolated mitochondria, in perfused rat liver, the oxidation of *trans* fatty acids to ketonebodies is much faster than that for *cis* isomers. This applied for elaidic versus oleic acid and linolelaidic versus linoleic acid (Ide and Sugano, 1984, 1986a,b).

1.4.2. In vivo studies

Incorporation and conversions of *trans* fatty acids

Feeding *trans* fatty acid isomers generally results in incorporation in various tissue lipid classes. In most experiments partially hydrogenated vegetable oils (PHVO) have been used, thus supplying rats with various *trans* isomers. Except under extreme conditions, hydrogenated fat has generally little influence on tissue lipid class composition (Emken, 1984). *Trans* octadecenoates were found in all tissues, but concentrations were dependent on dietary level, tissue and lipid class. A high dietary supply of these acids may give rise to a deposition of 15 - 20% in tissue lipids (Wood, 1979; Moore et al, 1980; Masuzawa et al, 1987). As generally similar maximum values are reported, this could indicate that a limit to the incorporation of *trans* fatty acids isomers in lipids exists. The incorporation of *trans* fatty acids in heart phospholipid increased with increasing content in the diet, but seemed to level off at a dietary level of about 16 en% (Hill et al, 1979). The degree of incorporation of *trans*-monoenes was not influenced by the linoleic acid content of the diet (Hill et al, 1982; Blomstrand and Svensson, 1983; Blomstrand et al, 1985). Brain lipid was generally found to incorporate the lowest amount of *trans* fatty acids, liver and adipose tissue the highest. Wood and co-workers and Reichwald-

Hacker and co-workers have performed elaborate rat feeding studies with partially hydrogenated safflower oil and soybean oil respectively, analyzing incorporation of positional isomers of *trans* monoenoic acids in a number of tissues (Wood and Chumbler, 1978; Reichwald-Hacker et al, 1979; Wood, 1979). The distribution of *trans* monoenoic acids in triacylglycerols resembled that of the diet. Generally, adipose tissue fatty acid compositon reflects the composition of the dietary fatty acids reasonably well and does not selectively incorporate or exclude any specific isomeric fatty acid (Emken, 1984). In contrast, the percentage distribution in phospholipid classes differed dramatically from the diet. Most tissues incorporated much less δ10-isomer than could be expected from the dietary composition. The δ12-, δ13- and δ14-isomers were incorporated to a higher extent than expected. The difference in isomer pattern between the two main phospholipid classes, PC and PE, was small, although PE incorporated significantly more *t*-18:1 than PC in heart mitochondria (Blomstrand and Svensson, 1983). *Trans* monoenoic acids, like saturated fatty acids, were incorporated predominantly at the 1-position of both PC and PE in liver and heart. Small amounts of δ9 to 12-isomers, however, were found at the 2-position. In the triacylglycerols *trans* octadecenoates were preferentially esterified at the 1,3-positions. Also cholesterolesters may contain relatively large proportions of *trans* octadecenoates, of which the *trans*-9-octadecenoyl moieties were found to be the most predominant constituents.

The relative distribution of *t*-18:1 isomers in total lipids of rat liver mitochondria did not depend on the level of partially hydrogenated peanut oil in the diet (Høy and Hølmer, 1979).

In agreement with in vitro acyltransferase activities, the most common *trans* fatty acids seem to compete mainly with saturated fatty acids for incorporation into phospholipids. In many studies, the increase in deposition of *trans* fatty acids is largely compensated for by a decrease in saturated fatty acids (Blomstrand and Svensson, 1983; Blomstrand et al, 1985; Masazuwa et al, 1987).

In animals fed a diet containing *trans* octadecenoic acids without detectable amounts of hexadecenoates, the 16:1 fraction from the lipid classes of all the tissues was nevertheless composed of 10 - 70% of *trans* isomers, indicating chain shortening of the dietary *trans*-octadecenoates (Wood, 1979). *Cis*-octadecenoates were apparently not shortened. C18 fatty acids with one or more *trans* double bonds, arising from dietary *trans* monoenoic fatty acids from PHVO or trielaidate in vivo, were found (Lemarchal and Munsch, 1965; Guo and Alexander, 1974; Lawson et al, 1982). 5*c*,9*t*-18:2 was identified as the main product of the conversion of *trans* monoenoic fatty acids (Lemarchal and Munsch, 1965), incorporated at a level of about 5% in liver phospholipid in EFAD-rats. This level decreased much when linoleic acid was supplied to the diet (Guo and Alexander, 1974).

Feeding *trans* isomers of linoleic acid (either as free fatty acid or methyl-ester or via PHVO) also resulted in incorporation in tissue lipids. Linolelaidic acid was deposited in phospholipids, cholesterol-esters and triglycerides of all tissues examined (Privett and Blank, 1964). The level in liver lipids was proportional to that of the diet (Anderson et al, 1975) and reached a maximum of 5% in liver lipids (with a dietary level of 8 en%). When a mixture of 9*c*,12*t*- and 9*t*,12*c*-18:2 was fed, in the presence of a marginal amount of linoleic acid, the *c*,*t*-18:2 isomer was only slightly incorporated in liver lipids, while the *t*,*c*-isomer was readily deposited. Earlier work had already shown that in agreement with in vitro acyltransferase specificities, 9*c*,12*t*-18:2 is predominantly esterified in the 2-position of PC in EFAD-rats, while *t*,*t*-18:2 was incorporated at the 1-position (Privett et al, 1966). Probably the former isomer competes only weakly with polyunsaturated fatty acids and consequently is almost totally excluded from liver phospholipid. The *t*-18:2 isomers were deposited in adipose tissue at levels proportional to their levels in the diet (Anderson et al, 1979).

Linolelaidic acid was not converted into arachidonic acid in the live rat (even under EFAD-conditions) (Privett and Blank, 1964; Privett et al, 1967). However, Knipprath and Mead (1964) reported a labelled 20:4 isomer in rats fed radiolabelled linolelaidic acid. The formation of low amounts of radiolabelled 20:4 from *t,t*-18:2 in developing rat brain was confirmed by Cook (1980). However, these findings with radio-labelled fatty acids could be explained by elongation of γ-linolenic acid with radiolabelled acetate derived from β-oxidation of *t,t*-18:2. A *trans* isomer of 20:4 (presumably 5*c*, 8*c*, 11*c*, 14*t*) was found in EFAD-rats fed 9*c*,12*t*-18:2 (Blank and Privett, 1963; Privett et al, 1967). However, this conversion was not nearly as efficient as that of 9*c*,12*c*-18:2. The 9*t*,12*t*-isomer did not yield significant levels of *t*-20:4. A *trans* isomer of linolenic acid was converted to a *trans*-20:5. Even in non-EFAD conditions (about 0.8 en% linoleic acid), a mixture of 9*c*,12*t*/9*t*,12*c*-18:2 gave rise to formation of *t*-20:4 in liver lipids: levels of about 2% were deposited in these lipids (Anderson et al, 1975).
Despite possible conversions of *t*-18:2 isomers, already in the fifties it was demonstrated that *trans* isomers of linoleic acid do not have essential fatty acid activity (Holman, 1951; Privett et al, 1955; Holman and Aaes-Jørgensen, 1956; Mattson, 1960).

Only a few *trans* fatty acid isomers are incorporated in tissue lipids at a higher level than present in the diet. However, none of these isomers accumulated, in the sense that after prolonged feeding of *trans* fatty acids some lipid species with mainly one *trans* isomer appear. With the exception of adipose tissue, where changes in fatty acid composition generally occur slowly, 8 weeks after cessation of *trans* fatty acids-feeding, only neglegible amounts of these isomeric fatty acids were present in rat lipids (Moore et al, 1980), showing that tissues readily metabolize *trans* fatty acids.

Effects of *trans* fatty acids on polyunsaturated fatty acid metabolism and prostaglandin synthesis

Microsomes of rats fed *trans* fatty acids have been used to investigate effects of dietary *trans* fatty acids on the microsomal Δ5-, Δ6- and Δ9-desaturation and elongation. Dietary linolelaidic acid, in the form of methyl- or ethyl-ester or as trilinolelaidate, is repeatedly reported to inhibit the Δ6-desaturation of linoleic acid at relatively low dietary levels of linoleic acid. Effects on the Δ9- and Δ5-desaturation are less consistent.

Shimp and co-workers (1982) fed rats increasing amounts of trilinolelaidate (0 to 5.5 en%) with a constant amount of linoleic acid (1.1 en%) while the remainder of the fat consisted of fully hydrogenated tallow. The Δ6-desaturation of linoleic acid was increasingly inhibited by increasing levels of dietary trilinolelaidate when compared to hydrogenated tallow. However, compared to chow fed animals, only the animals fed the highest dose of trilinolelaidate had a lower Δ6-desaturase activity. The Δ5-desaturation of 20:3 was not influenced by levels of dietary trilinolelaidate up to 2.8 en%; at the highest level (5.5 en%) an increase in activity was found. The authors concluded that it is unlikely that at the levels currently consumed in the U.S, 9*t*,12*t*-18:2 adversely affects essential fatty acid metabolism in man.

Both in essential fatty acid deficient and non-EFAD rats, a mixture of the ethylesters of 9*t*,12*t*-18:2 (ca. 50%), 9*t*-18:1 (ca. 20%) and with or without about 18% *t*,*c*/*c*,*t*-18:2 fed at a level of 10 en%, inhibited the linoleic acid desaturation in isolated liver microsomes under saturating conditions (De Schrijver and Privett, 1982b; Kurata and Privett, 1981). The Δ9-desaturation of stearic acid was increased by the *trans* concentrate, probably as a consequence of its reduced linoleic acid level (De Schrijver and Privett, 1982b). Brenner and co-workers (De Alaniz et al, 1986; De Gomez Dumm et al, 1983) investigated effects of specific fatty acids on the Δ9- and Δ5-desaturation of microsomes of rats fed a fat-free diet supplied with 3 en% methyl esters for only 48 h. Linolelaidic acid did not increase the Δ5-desaturation and Δ9-desaturation compared to polyunsaturated fatty

acids with 9*c* and 12*c* double bonds. The modulation of Δ9- and Δ5-desaturation by dietary n-6 unsaturated fatty acids depends on the presence of the *cis* double bond configuration of these acids.

Microsomes of animals fed a mixture of 9*t*,12*t*-18:2 and 9*t*-18:1 were more sensitive to inhibition of linoleic acid desaturation with exogenous fatty acids than those of hydrogenated coconut oil- or safflower oil-fed animals. In the first group of animals, palmitic acid inhibited the Δ6-desaturation most and elaidic acid least (16:0 > 9*t*,12*t*-18:2 > 18:0 > 9*c*-18:1 > 9*t*-18:1). In the safflower oil-fed animals, 9*t*,12*t*-18:2 gave the highest inhibition, followed by 9*t*-18:1 (Kurata and Privett, 1980). Thus, it appears that feeding the *trans* mixture altered the physical properties of the Δ6-desaturase enzyme system, probably via a change in composition of the microsomal membrane. Linolelaidic acid generally decreases Δ6-desaturase activity, probably in a dose-dependent way. This effect is not only apparent in EFAD. The other desaturases, Δ9 and Δ5, are not significantly affected. Insufficient information is available on effects of the *c*,*t*/*t*,*c*-18:2 isomers on these systems.

Numerous studies have shown that levels of polyunsaturated fatty acids in tissue lipids can be influenced by isomeric fatty acids. But of course, tissue polyunsaturated fatty acid compositions highly depend on their dietary intake. Therefore, effects of *trans* fatty acids on polyunsaturated fatty acid composition in EFAD-rats are of little use in evaluating consequences of consumption of these isomeric fatty acids. A minimum and constant level of essential fatty acids should be present in the diet in order to study effects on polyunsaturated fatty acid compositions in a relevant way. In only a few studies these criteria were met.

Increasing levels of *t*,*t*-18:2, fed as triglyceride inter-esterified with other fatty acids, in the presence of about 1 en% linoleic acid, caused a decrease in arachidonic acid levels in liver, while linoleic acid levels were increased (Anderson et al, 1975). The ratio of arachidonic acid/linoleic acid decreased linearly with

decreasing ratio of dietary linoleic acid/*t*,*t*-18:2. A mixture of *c*,*t*/*t*,*c*-18:2 did not affect liver arachidonic acid levels. Both the *t*,*t*-and the *t*,*c*/*c*,*t*-18:2 decreased the levels of 20:3 n-9 in liver lipids. Also in lungs and heart, arachidonic acid level in the phospholipid fraction was decreased, when rats were fed trilinolelaidate at a level of 0.6 to 6.3 en% (with 1.1 en% linoleic acid) (Bruckner et al, 1983, 1984). When the dietary ratio of *t*,*t*-18:2/*c*,*c*-18:2 surpassed 0.4, arachidonic acid levels in platelet phospholipid were significantly reduced (Hwang et al, 1982). Again, linoleic acid was (slightly) increased.

When it was established that *trans* fatty acids could influence prostaglandin-precursor levels, interest in effects of *trans* fatty acids on prostaglandin biosynthesis developed.
Hwang and Kinsella (1978, 1979) were among the first to report effects of linolelaidate on prostaglandin biosynthesis in rats. A dietary mixture of equal amounts of *trans*,*trans*-linoleate and *cis*,*cis*-linoleate (both 5.5 en%) reduced the blood levels of $PGF_{2\alpha}$, PGE_1 and PGE_2 (determined by radioimmuno assay (RIA)) compared to *cis*,*cis*-linoleate alone. The levels of arachidonic acid in serum and those of eicosatrienoic and arachidonic acid in platelet lipid had also decreased but to a lesser extent. It was concluded that the decrease in precursor level (eicosatrienoic acid, arachidonic acid) probably resulted in the decreased biosynthesis of these prostaglandins. The replacement of 2 en% hydrogenated coconut oil by *trans*, *trans*-linoleate (with 5.5 en% *cis*,*cis*-linoleic acid) diminished the synthesis of TXB_2, $PGF_{2\alpha}$ and 12-HETE (all determined by RIA) by rat platelets slightly but non-significantly (Hwang et al, 1982). Serum levels of TXB_2 were more affected by high doses of *t*,*t*-18:2 than those of 6keto-$PGF_{2\alpha}$ (Bruckner et al, 1983).
Goswami et al (1983) fed rats trilinolelaidate with 1.1 en% linoleate and measured the synthesis of PGE_2, 6keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, TXB_2 and PGD_2 from added [1-^{14}C]arachidonic acid by brain, liver and stomach fundus homogenates in vitro. The synthesis of labelled prostaglandins was

increased in all tissues in this group compared to the group fed no *t,t*-18:2. This apparent increase in prostaglandin synthesis could be due to the reduced amount of substrate arachidonic acid in the tissues (less dilution of the radiolabelled arachidonic acid). These results also suggest that *t,t*-18:2 does not directly inhibit prostaglandin synthetase by competing with the substrates for the active site. The inhibiton of prostaglandin biosynthesis in the above mentioned studies can be explained by diminished levels of substrate fatty acids.

As the *trans* fatty acids in human diets in western societies are largely supplied by partially hydrogenated vegetable oils and only low levels of *trans* dienoic isomers are present, feeding experiments with partially hydrogenated vegetable oils as the source of *trans* fatty acids are more interesting from a nutritional point of view than the experiments with large doses of *trans* dienes. In 1972 Egwim and Kummerow reported an extensive study on the influence of hydrogenated fat on polyunsaturated fatty acid concentrations in various rat tissues. They did not supply the hydrogenated fat with linoleic acid and concluded that the linoleate-deficient nature of this fat rather than its content of *trans* fatty acids decreased the incorporation of n-6 polyunsaturated fatty acids. In moderately EFA-deficient rats, fed only 0.18 en% linoleic acid, partially hydrogenated soybean oil (PHSO) decreased the Δ6- and Δ9-desaturation in liver microsomes, compared to hydrogenated coconut oil by 30 and 19% respectively (Hill et al, 1982). The Δ5-desaturation, however, was not affected. In this report emphasis was put on changes in polyunsaturated fatty acid composition of liver phospholipids. Arachidonic acid was somewhat lowered by the PHSO compared to the coconut oil and linoleic acid was increased. The authors tried to correlate the shifts in the ratio's of polyunsaturated fatty acid products/precursors with the desaturase activities measured in vitro. They concluded that the "status of desaturase" was more precisely shown by GLC-analysis of tissue polyunsaturated fatty acid composition than by enzymatic desaturase assays. However, it should be borne in mind that the animals in the latter experiment were EFAD, which was

manifested in the extremely low levels of polyunsaturated fatty acids in liver phospholipid. In a second experiment in rats fed two levels of PHSO (10 and 20 en%), an increase in total n-6 fatty acids in heart phospholipid with increasing dietary level of linoleic acid (0.5 to 7.5 en%) was observed (Hill et al, 1982). The higher level of PHSO (20 en%) caused lower n-6 fatty acid levels, irrespective of the dietary level of linoleic acid. The deposition of Mead's acid (20:3 n-9) decreased with increasing dietary linoleic acid and no 20:3 n-9 was found with 3 en% linoleic acid. In another study of the same group (Hill et al, 1979), the intake of *trans* fatty acids from margarine stock was varied from 0 to 11%, while keeping the linoleic acid intake constant (but very low), via provision of 0.5% corn oil. The incorporation of n-6 polyunsaturated fatty acids in heart phospholipids decreased, according to the authors, in a lineair fashion. But a sudden drop in n-6 level, at a dietary level of more than 16 en% *trans* fatty acids could also be concluded from the data. The Δ5-desaturase activity in liver microsomes augmented as the *trans* fatty acid level in the diet increased.

This decade some isomeric fatty acid-feeding studies were performed with adequate linoleic acid levels.
Three dietary experiments with rats have been reported in which partially hydrogenated peanut oil (PHPO), containing about 40% *trans* fatty acids, mainly octadecenoic acids, was compared with unhydrogenated peanut oil. Although the hydrogenated oils in these experiments were suppleted with vegetable oils to supply an adequate amount of linoleic acid, the linoleic acid level in the unhydrogenated peanut oils was still much higher (maximally about 2 en% versus 16 en%). In the animals fed the PHPO the Δ6-desaturase activity in liver microsomes was decreased, either significantly (by about 35%) (Kirstein et al, 1983; Hølmer et al, 1982) or not-significantly (by about 20%) (Svensson, 1983). The Δ5-desaturation was either slightly increased (Svenson, 1983) or not influenced by the hydrogenated oil (Kirstein et al, 1983). Analysis of the desaturation capacity under saturating or non-saturating conditions did not change the results (Kirstein et al, 1983). The most striking effect was seen with the Δ9-desaturation activity, which was

increased 4-fold by the PHPO (Svensson, 1983). However, this is probably mainly due to the low linoleic acid content in the diet with this oil.
A PHSO fed at a 30 en% level caused a decrease in the Δ6-desaturation activity compared to an oleic acid-rich sunflower seed oil (with 76% oleic acid) or lard (with 40% saturated fatty acids), all with 10 to 15% linoleic acid (Mahfouz et al, 1984). The Δ5-desaturation was not changed and the Δ9-desaturation was not significantly inhibited compared to the high-oleic-acid sunflower seed oil or lard. The ratio of arachidonic acid/linoleic acid in liver microsomal total lipid and phospholipid was significantly lower than that in the other two groups. This was explained by the decreased Δ6-desaturase activity. The PHSO also decreased the total n-6 fatty acid level compared to the high-oleic acid oil. It was concluded that *trans* octadecenoic acids in partially hydrogenated soybean oil had a more inhibitory effect on EFA metabolism than saturated fatty acids and oleic acid even in the presence of adequate amounts of linoleic acid. However, the PHSO used probably also contained a few percent of *t*-and *c*-dienoic isomers, formed during hydrogenation of α-linoleic acid or linoleic acid.
These results are clearly different from those of Blomstrand et al. (1985). They compared a partially hydrogenated low-erucic acid rapeseed oil (PHRO) (with 36% *t*-18:1, and less than 1% *t*-18:2 isomers), suppleted with sunflower seed oil to increase the linoleic acid content to 12%, with an olive oil with the same linoleic acid level, thus giving diets with 4.6 en% linoleic acid. No significant differences in liver microsomal Δ6-desaturation were detected. The *trans* fatty acids did increase the Δ5-desaturation. The arachidonic acid content of liver microsomal lipid was equal in both groups; linoleic acid was almost double in the PHRO-group. Also in platelet total lipid and various phospholipids, no effect of *trans* fatty acids on arachidonic acid level was observed. So it appears that *t*-monoenoic acids, in the presence of adequate levels of linoleic acid, do not always alter the Δ6-desaturase capacity as measured in vitro. The effect on the Δ5-desaturase is not consistent; the Δ9-desaturase is probably much more sensitive to the dietary level of linoleic acid than to that of *trans* fatty acids.

Comparing a PHSO with beef tallow, in diets with 3 en% linoleic acid and 1 en% linolenic acid, showed that the PHSO generally reduced arachidonic acid levels in liver phospholipid classes (except PS), as well as in heart and testes phospholipid (Lawson et al, 1983). When arachidonic acid levels in phospholipids were correlated with the deposition of isomeric fatty acids, a significant negative correlation was only found for the 12*cis*- and 13*cis*-18:1 isomers. Elaidic acid on the other hand, did not contribute at all to the arachidonic acid reduction. In an attempt to differentiate between effects of *trans* isomeric octadecenoates and *cis*-octadecenoates, a fraction of mainly *t*-18:1 was crystallized from PHSO dissolved in acetone (Lawson et al, 1985). A *cis*-fraction was obtained by precipitation at -20°C. These concentrates were compared with the original PHSO and beef tallow. All these fats were mixed with vegetable oils to provide 3.5 en% linoleic acid and 1.1 en% linolenic acid in the total diet. Compared to the beef tallow, all fats lowered liver PC arachidonic acid levels; linoleic acid was increased by the *trans* fatty acids-containing diets, but decreased in the *cis*-concentrate-fed group. The *cis*-concentrate also lowered n-3 fatty acid levels and increased 20:3 n-9 content. In liver PE, the *cis*-concentrate increased arachidonic acid content, while *trans* fatty acids diminished the level of this acid. The polyunsaturated fatty acid pattern of PE was similarly influenced by *trans* fatty acids as that of PC. The final conclusion was that *trans* octadecenoates inhibit polyunsaturated fatty acid synthesis, whereas *cis*-18:1 isomers appeared to compete with mainly n-3 polyunsaturated fatty acids for acylation at the 2-position of phospholipids.

In one report (Ide et al, 1987) the influence of *trans* fatty acids on liver PC fatty acid composition was compared between two strains of rats: Wistar and Spraque-Dawley. Although the fatty acid pattern differed somewhat in the two species, the responses to *trans* fatty acids were similar (reduction in arachidonic acid and increase in linoleic acid). There is no reason to assume that the influence of isomeric fatty acids on polyunsaturated fatty acid metabolism will differ much between various rat species.

The experiments discussed clearly demonstrated the difficulty in evaluating the influence of dietary *trans* fatty acids on polyunsaturated fatty acid metabolism. The linoleic acid content of the diet, as well as the level of linolenic acid, plays a more important role in this respect than dietary *trans* fatty acids. The experiments with PHVO of course do not allow differentiation of effects between individual isomeric fatty acids, although the results of Lawson et al. (1983), pointing to a dominant role of 12*c*-18:1, are interesting. It thus seems that high levels of dietary *trans* monoenoic acids do not interfere seriously with arachidonic acid synthesis as long as sufficient linoleic acid is available. However, when the content of linoleic acid is lowered, PHVO increase the levels of linoleic acid in tissue phospholipid and decrease arachidonic acid deposition. This could be explained by an inhibited desaturase/elongation activity. However, other enzyme systems like acyltransferases may also play an important role.

Only one experiment investigating the effect of PHVO on prostaglandin synthesis, has been reported. Thrombin-stimulated platelets from rats fed PHRO with 4.6 en% linoleic acid did not produce less 12-HETE and HHT (measured by HPLC) than those of rats fed olive oil (same level of linoleic acid) (Blomstrand et al, 1985). No statistical differences in 6keto-$PGF_{1\alpha}$-production from aorta pieces between those groups were noted. It was concluded that high dietary levels of *trans* isomers of monoenoic acids do not interfere with platelet cyclooxygenase or lipoxygenase activity and 6keto-$PGF_{1\alpha}$ production in aorta, again, provided sufficient linoleic acid is available.

Catabolism of *trans* fatty acids

The catabolism of *trans* fatty acids was investigated with the use of radiolabelled isomers. In rats on an EFA-adequate diet, the total recovery of expired $^{14}CO_2$ after feeding ^{14}C-fatty acids, was followed during 50 h. The recovery of CO_2 from elaidic acid was similar to that of oleic acid and palmitate and higher than that of stearic acid (Coots, 1964a). Linolelaidic acid and a mixture of *c*,*t*/*t*,*c*-18:2 were catabolized

faster than linoleic acid (Coots, 1964b). The difference in catabolism between linoleic acid and its isomers was inversly correlated with the deposition in carcass lipids. The final conclusion was that *trans* isomers were apparently catabolized in an efficient and normal way. In another experiment no significant differences were found in the recovery of radiolabelled CO_2 from elaidic or oleic acid and linoleic or linolelaidic acid administered intravenously (Ono and Frederickson, 1964). Anderson and Coots reported a catabolism of oleic acid which was somewhat faster than that of elaidic acid (Anderson and Coots, 1967). The differences between the catabolism to CO_2 of three isomers of 18:2 were small. In all these experiments fasting rats were used. The fasting may have accentuated differences between the rates of oxidation of the various isomers.

In heart homogenates of male and female rats on stock diet, radiolabelled palmitate was more rapidly oxidized to CO_2 than elaidate, which in turn was oxidized faster than oleate (Menon and Dhopeshwarkar, 1983). When rats were fed a diet rich in *trans* fatty acids (via a partially hydrogenated corn oil), with 1 en% linoleic acid, the oxidation rate of all substrates more than doubled. The *trans* fat increased the oxidation rates more than a fat rich in *cis* monoenes (olive oil). However, Ide et al. (1987), using similar diets, concluded that differences in geometry of dietary fatty acids had only a marginal effect in modulating the hepatic fatty acid oxidation system, in spite of marked differences in the metabolic behaviour of *cis* and *trans* fatty acids in cell-free preparations and perfused liver.
Thomassen et al. (1982) suggested that diets rich in *trans* fatty acids lead to increased peroxisomal β-oxidative capacity of the liver. A PHSO induced a higher peroxisomal β-oxidative activity than the unhydrogenated soybean oil. On the other hand, with the same type of oils, Norseth and Thomassen later found no stimulatory effect of *trans* fatty acids on heart microperoxisomal β-oxidative activity (Norseth and Thomassen, 1983).

1.4.3. Conclusions

Summing up, a number of conclusions on effects of *trans* fatty acids, derived from the in vitro and in vivo studies mentioned earlier, are given below. Special reference is made to effects of *trans* fatty acids, compared to other fatty acids, on the linoleic acid metabolism.
In the human diet in western, industrialized societies, the content of *trans* fatty acids is estimated at about 2.5 en%. By far the largest part of this intake is monoenoic fatty acids with double bonds at positions 8 to 11. *Trans* isomers of linoleic acid do not constitute more than 10% of the total *trans* intake. The consumption of 9*t*,12*t*-18:2 is very low and can be neglected.

In vitro experiments indicated that it is not likely that differences in metabolism of *trans* and *cis* fatty acids are due to differences in rate of activation.
Just like comparable *cis* fatty acids, *trans* fatty acids can be incorporated in all tissues investigated. No organs were found to specifically exclude *trans* fatty acids, although deposition in brain and testes is very low. There is some evidence that an upper limit to the incorporation of *trans* fatty acids in phospholipids exists. Even when very high levels of *trans* isomers are fed, deposition generally does not exceed 20% of all fatty acids.

Incorporation of *trans* fatty acids (except perhaps that of *c*,*t*/*t*,*c*-18:2) in tissue lipids does not seem to depend on the dietary linoleic acid level.

In accordance with acyltransferase specificities in vitro, most common *t*-octadecenoates and *t*,*t*-18:2 are mainly incorporated at the 1-position of tissue phospholipids (if animals are not essential-fatty-acid-deficient). The resulting competition with saturated fatty acids is often reflected in reduced levels of saturated fatty acids after feeding of *trans* fatty acids. The 9*cis*,12*trans*-18:2 isomer is found

at the 2-position and therefore competes with polyunsaturated fatty acids for incorporation, although the affinity for the 2-position is much lower than that of the common polyunsaturated fatty acids.

Not all positional *trans* fatty acid isomers are deposited in tissue phospholipids to the same degree. *Trans* octadecenoates with a double bond at the 12- to 14-position are incorporated to a higher extent than expected from the dietary level. The δ10-isomer is relatively excluded. The 9*c*,12*t*-18:2 isomer is also only slightly incorporated, while 9*t*,12*c*-18:2 is readily deposited.

When the supply of essential fatty acids is adequate, conversions of *trans* isomers into polyunsaturated fatty acids as demonstrated in vitro, are probably not of any significance. Especially the Δ6-desaturation of *trans* isomers, giving *trans* polyunsaturated fatty acids, is probably completely prevented under these conditions. Small amounts of Δ9-desaturation products may be formed (*xt*,9*c*-18:2 isomers). In feeding studies with sufficient essential fatty acids, generally no *trans* polyunsaturated fatty acids were observed.

Tissue-lipid-class compositions (i.e. relative distribution of phospholipid classes) is not influenced by *trans* fatty acids, except under extreme dietary conditions, e.g. EFA-deficiency.

Although it is repeatedly stated that *trans* fatty acids (both monoenes and dienes) inhibit the Δ6-desaturation in vitro, there is hardly any experimental evidence that *trans* fatty acids inhibit this desaturation more than e.g. oleic acid, the most common fatty acid. However, feeding studies with high doses of *t*,*t*-18:2, in combination with low doses of linoleic acid, show a decrease in Δ6-desaturation capacity in isolated microsomes. Effects on Δ5- and Δ9-desaturation are less consistent. At low levels of linolelaidic acid, no significant effects on desaturation enzymes are to be expected. The influence of *t*-octadecenoates (as present in PHVO) on desaturation

capacity was compared to that of oleic acid in two experiments with adequate and equal levels of linoleic acid. The Δ6-desaturation was either inhibited or remained unchanged, the Δ5-desaturation was either unchanged or increased. In essential-fatty-acid-deficient rats, *t*-monoenes were repeatedly found to inhibit the Δ6-desaturase, compared to saturated or *cis*-monounsaturated fatty acids.

Both monoenoic and dienoic *trans* fatty acids can influence polyunsaturated fatty acid deposition in tissue phospholipids. Linoleic acid level is consistently increased. This is not due to the appearance of *t*-isomers of 18:2, as detailed analyses demonstrated the increase in 9*c*,12*c*-18:2. Arachidonic acid levels are decreased in most cases, depending on reference diet, dietary level of linoleic acid and organ and phospholipid class investigated. At higher levels of dietary linoleic acid, effects of *trans* fatty acids on arachidonic acid level seem to diminish. PE responds generally less than PC. Also n-3 fatty acid deposition may be altered. Inhibition of the Δ6-desaturase system is the most frequently used explanation for the decrease in arachidonic acid/linoleic acid ratio. However, other enzyme systems also play a significant role in tissue-fatty-acid-composition and could also be influenced by *trans* fatty acids. It should not be forgotten, however, that small amounts of polyunsaturated fatty acids of the n-3 family (e.g. α-linolenic acid and eicosapentaenoic acid) have a much greater effect on the metabolism of n-6 fatty acids than the high amounts of *trans* fatty acids commonly used in animal studies.

Available evidence from in vivo and in vitro studies indicates that prostaglandin-biosynthesis is not directly inhibited by common *trans* fatty acids. However, *trans* fatty acids can influence the precursor levels of prostaglandins in tissues and consequently may decrease the production of prostaglandins via reduction of precursor availability. But as stated above, the n-3 polyunsaturated fatty acids have a much more profound effect on prostaglandin-synthesis. Moreover, in most experiments on the effects of dietary fats on prostaglandin-synthesis, the maximum capacity of a tissue to produce prostaglandins is measured in vitro. It is doubtful whether this maximum capacity is ever needed under normal physiological conditions.

Although rates of oxidation of *trans* fatty acids in isolated mitochondria are lower than those of the corresponding *cis* isomers, feeding experiments indicate that *trans* fatty acids (both 18:1 and 18:2 isomers) are apparently catabolized efficiently and at rates similar to that of saturated fatty acids or *cis* isomers. This is reflected in the disappearance of *trans* fatty acids from tissue lipids after discontinuation of their consumption. No specific isomers accumulate.

The geometry of fatty acids possibly has a different effect on hepatic or cardiac fatty acid oxidation capacity. However, the available data are not consistent. *Trans* fatty acids (as present in PHVO) may induce peroxisomes in liver, but not in heart.

These conclusions indicate that *trans* fatty acids as present in partially hydrogenated vegetable oils do not have specific biochemical effects: their behaviour and effects on polyunsaturated fatty acid metabolism in rats are comparable to other fatty acids.

1.5. References

Aaes-Jørgensen, E. and Hølmer, G. (1969) Essential fatty acid-deficient rats: 1. Growth and testes development. Lipids 4, 501-506.

Adlof, R.O. and Emken, E.A. (1986) Distribution of hexadecenoic, octadecenoic and octadecadienoic acid isomers in human tissue lipids. Lipids 21, 543-547.

Anderson, R.L. (1967) Oxidation of the geometric isomers of δ9-octadecenoic acid by rat liver mitochondria. Biochim. Biophys. Acta 144, 18-24.

Anderson, R.L. (1968) Oxidation of the geometric isomers of δ9,12-octadecadienoic acid by rat liver mitochondria. Biochim. Biophys. Acta 152, 531-538.

Anderson, R.L. and Coots, R.H. (1967) The catabolism of the geometric isomers of uniformly ^{14}C-labeled δ9-octadecenoic acid and uniformly ^{14}C-labeled δ9,12-octadecadienoic acid by the fasting rat. Biochim. Biophys. Acta 144, 525-531.

Anderson, R.L., Fullmer, C.S. and Hollenbach, E.J. (1975) Effects of the *trans* isomers of linoleic acid on the metabolism of linoleic acid in rats. J. Nutr. 105, 393-400.

Åkesson, B., Johansson, B.M., Eng, M., Svensson, M. and Ockerman, P.A. (1981) Content of *trans*-octadecenoic acid in vegetarian and normal diets in Sweden, analyzed by the Duplicate Portion Technique. Am. J. Clin. Nutr. 34, 2517-1520.

Beare-Rogers, J.L. (1983) *Trans*- and positional isomers of common fatty acids. In: Advances in nutritional research (Draper, H. ed.) Vol. 5., pp. 171-200, Plenum Publishing Corporation, New York.

Beerthuis, R.K., Nugteren, D.H., Pabon, H.J.J., Steenhoek, A. and Van Dorp, D.A. (1971) Synthesis of a series of polyunsaturated fatty acids, their potencies as essential fatty acids and as precursors of prostaglandins. Rec. Trav. Chim. 90, 943-960.

Bernert, J.T. Jr and Sprecher, H. (1975) Studies to determine the role rates of chain elongation and desaturation play in regulating the unsaturated fatty acid composition of rat liver lipids. Biochim. Biophys. Acta 398, 354-363.

Blank, M.L. and Privett, O.S. (1963) Studies on the metabolism of *cis,trans* isomers of methyl linoleate and linolenate. J. Lipid Res. 4, 470-476.

Blankenship, J.W., Miller, T. and Boucek, R.J. (1986) Dietary fat and cholesterol modification of rat abdominal aorta prostacyclin and leukotriene production (in vitro). Prost. Leukotr. Med. 23, 45-52.

Blomstrand, R. and Svensson, L. (1983) The effects of partially hydrogenated marine oils on the mitochondrial function and membrane phospholipid fatty acids in rat heart. Lipids 18, 151-170.

Blomstrand, R., Diczfalusy, U., Sisfontes, L. and Svensson, L. (1985) Influence of dietary partially hydrogenated vegetable and marine oils on membrane composition and function of liver microsomes and platelets in rats. Lipids 20, 283-295.

Bremer, J. (1977) Carnitine and its role in fatty acid metabolism. Trends Biochem. Sci. September 1977, 207-209.

Bremer, J. and Osmundsen, H. (1984) Fatty acid oxidation and its regulation. In: Fatty acid metabolism and its regulation (S. Numa, ed.) pp. 113-154, Elsevier Science Publishers, Amsterdam.

Brenner, R.R. (1971) The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. Lipids 6, 567-575.

Brenner, R.R. (1981) Nutritional and hormonal factors influencing desaturation of essential fatty acids. Progr. Lipid Res. 20, 41-47.

Brenner, R.R. and Peluffo, R.O. (1966) Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic, and linolenic acids. J. Biol. Chem. 22, 5213-5219.

Brenner, R.R. and Peluffo, R.O. (1969) Regulation of unsaturated fatty acid biosynthesis. I. Effect of unsaturated fatty acid of 18 carbons on the microsomal desaturation of linoleic acid into γ-linolenic acid. Biochim. Biophys. Acta 176, 471-479.

Brisson, G.D. (1981) In: Lipids in human nutrition, p.p. 41-71, MTP Press, New Jersey.

Bruckner, G., Trimbo, S., Goswami, S. and Kinsella, J.E. (1983) Dietary trilinoelaidate: Effects on hematological parameters, serum eicosanoids and tissue fatty acid composition in rats. J. Nutr. 113, 704-713.

Bruckner, G., Goswami, S. and Kinsella, J.E. (1984) Dietary trilinoelaidate: Effects on organ fatty acid composition, prostanoid biosynthesis and platelet function in rats. J. Nutr. 114, 58-67.

Brussaard, J.H. (1986) Hoeveel transvetzuren bevat de Nederlandse voeding? Voeding 47, 108-111.

Chang, H.-C., Janke, J., Pusch, F. and Holman, R.T. (1973) Effect of double and triple bonds in fatty acid inhibitors upon desaturation of stearic acid by rat liver microsomes. Biochim. Biophys. Acta 306, 21-25.

Christensen, E., Hagve, T.-A. and Christophersen, B.O. (1986) Mitochondrial and peroxisomal oxidation of arachidonic and eicosapentaenoic acid studied in isolated liver cells. Biochim. Biophys. Acta 879, 313-321.

Cook, H.W. (1978) In vitro formation of polyunsaturated fatty acids by desaturation in rat brain: some properties of the enzymes in developing brain and comparisons with liver. J. Neurochem. 30, 1327-1334.

Cook, H.W. (1980) Metabolism of positional distribution of isomers of 18-carbon dienoic fatty acids containing *trans* and *cis* double bonds by developing rat brain. Can. J. Biochem, 58, 121-127.

Coots, R.H. (1964a) A comparison of the metabolism of elaidic, oleic, palmitic and stearic acids in the rat. J. Lipid Res. 5, 468-472.

Coots, R.H. (1964b) A comparison of the metabolism of *cis,cis*-linoleic *trans,trans*-linoleic and a mixture of *cis,trans*- and *trans,cis*-linoleic acids in the rat. J. Lipid Res. 5, 473-476.

Coots, R.H. (1965) Metabolism of arachidonic-1-^{14}C in the rat. J. Lipid Res. 6, 494-497.

De Alaniz, M.J.T., De Gomez Dumm, I.N.T. and Brenner, R.R. (1986) Effect of different acids with δ9,12-dienoic structures on Δ9 desaturation activity in rat liver microsomes. Lipids 21, 425-429.

De Gomez Dumm, I.N.T., Peluffo, R.O. and Brenner, R.R. (1972) Comparative effect of a protein diet on the desaturation, elongation and simultaneous desaturation and elongation of linoleic acid. Lipids 7, 590-592.

De Gomez Dumm, I.N.T., De Alaniz, M.J.T. and Brenner, R.R. (1983) Effect of dietary fatty acids on Δ5-desaturase activity and biosynthesis of arachidonic acid in rat liver microsomes. Lipids 18, 781-788.

De Gomez Dumm, I.N.T., De Alaniz, M.J.T. and Brenner, R.R. (1984) Daily variations of the biosynthesis and composition of fatty acids in rats fed on complete and fat-free diets. Lipids 19, 91-95.

De Schrijver, R. and Privett, O.S. (1982a) Effects of dietary long-chain fatty acids on the biosynthesis of unsaturated fatty acids in the rat. J. Nutr. 112, 619-626.

De Schrijver, R. and Privett, O.S. (1982b) Interrelationship between dietary *trans* fatty acids and the 6- and 9-desaturases in the rat. Lipids 17, 27-34.

De Schrijver, R. and Privett, O.S. (1983) Hepatic fatty acids and acyl desaturases in rats: effects of dietary carbohydrate and essential fatty acids. J. Nutr. 113, 2217-2222.

De Tomás, M.E. and Brenner, R.R. (1970) Effects of the polar groups of lysophospholipids upon acyltransferase activity with coenzyme A thiol esters. Biochim. Biophys. Acta 202, 184-186.

Deuel, H.J., Hallman, L.F. and Movitt, E. (1945) Studies on the comparative nutritive value of fats. VI Growth and reproduction over 10 generations on Sherman diet B where butterfat was replaced by a margarine fat. J. Nutr. 29, 309-320.

Dutton, H.J. (1979) Hydrogenation of fats and its significance. In: Geometric and positional fatty acid isomers (Emken, E.A. and Dutton, H.J., eds), pp. 1-16, American Oil Chemists' Society, Champaign, Il.

Egwim, P.O. and Kummerow, F.A. (1972) Influence of dietary fat on the concentration of long-chain unsaturated fatty acid families in rat tissues. J. Lipid Res. 13, 500-510.

Emken, E.A. (1981) Influence of *trans*-9-, *trans*-12-, and *cis*-12-octadecenoic acid isomers on fatty acid composition of human plasma lipids. Prog. Lipid Res. 20, 135-141.

Emken, E.A. (1983) Biochemistry of unsaturated fatty acid isomers. J. Am. Oil Chem. Soc. 60, 995-1004.

Emken, E.A. (1984) Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. Ann. Rev. Nutr. 4, 339-376.

Enig, M.G., Munn, R.J. and Keeney, M. (1978) Dietary fat and cancer trends: a critique. Fed. Proc. 37, 2215-2220.

FASEB (1985) Health aspects of dietary *trans* fatty acids. Report of the ad-hoc Committee of the Federation of American Societies for Experimental Biology (Senti, F.R., ed.) pp. 27-31, FDA223-83-2020.

Garg, M.L., Keelan, M., Thomson, A.B.R. and Clandinin, M.T. (1988) Fatty acid desaturation in the intestinal mucosa. Biochim. Biophys. Acta 958, 139-141.

Goswami, S.K., Bruckner, G. and Kinsella, J.E. (1983) Dietary trilinoelaidate: Effects on the in vitro biosynthesis of different prostaglandins in brain, lung and stomach fundus of rats. Prost. Leukotr. Med. 10, 19-26.

Gottenbos, J.J. (1983) Biological effects of *trans* fatty acids. In: Dietary fats and health (Perkins, E.G. and Visek, W.J., eds.) pp. 375-390, American Oil Chemists' Society, Champaign, Il.

Granström, E., Diczfalusy, U., Hamberg, M., Hansson, P., Malmsten, C. and Samuelsson, B. (1982) Thromboxane A_2: Biosynthesis and effects on platelets. Adv. Prostaglandin, Thromboxane and Leukotriene Res. 10, 15-58.

Guo, L.S.S. and Alexander, J.C. (1974) Comparative studies on composition of liver phospholipids from rats fed oleic or elaidic acid. Nutr. Metabol. 16, 51-63.

Gurr, M.I. (1983) *Trans* fatty acids. International Dairy Federation Document 166, 5-18.

Hølmer, G., Høy, C.-E. and Kirstein, D. (1982) Influence of partially hydrogenated vegetable and marine oils on lipid metabolism in rat liver and heart. Lipids 17, 585-593.

Høy, C.-E and Hølmer, G. (1979) Incorporation of *cis*- and *trans*-octadecenoic acids into the membranes of rat liver mitochondria. Lipids 14, 727-733.

Heckers, H., Melcher, F.W. and Dittmar, K. (1979) Zum täglichen Verzehr *trans*-isomere Fettsäuren. Eine Kalkulation unter Zurgrundelegung der Zusammensetzung handelsüblicher Fette und verschiedener menschlicher Depotfette. Fette Seife Anstrichm. 6, 217-226.

Higgs, E.A., Moncada, S. and Vane, J.R. (1986) Prostaglandins and thromboxanes from fatty acids. Prog. Lipid Res. 25, 5-11.

Hill, E.G. and Lands, W.E.M. (1968) Incorporation of long-chain and polyunsaturated acids into phosphatidate and phosphatidylcholine. Biochim. Biophys. Acta 152, 645-648.

Hill, E.G., Johnson, S.B. and Holman, R.T. (1979) Intensification of essential fatty acid deficiency in the rat by dietary *trans* fatty acids. J. Nutr. 109, 1759-1766.

Hill, E.G., Johnson, S.B., Lawson, L.D., Mahfouz, M.M. and Holman, R.T. (1982) Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. Proc. Natl. Acad. Sci. USA 79, 953-957.

Hiltunen, J.K., Osmundsen, H. and Bremer, J. (1983) β-Oxidation of polyunsaturated fatty acids having double bonds at even-numbered positions in isolated rat liver mitochondria. Biochim. Biophys. Acta 752, 223-232.

Hiltunen, J.K., Kärki, T., Hassinen, I.E. and Osmundsen, H. (1986) β-Oxidation of polyunsaturated fatty acids by rat liver peroxisomes. J. Biol. Chem. 261, 16484-16493.

Holman, R.T. (1951) Metabolism of isomers of linoleic and linolenic acid. Proc. Soc. Exp. Bio. Med. 76, 100-102.

Holman, R.T. (1981) Effect of dietary *trans* fatty acids upon prostaglandin precursors. In: Nutritional factors: modulating effects on metabolic processes (Beers, R.F. and Bassett, E.G., eds.) pp. 523-537, Raven Press, New York.

Holman, R.T. and Aaes-Jørgensen, E. (1956) Effects of *trans* fatty acid isomers upon essential fatty acid deficiency in rats. Proc. Soc. Exp. Biol. Med. 93, 175-179.

Holman, R.T., Mahfouz, M.M., Lawson, L.D. and Hill, E.G. (1983) Metabolic effects of isomeric octadecenoic acids. In: Dietary fats and health, (Perkins, E.G. and Visek, W.J., eds.), pp. 320-339, American Oil Chemists' Society, Champaign, Il.

Hornstra, G., Haddeman, E., Kloeze, J., and Verschuren, P.M. (1983) Dietary-fat-induced changes in the formation of prostanoids of the 2 and 3 series in relation to arterial thrombosis (rat) and atherosclerosis (rabbit). In: Advances in Prostaglandins, Thromboxane, and Leukotriene Research, Vol. 12 (Samuelsson, B., Paoletti, R. and Ramwell, P., eds.) pp. 193-202, Raven Press, New York.

Horrobin, D.F. (1981) Loss of delta-6-desaturase activity as a key factor in aging. Med. Hypotheses 7, 1211-1220.

Houtsmuller, U.M.T. (1978) Biochemical aspects of fatty acids with *trans* double bonds. Fette Seifen Anstrichm. 80, 162-169.

Hunter, J.E. and Applewhite, T.H. (1986) Isomeric fatty acids in the US diet: levels and health perspectives. Am. J. Clin. Nutr. 44, 707-717.

Hwang, D.H., Mathias, M.M., Dupont, J. and Meyer, D.L. (1975) Linoleate enrichment of diet and prostaglandin metabolism in rats. J. Nutr. 105, 995-1002.

Hwang, D.H. and Kinsella, J.E. (1978) The effects of *trans* fatty linoleic acid on the concentration of serum prostaglandin $F_{2\alpha}$ and platelet aggregation. Prostagl. Med. 1, 121-130.

Hwang, D.H. and Kinsella, J.E. (1979) The effects of *trans trans* methyl linoleate on the concentration of prostaglandins and their precursors in rat. Prostaglandins 17, 543-559.

Hwang, D.H. and Carroll, A.E. (1980) Decreased formation of prostaglandins derived from arachidonic acid by dietary linolenate in rats. Am. J. Clin. Nutr. 33, 590-597.

Hwang, D.H., Chanmugam, P. and Anding, R. (1982) Effects of dietary 9-*trans*,12-*trans* linoleate on arachidonic acid metabolism in rat platelets. Lipids 17, 307-313.

Ide, T. and Sugano, M. (1984) Oxidation and esterification of *cis*- and *trans*-isomers of octadecenoic and octadecadienoic acids in isolated rat liver. Biochim. Biophys. Acta 794, 281-291.

Ide, T. and Sugano, M. (1986a) Strain dependence of the metabolism of *cis*- and *trans*-isomers of 9-octadecenoic in perfused rat liver and cell-free preparation in rats. Biochim. Biophys. Acta 877, 104-111.

Ide, T. and Sugano, M. (1986b) Oxidation and esterification of geometrical and positional isomers of octadecenoic acids in perfused rat liver. J. Biochem. 100, 1561-1568.

Ide, T., Watanabe, M., Sugano, M. and Yamamoto, I. (1987) Activities of liver mitochondria and peroxisomal fatty acid oxidation enzymes in rats fed *trans* fat. Lipids 22, 6-10.

Inkpen, C.A., Harris, R.A. and Quackenbush, F.W. (1969) Differential responses to fasting and subsequent feeding by microsomal systems of rat liver: 6- and 9-desaturation of fatty acids. J. Lipid Res. 10, 227-282.

Jeffcoat, R. and James, A.T. (1977) Interrelationships between the dietary regulation of fatty acid synthesis and the fatty acyl-CoA desaturases. Lipids 12, 469-474.

Jeffcoat, R., Brown, R.R., Safford, R. and James, A.T. (1977) Properties of rat liver microsomal stearoyl-coenzyme A desaturase. Biochem. J. 161, 431-437.

Kameda, K., Valicenti, A.J. and Holman, R.T. (1980) Chain elongation of *trans*-octadecenoic acid isomers in rat liver microsomes. Biochim. Biophys. Acta 618, 13-17.

Kawashima, Y., Hirose, A., Adachi, T. and Kozuka, H. (1985) Role of stearoyl-CoA desaturase and 1-acyl-glycerophosphorylcholine acyltransferase in the regulation of the acyl compositon of phosphatidylcholine in rat liver. Biochim. Biophys. Acta 837, 222-229.

Kinsella, J.E., Bruckner, G., Mai, J. and Shimp, J. (1981) Metabolism of *trans* fatty acids with emphasis on the effects of *trans*, *trans*-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview. Am. J. Clin. Nutr. 34, 2307-2318.

Kirstein, D., Høy, C.-E. and Hølmer, G. (1983) Effect of dietary fats on the Δ6- and Δ5-desaturation of fatty acids in rat liver microsomes. Br. J. Nutr. 50, 749-756.

Kivits, G.A.A. and Nugteren, D.H. (1988) The urinary excretion of prostaglandins and their corresponding tetranor metabolites by rats fed a diet rich in eicosapentaenoate. Biochim. Biophys. Acta 958, 289-299.

Knipprath, W.G. and Mead, J.F. (1964) The metabolism of *trans,trans*-octadecadienoic acid. Incorporation of *trans,trans*-octadecadienoic acid into C20 polyunsaturated acids of the rat. J. Am. Oil Chem. Soc. 41, 437-440.

Kurata, N. and Privett, O.S. (1980) Effect of dietary fatty acid composition on the biosynthesis of unsaturated fatty acids in rat liver microsomes. Lipids 15, 512-518.

Kurata, N. and Privett, O.S. (1981) Effects of dietary *trans* acids on the biosynthesis of arachidonic acid in rat liver microsomes. Lipids 15, 1029-1036.

Lands, W.E.M. (1966) Effects of double bond configuration on lecithin synthesis. J. Am. Oil Chem. Soc. 42, 465-467.

Lands, W.E.M. (1979) Selective recognition of geometrical and positional isomers of fatty acids in vitro and in vivo. In: Geometrical and positional fatty acid isomers (Emken, E.A. and Dutton, H.J., eds.) pp. 181-212, American Oil Chemists' Society, Champaign, Il.

Lands, W.E.M, Blank, M.L., Nutter, L.J. and Privett, O.S. (1966) A comparison of acyltransferase activities in vitro with the distribution of fatty acids in lecithins and triglycerides in vivo. Lipids 1, 224-229.

Lands, W.E.M., Inoue, M., Sugiura, Y. and Okuyama, H. (1982) Selective incorporation of polyunsaturated fatty acids into phosphatidylcholine by rat liver microsomes. J. Biol. Chem. 257, 14968-14972.

Lanser, A.C., Emken, E.A. and Ohlrogge, J.B. (1986) Oxidation of oleic and elaidic acids in rat and human heart homogenates. Biochim. Biophys. Acta 875, 510-515.

Lawson, L.D. and Kummerow, F.A. (1979) β-Oxidation of the coenzyme A esters of elaidic, oleic, and stearic acids and their full-cycle intermediates by rat heart mitochondria. Biochim. Biophys. Acta 573, 245-254.

Lawson, L.D. and Holman, R.T. (1981) β-Oxidation of the geometric and positional isomers of octadecenoic acid by rat heart and liver mitochondria. Biochim. Biophys. Acta 665, 60-65.

Lawson, L.D., Hill, E.G. and Holman, R.T. (1982) Biosynthesis of octadecadienoic acids from octadecenoic acids present in partially hydrogenated vegetable oil. J. Am. Oil Chem. Soc. 59, 307A.

Lawson, L.D., Hill, E.G. and Holman, R.T. (1983) Suppression of arachidonic acid in lipids of rat tissues by dietary mixed isomers of *cis* and *trans* octadecenoates. J. Nutr. 113, 1827-1835.

Lawson, L.D., Hill, E.G. and Holman, R.T. (1985) Dietary fats containing concentrates of *cis* or *trans* octadecenoates and the patterns of polyunsaturated fatty acids of liver phosphatidylcholine and phosphatidylethanolamine. Lipids 20, 262-267.

Lemarchal, P. and Munsch, N. (1965) Etude sur le métabolisme de l'acide élaidique chez des rats carencés en acides gras indispensables. C.R. Soc. Biol. 260, 714-716.

Leyton, J., Drury, P.J. and Crawford, M.A. (1987) Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. Br. J. Nutr. 57, 383-393.

Lippel, K. (1973) Activation of long chain fatty acids by subcellular fractions of rat liver: I. Activation of *trans*-unsaturated acids. Lipids 8, 111-118.

Lippel, K., Gunstone, F.D. and Barve, J.A. (1973) Activation of long chain fatty acids by subcellular fractions of rat liver: II. Effect of ethylenic bond position on acyl-CoA formation of *trans*-octadecenoates. Lipids 8, 119-123.

Ludwig, S.A. and Sprecher, H. (1979) Substrate specificity studies on the malonyl-CoA-dependent chain elongation of all-*cis* polyunsaturated fatty acids by rat liver microsomes. Arch. Biochem. Biophys. 197,333-341.

Mahfouz, M.M., Johnson, S. and Holman, R.T. (1980a) The effect of isomeric *trans*-18:1 acids on the desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids by rat liver microsomes. Lipids 15, 100-107.

Mahfouz, M.M., Valicenti, A.J. and Holman, R.T. (1980b) Desaturation of isomeric *trans*-octadecenoic acid by rat liver microsomes. Biochim. Biophys. Acta 618, 1-12.

Mahfouz, M., Johnson, S. and Holman, R.T. (1981) Inhibition of desaturation of palmitic, linoleic and eicosa-8,11,14-trienoic acids in vitro by isomeric *cis*-octadecenoic acids. Biochim. Biophys. Acta 663, 58-68.

Mahfouz, M.M., Smith, T.L. and Kummerow, F.A. (1984) Effect of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat-liver microsomes. Lipids 19, 214-222.

Marcel, Y.L. and Suzue, G. (1972) Kinetic studies on the specificity of long chain acyl coenzyme A synthetase from rat liver microsomes. J. Biol. Chem. 247, 4433-4436.

Marshall, P.J., Kulmacz, R.J. and Lands, W.E.M. (1987) Constraints on prostaglandin biosynthesis in tissues. J. Biol. Chem. 262, 3510-3517.

Masters, C. and Crane, D. (1984) The role of peroxisomes in lipid metabolism. Trends Pharmacol. Sci. July 1984, 314-317.

Masuzawa, Y., Prasad, M.R. and Lands, W.E.M. (1987) Distribution of dietary *trans*-octadecenoate among acyl-CoA and other lipid fractions of rat liver and heart. Biochim. Biophys. Acta 919, 297-306.

Mathias, M.M and Dupont, J. (1985) Quantitative relationships between dietary linoleate and prostaglandin (eicosanoid) biosynthesis. Lipids 20, 791-801.

Mattson, F.H. (1960) An investigation of the essential fatty acid activity of some of the geometrical isomers of unsaturated fatty acids. J. Nutr. 71, 366-370.

McGarry, D.J., Meier, J.M. and Foster, D.W. (1973) The effects of starvation and refeeding on carbohydrate and lipid metabolism in vivo and in the perfused rat liver. J. Biol. Chem. 248, 270-278.

Menon, N.K. and Dhopeshwarkar, G.A. (1983) Differences in the fatty acid profile and β-oxidation by heart homogenates of rats fed *cis* and *trans* octadecenoic acids. Biochim. Biophys. Acta 751, 14-20.

Mohrhauer, H. and Holman, R.T. (1963) Effect of linolenic acid upon the metabolism of linoleic acid. J. Nutr. 81, 67-74.

Moncada, S and Vane, I.R. (1978) Unstable metabolites of arachidonic acid and their role in haemostasis and thrombosis. Br. Med. Bull. 34, 129-135.

Moore, C.E., Alfin-Slater, R.B. and Aftergood, L. (1980) Incorporation and disappearance of *trans* fatty acids in rat tissues. Am. J. Clin. Nutr. 33, 2318-2323.

Neat, C.E., Thomassen., M.S. and Osmundsen, H. (1981) Effects of high-fat diets on hepatic fatty acid oxidation in the rat. Biochem. J. 196, 149-159.

Normann, P.T. and Flatmark, T. (1980) Acyl-CoA synthetase activity of brown adipose tissue mitochondria. Substrate specificity and its relation to the endogenous pool of long-chain fatty acids. Biochim. Biophys. Acta 619, 1-10.

Normann, P.T., Thomassen, M.S., Christiansen, E.N. and Flatmark, T. (1981) Acyl-CoA synthetase activity of rat liver microsomes. Substrate specificity with special reference to very-long-chain and isomeric fatty acids. Biochim. Biophys. Acta 664, 416-427.

Norseth, J. and Thomassen, M.S. (1983) Stimulation of microperoxisomal β-oxidation in rat heart by high-fat diets. Biochim. Biophys. Acta, 751, 312-320.

Nugteren, D.H. (1970) Inhibition of prostaglandin biosynthesis by 8*cis*,12*trans*,14*cis*-eicosatrienoic acid and 5*cis*,8*cis*,12*trans*,14*cis*-eicosatetraenoic acid. Biochim. Biophys. Acta 210, 171-176.

Nugteren, D.H., Beerthuis, R.K. and Van Dorp, D.A. (1966) The enzymic conversion of all-*cis* 8,11,14-eicosatrienoic acid into prostaglandin E_1. Rec. Trav. Chim. 85, 405-419.

Nugteren, D.H., Vanevert, W.C., Soeting, W.J. and Spuy, J.H. (1980) Effects of different amounts of linoleic acid in the diet on the excretion of urinary prostaglandin metabolites in the rat. Adv. Prostaglandin and Thromboxane Res. 8, 1793-1798.

Nugteren, D.H. and Christ-Hazelhof, E. (1987) Naturally occurring conjugated octadecatrienoic acid are strong inhibitors of prostaglandin biosynthesis. Prostaglandins 33, 403-417.

Ohlrogge, J.B. (1983) Distribution in human tissues of fatty acid isomers from hydrogenated oils. In: Dietary fats and health (Perkins, E.G. and Visek, W.J., eds.) pp. 359-374, American Oil Chemists's Society, Campaign, Il.

Okuyama, H., Lands, W.E.M., Gunstone, F.D. and Barve, J.A. (1972) Selective transfers of *trans*-ethylenic acids by acyl coenzyme A phospholipid acyltransferases. Biochemistry 11, 4392-4398.

Ono, K. and Frederickson, D.S. (1964) The metabolism of ^{14}C-labeled *cis* and *trans* isomers of octadecenoic and octadecadienoic acids. J. Biol. Chem. 239, 2482-2488.

Osmundsen, H. (1985) Mitochondria, peroxisomes and hepatic fatty acid oxidation. Trends Biochem. Sci. Februari 1985, 61.

Osmundsen, H., Neat, C.E. and Norum, K.R. (1979) Peroxisomal oxidation of long chain fatty acids. FEBS Letters 99, 292-296.

Pace-Asciak, C. and Wolfe, L.S. (1968) Inhibition of prostaglandin synthesis by oleic, linoleic and linolenic acids. Biochim. Biophys. Acta 152, 784-787.

Pace-Asciak, C.R. and Rangaraj, G. (1977) Distribution of prostaglandin biosynthetic pathways in several rat tissues, formation of 6-ketoprostaglandin $F_{1\alpha}$. Biochim. Biophys. Acta 486, 579-582.

Parodi, P.W. (1976) Distribution of isomeric octadecenoic fatty acids in milk fat. J. Dairy Sci. 59, 1870-1873.

Pascaud, M. and Strouvé, C. (1968) Devenir métabolique des acides ^{14}C linoléique et palmitéique chez le rat en croissance. II. Retention et dérivations. Bull. Soc. Chim. Biol. 50, 579-589.

Pollard, M.R., Gunstone, F.D., James, A.T. and Morris, L.J. (1980a) Desaturation of positional and geometric isomers of monoenoic fatty acids by microsomal preparations from rat liver. Lipids 15, 306-314.

Pollard, M.R., Gunstone, F.D., Morris, L.J. and James, A.T. (1980b) The Δ5 and Δ6 desaturation of fatty acids of varying chain length by rat liver: a preliminary report. Lipids 15, 690-693.

Privett, O.S., Pusch, F.J. and Holman, R.T. (1955) Polyethenoid fatty acid metabolism. VIII. Non-potency of *cis*-9,*trans*-12-linoleate as essential fatty acid. Arch. Biochem. Biophys. 57, 156-162.

Privett, O.S. and Blank, M.L. (1964) Studies on the metabolism of linoelaidic acid in the essential fatty acid-deficient rat. J. Am. Oil Chem. Soc. 41, 292-297.

Privett, O.S., Nutter, L.J. and Lightly, F.S. (1966) Metabolism of *trans* acids in the rat: Influence of the geometric isomers of linoleic acid on the structure of liver triglycerides and lecithins. J. Nutr. 89, 257-264.

Privett, O.S., Stearns, E.M. and Nickel, E.C. (1967) Metabolism of the geometric isomers of linoleic acid in the rat. J. Nutr. 92, 303-310.

Pugh, E.L. and Kates, M. (1979) Membrane-bound phospholipid desaturases. Lipids 14, 159-165.

Pugh, E.L. and Kates, M. (1984) The dietary regulation of acyltransferase and desaturase activities in microsomal membranes of rat liver. Lipids 19, 48-55.

Ramesha, C.S., Gronke, R.S., Sivarajan, M. and Lands, W.E.M. (1985) Metabolic products of arachidonic acid in rats. Prostaglandins 29, 991-1008.

Reichwald-Hacker, I., Grosse-Oetringhaus, S., Kiewitt, I. and Mukherjee, K.D. (1979) Incorporation of positional isomers of *cis*- and *trans*-octadecenoic acids into acyl moieties of rat tissue lipids. Biochim. Biophys. Acta 575, 327-334.

Reitz, D.C., El-Sheikh, M., Lands, W.E.M., Ismail, I.A. and Gunstone, F.D. (1969) Effects of ethylenic bond position upon acyltransferase activity with isomeric *cis*-octadecenoyl coenzyme A thiol esters. Biochim. Biophys. Acta 176, 480-490.

Sabatier, P. and Senderens, J.B. (1897) Compt. Rend. 124, 616. Translation by E.E. Reid (1922) Van Nostrand, New York.

Samuelsson, B. (1986) Leukotrienes and other lipoxygenase products. Prog. Lipid Res. 25, 13-18.

Schulz, H. and Kunau, W.-H. (1987) Beta-oxidation of unsaturated fatty acids: a revised pathway. Trends Biochem. Sci. 12, October, 403-406.

Shimp, J.L., Bruckner, G. and Kinsella, J.E. (1982) The effects of dietary trilinoelaidin on fatty acid and acyl desaturases in rat liver. J. Nutr. 112, 722-735.

Sinclair, A.J. (1975) Incorporation of radioactive polyunsaturated fatty acids into liver and brain of developing rats. Lipids 10, 175-184.

Sprecher, H. (1981) Biochemistry of essential fatty acids. Prog. Lipid Res. 20, 13-22.

Sprecher, H. (1986) The metabolism of (n-3) and (n-6) fatty acids and their oxygenation by platelet cyclooxygenase and lipoxygenase. Prog. Lipid Res. 25, 19-28.

Stakkestad, J.A. and Lund, H. (1984) The effect of nutritional and thyroid state on the distribution of fatty acids between oxidation and esterification in isolated rat hepatocytes. Biochim. Biophys. Acta 793, 1-9.

Svensson, L. (1983) The effect of dietary partially hydrogenated marine oils on desaturation of fatty acids in rat liver microsomes. Lipids 18, 171-178.

Ten Hoor, F., de Deckere, E.A.M., Haddeman, E., Hornstra, G. and Quadt, J.F.A. (1980) Dietary manipulation of prostaglandin and thromboxane synthesis in heart, aorta and blood platelets of the rat. Adv. Prostaglandin and Thromboxane Res. 8, 1771-1781.

Thomassen, M.S., Christiansen, E.N. and Norum, K.R. (1982) Characterization of the stimulatory effect of high-fat diets on peroxisomal β-oxidation in rat liver. Biochem. J. 206, 195-202.

Thompson, G.A. jr. and Martin, C.E. (1976) Regulation of phospholipid fatty acid composition. In: Regulation of membrane fluidity (Shinitzky, M., ed.) pp. 99-129, CRC Press, Boca Raton, Fl.

Van Den Bosch, H., Van Golde, L.M.G.; Slotboom, A.J. and Van Deenen, L.L.M. (1968) The acylation of isomeric monoacylphosphatidylcholines. Biochim. Biophys. Acta 152, 694-703.

Van Den Reek, M.M., Craig-Schmidt, M.C., Weete, J.D. and Clark, A.J. (1986) Fat in the diets of adolescent girls with emphasis on isomeric fatty acids. Am. J. Clin. Nutr. 43, 530-537.

Van Dorp, D.A., Beerthuis, R.K., Nugteren, D.H. and Vonkeman, H. (1964) The biosynthesis of prostaglandins. Biochim. Biophys. Acta 90, 204-207.

Vles, R.O. and Gottenbos, J.J. (1972a) Long-term effects of feeding butterfat, coconut oil, and hydrogenated or non hydrogenated soya-bean oils. I. Eighteen-month experiment in mice. Voeding 33, 428-433.

Vles, R.O. and Gottenbos, J.J. (1972b) Long-term effects of feeding butterfat, coconut oil, and hydrogenated or non hydrogenated soya-bean oils. II. Life-span experiments in rats. Voeding 33, 455-465.

Vles, R.O., Gottenbos, J.J. and van Pijpen, P.L. (1977) Aspects nutritionels des huiles de soja hydrogenees et de leur acides gras insatures isomeriques. Bibl. Nutr. Dieta 25, 186-196.

Waku, Keizo and Lands, W.E.M. (1968) Control of lecithin biosythesis in erythrocyte membranes. J. Lipid Res. 9, 12-18.

Willebrands, A.F. and Van Der Veen, K.J. (1966) The metabolism of elaidic acid in the perfused rat heart. Biochim. Biophys. Acta 116, 583-585.

Wolfe, L.S. (1982) Eicosanoids: prostaglandins, thromboxanes, leukotrienes and other derivatives of carbon-20 unsaturated fatty acids. J. Neurochem. 38, 1-14.

Wood, R. (1979) Incorporation of dietary *cis* and *trans* octadecenoate isomers in the lipid classes of various rat tissues. Lipids 14, 975-982.

Wood, R. and Chumbler, F. (1978) Distribution of dietary octadecenoate isomers at the 1- and 2-positions of hepatoma and liver phospholipids. Lipids 13, 75-84.

Zijlstra, F.J. and Vincent, J.E. (1985) Incorporation into the tissues and turnover of arachidonic acid after administration to normal and essential fatty acid deficient rats. Prost. Leukotr. Med. 20, 287-297.

Chapter 2.

LINOLEIC ACID REQUIREMENT OF RATS FED *TRANS* FATTY ACIDS. A NUTRITIONAL STUDY

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Linoleic acid requirement of rats fed *trans* fatty acids.
A nutritional study
Submitted to the Journal of Nutrition

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ABSTRACT

It has been reported that *trans* fatty acids from hydrogenated vegetable oils and fats do not induce detrimental changes in rats, like poor growth, aggravation of essential fatty acid deficiency, impairment of spermatogenesis and deteriorated skin, provided sufficient linoleic acid is present in the diet. A description is given of two rat feeding studies aimed at defining the minimum level of linoleic acid required to prevent undesirable effects of C18 *trans* fatty acids. In the first experiment 6 groups of animals were fed a constant, high amount (20% of energy) of *trans* fatty acids, as present in a specially prepared, partially hydrogenated soybean oil, and increasing amounts of linoleic acid (0.4 to 7.1% of energy). Two reference groups received diets with 2 or 5% of energy as linoleic acid and 0% *trans* fatty acids. The second rat trial comprised 4 groups fed different fat blends, all containing 2% of energy as linoleic acid. In this study *trans* fatty acids were compared with saturated and *cis*-monounsaturated fatty acids of the same chain length. Bodyweights, food and water consumption were not significantly different between the groups in both experiments. Hematology, clinical chemistry and histopathology revealed no significant treatment related differences. From these studies it can be concluded that a diet with about 20% of energy as *trans* fatty acids does not cause detrimental effects when linoleic acid is present as 2% of energy. These studies support the conclusion that there is little reason for concern with respect to the safety of dietary *trans* fatty acids at the present and expected levels of consumption of linoleic acid. J. Nutr. : , 1988.

INDEXING KEY WORDS

•*trans* fatty acids •linoleic acid requirement •rat feeding trial •post-mortem studies.

Trans fatty acids from partially hydrogenated oils and ruminant fats are are part of the human diet. Through the years there has been a marked interest in the biological effects of *trans* fatty acids. In our laboratory, a short-term toxicity study with various partially hydrogenated vegetable oils containing substantial amounts of *trans* fatty acids has been performed in rats (1). High amounts of hydrogenated fats of extremely divergent composition were not found to induce adverse effects. Subsequently, long-term feeding studies with soybean oils, hardened to various degrees, were carried out in rats, mice and rabbits (2-4). Animals were fed diets containing high amounts of (partially) hydrogenated soybean oils differing widely in amounts of saturated, mono-unsaturated and isomeric fatty acids. The dietary linoleic acid content varied from 1.3 to 8.2% of energy (en%) and the *trans* fatty acids content ranged from 8 up to 36 en%. It was concluded that none of the partially hydrogenated soybean oils, contrary to butter or coconut oil, induced special adverse effects, except for an enhanced atherosclerosis in rabbits fed the diet with the lowest amount of linoleic acid. These studies (1-4) indicated that no pathological changes were induced by the feeding of *trans* fatty acids provided an adequate level of linoleic acid was present in the diet.

Presently, reviewers in the area of *trans* fatty acids generally conclude that *trans* fatty acids formed during the partial hydrogenation of vegetable oils do not exert undesirable effects when compared to saturated or *cis*-monounsaturated fatty acids, provided sufficient linoleic acid is present in the diet (5-9). In spite of this general conclusion, it has been reported that *trans* fatty acids aggravate symptoms of essential fatty acid deficiency in experimental animals.

These symptoms are poor growth, deteriorated skin, impairment of spermatogenesis and alterations in fatty acid composition (10,11). Our aim was to define the amount of linoleic acid required to prevent these undesirable effects of *trans* fatty acids in partially hydrogenated vegetable oils.

Two feeding experiments with rats were performed in which the effects of *trans* fatty acids, as present in a partially hydrogenated soybean oil, were investigated. In the first experiment the effects of diets with a constant amount of *trans* fatty acids and increasing amounts of linoleic acid were investigated. In the second experiment the effects of *trans* fatty acids in the presence of 2 en% linoleic acid were compared with those of (mainly) long-chain saturated or *cis*-monounsaturated fatty acids. During the in-life phase, bodyweight, food and water consumption were recorded and the trans epidermal water loss was measured. After the experimental feeding periods the rats were sacrificed and gross pathology, histopathology, histochemistry, hematology and serum chemistry were performed. The work described in this paper, is part of an extensive study on the biological effects of *trans* fatty acids.

MATERIALS AND METHODS

Materials

Partially hydrogenated soybean oil (PHSO) was obtained from van den Bergh and Jurgens (Rotterdam, The Netherlands); hydrogenated coconutoil (HCNO) from Chempry BV (Raamsdonkveer, The Netherlands), sunflowerseed oil (SSO) from Union (Antwerpen, Belgium) and cocoabutter (CB) from J. Schoenmaker BV (Zaandam, The Netherlands). Olive oil (OV) was purchased from Fol Jr and Co (Krimpen aan de IJssel, The Netherlands) and the low-linoleic acid olive oil (OV-LL) was a gift from Elais SA (Piraeus, Greece). All commercial kits for automatic determination of serum enzymes and other components levels were supplied by Boehringer (Mannheim, FRG), except for the kits for calcium and albumin which were from Pierce Diagnostics, obtained from Hicol BV (Rotterdam, The Netherlands).

TABLE 1

Composition of experimental fats (First experiment)[1].

Dietary oil	Experimental fats							
	1	2	3	4	5	6	7[2]	8[2]
PHSO	30.0	30.0	30.0	30.0	30.0	30.0	-	-
HCNO	7.3	5.0	5.0	5.0	3.0	-	22.6	-
OV	2.7	4.8	2.7	1.0	-	-	17.4	39.3
SSO	-	0.2	2.3	4.0	7.0	10.0	-	0.7
LA[3]	0.4	0.8	2.0	3.0	5.0	7.1	2.0	5.0

1) Values indicate energy% (en%); all diets contained 40 en% fat.

2) Reference groups.

3) Calculated linoleic acid content in en% of the final diet.

Animals and diets

Male SPF-Wistar rats of 3 weeks of age, were purchased from CPB/WU, Central Breeding Station TNO (Zeist, The Netherlands). The animals were housed individually in a climatized animal room (mean temperature 23.0°C and relative humidity 55%) with a day/night cycle of 12 h; they had free access to water and food. In both experiments the animals received semi-synthetic diets containing 40 en% fat.

The diets were composed of (in $g.MJ^{-1}$ of the total diet): casein 14.8; vitamin mixture[1] 0.2; salt mixture[1] 1.3; cellulose 3.8; maize starch 25.2; experimental fat 10.3.

In the first experiment, 8 groups (7 of 12 animals each and one of 24 animals: group nr. 3) were fed diets on the basis of PHSO, HCNO, OV and SSO. The oils were mixed in such a way as to yield 6 diets having an increasing linoleic acid content (0.4 -7.1 en%), but a constant *trans* fatty acid level of 20 en% (Table 1). Two fats served as references, one consisting of HCNO and OV (2 en% linoleic acid), another mainly of OV (5 en% linoleic acid). Table 2 shows the calculated fatty acid compositions of the experimental fats. The fatty acid composition of the fats and oils used was determined as described before (12).

In the second experiment 4 groups of 24 rats each were fed diets rich in *trans* fatty acids (PHSO), or saturated fatty acids (CB), or *cis*-monounsaturated fatty acids (OV-LL), or a mixture of these fatty acids (PHSOmix) (Table 3). All these diets contained 2 en% linoleic acid. The fatty acid compositions are given in Table 4.

Observations during the experiment

The animals were weighed and examined for general health weekly. Food and water consumption were recorded during 2 or 3 days at various times during the feeding period. In the first experiment the trans epidermal water loss was measured after 10 weeks of feeding as described by Houtsmuller and van der Beek (13).

Post-mortem studies

At the end of the feeding periods (14 and 12 weeks in the first and second experiment respectively) the animals were fasted overnight, weighed and sacrificed by aorta cannulation under ether anesthesia. Blood was collected for serum chemistry. After macroscopic examination,

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[1] Footnote see p. 93

TABLE 2

Calculated fatty acid composition of the experimental fats (First experiment).

fatty acid[1]	Group							
	1	2	3	4	5	6	7[2]	8[2]
6:0	0.1	-	-	-	-	-	0.2	-
8:0	1.2	0.8	0.8	0.8	0.5	-	3.8	-
10:0	1.0	0.7	0.7	0.7	0.4	-	3.2	-
12:0	8.9	6.1	6.1	6.1	3.7	0.1	27.0	-
14:0	3.6	2.5	2.5	2.5	1.6	0.2	10.3	-
16:0	10.3	10.4	10.1	9.9	9.6	9.4	10.5	12.2
16:1	0.3	0.3	0.3	0.2	0.2	0.2	0.4	1.0
17:0	0.1	0.1	0.1	0.1	0.1	0.2	-	0.1
17:1	-	-	-	-	-	0.1	0.1	0.2
18:0	8.5	7.9	8.1	8.1	7.8	7.2	8.0	2.9
18:1 *c*	16.5	20.3	17.5	15.3	14.8	16.1	30.7	69.7
18:1 *t*	39.1	39.1	39.1	39.1	39.1	39.1	-	-
18:2 9*c*,12*c*	1.0	2.0	5.0	7.5	12.5	17.7	5.0	12.5
18:2 *ct*,*tc* 9,12	0.6	0.6	0.6	0.6	0.6	0.6	-	-
18:2 9*t*,12*t*	0.5	0.5	0.5	0.5	0.5	0.5	-	-
18:2 other	7.7	7.7	7.7	7.7	7.7	7.7	-	-
18:3	-	-	-	-	-	0.1	0.2	0.5
20:0	0.3	0.3	0.3	0.3	0.4	0.4	0.2	0.2
20:1[3]	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0.3
22:0	0.3	0.4	0.3	0.3	0.4	0.5	0.1	0.3
actual LA-level[4]	1.1	n.d.	5.4	n.d.	n.d.	n.d.	5.2	n.d.

1) The shorthand notation used for the fatty acids indicates chain length: number of double bonds. *c*=*cis* isomers, *t*=*trans* isomers, other=non-9,12 isomers (*cis* and *trans*), -= not detected.
2) Reference groups.
3) Also contains conjugated 18:2 isomers.
4) Actual linoleic acid level (%) as determined by GLC, n.d.= not determined

TABLE 3

Composition of experimental fats (groups)
(Second experiment)[1].

Dietary oils	Experimental fats			
	CB	OV	PHSO	PHSOMix
PHSO	-	-	30.0	15.0
OV-LL (low LA)[2]	-	40.0	-	-
OV	-	-	2.7	15.0
CB	39.4	-	-	10.0
SSO	0.6	-	2.3	-
HCNO	-	-	5.0	-

1) Values indicate energy% (en%); all diets contained 40 en% fat.

2) LA=linoleic acid.

thymus, liver, kidneys, spleen and testes were excised, weighed and preserved in 10% formalin. In addition, lungs and mesenteric lymphnodes were fixed. Moreover, a sample of liver tissue was frozen in liquid N_2 and stored for (enzyme) histochemistry. All tissues were processed by conventional methods. Paraplast sections (5 μm) were stained with Harris' Azophloxin and Masson's trichrome stain.
Frozen sections of liver tissue (10 μm) were stained with Sudan III/IV (for the detection of neutral fat) and with Best Karmin (for the detection of glycogen) and the activity of glucose-6-phosphatase (EC 3.1.3.9; Wachstein and Meisel), 5'-nucleotidase (EC 3.1.3.5; Wachstein

TABLE 4

Fatty acid compositions of the experimental fats (Second experiment).

fatty acids[1]	CB	OV	PHSO	PHSOMix
	Experimental fats			
6:0	-	-	-	-
8:0	-	-	0.8	-
10:0	-	-	0.7	-
12:0	-	0.1	5.6	-
14:0	0.2	-	2.4	0.1
16:0	23.5	11.9	10.0	15.3
16:1	-	-	0.3	-
17:0	0.3	-	0.1	0.1
17:1	-	0.1	0.1	0.1
18:0	31.9	2.0	8.0	12.1
18:1 *c*	36.9	78.7	17.5	39.9
18:1 *t*	-	-	39.1	19.5
18:2 9*c*,12*c*	4.5	5.5	5.3	4.7
18:2 *ct*,*tc* 9,12	-	-	0.6	0.3
18:2 9*t*,12*t*	-	-	0.5	0.2
18:2 other	-	-	7.7	3.9
18:3	0.2	0.6	-	-
20:0	1.3	0.4	0.5	0.6
20:1	-	0.4	0.5[2]	0.4[2]
22:0	0.3	0.2	0.4	0.3

[1]) The shorthand notation used for the fatty acids indicates chain length: number of double bonds. *c*=*cis* isomers, *t*=*trans* isomers, other=non-9,12 isomers (*cis* and *trans*), -= not detected.

[2]) Also contains conjugated 18:2 isomers.

and Meisel), adenosine triphosphatase (EC 3.6.1.3; Padykurlas and Hermans), alkaline phosphatase (EC 3.1.3.1; Burstones) and acid phosphatase (EC 3.1.3.2; Burstones) was determined.

Serum chemistry

Serum was prepared by standard procedures (clotting: 30 min at 37°C; centrifuging: 10 min at 0°C at 1500xG). Within 48 h after serum preparation (serum was kept at +4°C), a number of enzyme activities and biochemical parameters were determined, using a programmable Vitatron PA 800 automatic analyzer (Vitatron, Dieren, The Netherlands). Sorbitol dehydrogenase was measured manually. Commercial test kits were used according to manufacturers prescription (see Materials). Total protein was determined with the Biuret method. In the first experiment the serum activity of alkaline phosphatase (AP; EC 3.1.3.1), alanine amino transferase (ALAT; EC 2.6.1.2), sorbitol dehydrogenase (SDH; EC 1.1.1.14) and the serum level of creatinine was measured. In the second experiment the activity of aspartate aminotransferase (ASAT; EC 2.6.1.1), isocitrate dehydrogenase (ICDH; EC 1.1.1.42), butyrylcholine esterase (BChE; EC 3.1.1.8), α-hydroxy-butyrate dehydrogenase (α-HBDH; EC 1.1.1.27) and creatine (phospho) kinase (CK; EC 2.7.3.2) and the levels of urea, total cholesterol, total triacylglycerol, glucose, anorganic phosphorus, total protein, albumin, magnesium and calcium in serum were also measured.

Hematology

In week 11 of the feeding period (prior to terminal sacrifice) of the second rat trial a 250 μl blood sample was taken from the tail vein of 10 animals per group. The samples were examined for red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), hemoglobin content (HB) and white blood cell count (WBC) using a Contraves Analyzer 8016 (Contraves, Zürich, Switzerland).

Statistical analysis

The data were analyzed using analysis of variance according to the randomized complete blocks design with days of sacrifice as blocks in the first experiment and litters in the second experiment. In the first experiment Dunnett's procedure has been applied to test which groups differed systematically from the standard group i.e. group 3 (20 en% *trans* fatty acids and 2 en% linoleic acid). In the second experiment the Student-Newman-Keuls multiple-range test was applied to locate systematic differences between the dietary treatments, if present.

RESULTS

Observations during the feeding period

No mortality occurred during the feeding periods, except for one animal in the second experiment which was sacrificed after an accident. The appearance of the animals was indicative of good health. The weekly check for general health revealed only slight abnormalities. These anomalies, commonly seen in Wistar rats, were not treatment-related. No clear symptoms of essential fatty acid (EFA)-deficiency could be detected in any of the groups.

The mean bodyweights of the test animals in the first experiment were not significantly different from those of standard group 3 (Table 5). In the second experiment the animals fed the CB-diet showed a slight growth retardation during the last weeks of the feeding period (Table 6). The differences between the groups, however, were not statistically significant.

The mean food and water consumption of the first experiment are included in Table 5. The mean food intake of group 8 was significantly (conf. > 90%) lower than that of group 3. Group 1 and group 8 showed a significantly (conf. > 95%) lower water consumption than group 3. The differences in water and food consumption could not be attributed to either the linoleic acid level nor to *trans* fatty acids.

TABLE 5

Bodyweights (g), water and food consumption (g/day) and trans epidermal water loss (TEWL)($1/cm^2/h$) of animals in the first experiment[1].

Group	T[2]	LA[3]	n	Bodyweight week 8	Bodyweight week 13	Water consumption	Food consumption	TEWL
1	20	0.4	12	305.3 ± 9.6	381.8 ± 12.9	18.7 ± 0.5[b]	16.3 ± 0.4	66.6 ± 12.2
2	20	0.8	12	322.0 ± 7.8	400.0 ± 10.4	20.9 ± 1.0	17.4 ± 0.4	35.6 ± 4.8
3	20	2.0	24	308.6 ± 5.5	385.6 ± 6.8	22.7 ± 0.9[c]	16.3 ± 0.3	45.7 ± 7.7
4	20	3.0	12	314.0 ± 7.9	390.0 ± 9.6	23.2 ± 1.3	16.2 ± 0.3	49.2 ± 6.6
5	20	5.0	12	321.7 ± 8.1	398.9 ± 10.3	22.6 ± 0.8	16.4 ± 0.4	46.8 ± 7.9
6	20	7.1	12	329.2 ± 10.0	403.6 ± 15.2	21.8 ± 1.4	16.8 ± 0.4	45.2 ± 6.7
7	0	2.0	12	305.8 ± 12.5	389.0 ± 13.9	21.6 ± 1.0	15.7 ± 0.5	56.1 ± 9.1
8	0	5.0	12	310.0 ± 6.3	386.0 ± 7.8	17.6 ± 1.0[b]	14.9 ± 0.3[a]	53.2 ± 8.1

1) Bodyweights are the means ± s.e.m. of n animals, measured in week 8 and 13 respectively.
Water- and food consumption are the means ± s.e.m. of the average consumption of n animals in week 4, 8 and 12. TEWL was determined in week 11.
a,b = Significantly different from values of group 3 (confidence >90%, conf. > 95% resp.) c = n=23.

2) T = Dietary level of *trans* fatty acids (en%).

3) LA = Dietary level of linoleic acid (en%).

In the second experiment no systematic differences were found in the mean food and water consumption between the groups at any time measured (data not shown).
The trans epidermal water loss (TEWL) showed a clear increase at the lowest level of linoleic acid in the diet (0.4 en%; see Table 5). There was no significant effect of *trans* fatty acids on TEWL.

Post-mortem findings

The relative spleen weights of the animals in group 6 were systematically higher than those in group 3, while the animals in group 7 displayed lower spleen weights than those in group 3 (confidence > 90%), see Table 7. In the second experiment no systematic differences between the groups at a confidence level of > 90% could be detected in the relative weights of the investigated organs (Table 7).

TABLE 6

Bodyweights (g) of animals in the second experiment[1].

Group	Bodyweight week 6	Bodyweight week 12
CB	284.6 ± 3.4	381.8 ± 12.0
OV	281.6 ± 3.2	400.4 ± 12.4
PHSO	291.1 ± 3.7	402.8 ± 12.7
PHSOMix	283.9 ± 3.6	401.0 ± 12.1

1) Bodyweights are the mean ± s.e.m. of 24 animals measured in week 6 and 12 respectively.

The incidence of the macroscopic findings of the animals in both experiments was recorded (data not shown). Both external and internal examination of a small number of animals throughout the groups revealed only slight deviations. The incidence of liver findings, suggestive of some fatty infiltration, seemed to increase with increasing linoleic acid intake in the animals fed *trans* fatty acids in the first

experiment. However, between the two reference groups 7 and 8 (2 and 5 en% linoleic acid resp.) no differences could be detected. Histopathological investigation of the livers of the animals of groups 1, 3 and 7 revealed that *trans* fatty acids (groups 1 and 3 compared to group 7) slightly decreased periportal fatty infiltration.

No pathological changes associated with the recorded differences in relative organ weights were observed. All other changes were either less important, incidental, or equally distributed among the groups.

TABLE 7

Relative organweights (mg/100 g bodyweight) of animals in both experiments[1].

Group	T[2]	LA[3]	n	Liver	Heart	Thymus	Kidneys	Brain	Adrenals	Testes	Spleen
1	20	0.4	12	2568 ± 64	n.d.	169 ± 6.3	670 ± 16.8	n.d.	n.d.	861 ± 27	169 ± 6.3
2	20	0.8	12	2740 ± 67	"	164 ± 4.9	642 ± 10.3	"	"	831 ± 25	164 ± 4.9
3	20	2.0	24	2509 ± 40	"	154 ± 4.4	628 ± 10.0	"	"	809 ± 27	154 ± 4.4
I 4	20	3.0	12	2606 ± 46	"	160 ± 6.2	637 ± 13.9	"	"	799 ± 40	160 ± 6.2
5	20	5.0	12	2758 ± 54	"	155 ± 5.3	609 ± 12.2	"	"	818 ± 21	155 ± 5.3
6	20	7.1	12	2828 ± 51	"	171 ± 6.3	642 ± 16.4	"	"	846 ± 31	171 ± 6.3[a]
7	0	2.0	12	2472 ± 45	"	136 ± 5.0	603 ± 12.5	"	"	806 ± 17	136 ± 5.0[a]
8	0	5.0	12	2529 ± 36	"	149 ± 5.2	642 ± 13.1	"	"	849 ± 17	149 ± 5.2
CB	0	2.0	10	3820 ± 65	319 ± 4.5	145 ± 4.7	632 ± 9.5	494 ± 13.4	14.0 ± 0.91	859 ± 23	164 ± 2.7
OV	0	2.0	10	3780 ± 69	316 ± 8.7	138 ± 6.8	635 ± 12.9	471 ± 18.0	13.2 ± 0.26	839 ± 39	154 ± 6.1
II PHSO	20	2.0	10	3880 ± 96	315 ± 6.4	129 ± 6.0	651 ± 14.5	475 ± 14.1	13.1 ± 0.55	882 ± 40	165 ± 5.7
PHSOMix	10	2.0	10	3750 ± 87	311 ± 4.9	138 ± 7.2	643 ± 12.1	472 ± 11.2	13.7 ± 0.77	888 ± 27	160 ± 5.6

1) Mean ± s.e.m. of n animals in experiment I and II are given. a = Values significantly different from those of group 3 with a confidence > 90%, n.d.= not determined.

2) T = Dietary level of trans fatty acids (en%).

3) LA= Dietary level of linoleic acid (en%).

In the second experiment, no significant pathological changes were found on macroscopic and histopathological examination in any of the treatment groups. On autopsy some OV-fed animals showed very slight

TABLE 8

Enzyme activity and glycogen and fat content in liver in the second experiment, determined by histochemistry[1].

Parameter	Location	CB	OV[2]	PHSO	PHSOMix
Alkaline phosphatase	periportal	1.5	2.3	2.6	2.0
	midzonal	1.1	1.6	1.7	1.3
	perivenular	0.3	0.8	1.0	0.6
Catalase	periportal	1.9	1.7	1.4	1.5
	midzonal	1.9	1.3	1.2	1.3
	perivenular	2.0	1.8	1.5	1.9
Glycogen	periportal	2.0	2.1	2.2	2.1
	midzonal	1.7	1.9	2.0	2.0
	perivenular	1.4	1.7	1.7	1.6
Neutral fat	periportal	0.3	1.2	0.1	0.1
	midzonal	0.3	1.3	0.0	0.4
	perivenular	1.0	1.4	0.4	0.9

1) Scores are means of individual scores on a scale 0-5.

2) Nine animals per group, all other groups 10 animals.

liver changes, which is suggestive of the infiltration of some fat. This finding could be confirmed by liver histochemistry (Table 8), which showed higher neutral-fat scores. Enzyme-histochemical investigation of the liver showed that the reaction to alkaline phosphatase in the animals fed CB was somewhat lower than that in the other animals.

Hematology

None of the hematological parameters revealed systematic differences with a confidence of > 90% between the groups (data not shown).

Serum chemistry

The mean values and s.e.m. of the activity of three enzymes and the concentration of creatinine in serum in the first experiment are given in Table 9. The data have been corrected for day-to day analytical differences; moreover they have been analyzed in two ways: untransformed and log-transformed. Except for creatinine, the same conclusions hold for the transformed and untransformed data. When applying Dunnett's analysis to the data, some statistical differences between the treatment groups and reference groups can be seen. The most striking finding is an increase in SDH with increased linoleic acid intake. None of the investigated parameters were significantly affected by *trans* fatty acids. In the second experiment the serum activity of a number of serum enzymes and the level of some serum components was measured (Table 10). The CB-group gave values deviating significantly from the other three groups; the levels of SDH and ALAT increased, whereas those of AP and α-HBDH both decreased. Furthermore the calcium levels found in this group were significantly lower than those in the other groups. The total cholesterol level was highest in the OV-group and lowest in the PHSO-group. No specific effects of *trans* fatty acids were apparent.

TABLE 9

Activity of enzymes and concentration of creatinine in serum (First experiment)[1]

Group	T[2]	LA[3]	n	Alkaline-phosphatase	Alanine-amino transferase	Sorbitol-dehydrogenase	Creatinine Un-transf.	Creatinine Log-transf[4]
1	20	0.4	12	3.17 ± 0.115	0.292 ± 0.0164	0.029 ± 0.0031	37.0 ± 1.30[b]	36.7 ; 3.7%[a]
2	20	0.8	12	3.06 ± 0.163	0.322 ± 0.0238	0.034 ± 0.0040	38.7 ± 0.66	38.6 ; 1.7%[a]
3	20	2.0	24	3.09 ± 0.140	0.284 ± 0.0064[5]	0.028 ± 0.0022	40.4 ± 1.17	40.2 ; 2.9%
4	20	3.0	12	3.04 ± 0.175	0.274 ± 0.0118	0.032 ± 0.0021	39.2 ± 0.86	39.1 ; 2.2%
5	20	5.0	12	2.71 ± 0.059	0.302 ± 0.0154	0.041 ± 0.0054[b]	40.5 ± 0.62	40.4 ; 1.6%
6	20	7.1	12	2.72 ± 0.085	0.282 ± 0.0103	0.040 ± 0.0033[b]	39.2 ± 0.78	39.1 ; 2.0%
7	0	2.0	12	2.75 ± 0.202	0.326 ± 0.0223	0.042 ± 0.0037[b]	38.2 ± 0.91	38.1 ; 2.3%[a]
8	0	5.0	12	2.97 ± 0.136	0.226 ± 0.0106[b]	0.040 ± 0.0039[b]	40.2 ± 0.87	40.1 ; 2.1%

1) Enzyme activities in μkatal/l; concentration of creatinine in μmol/l, mean ± s.e.m of 12 animals.
a,b = significantly different from values of group 3 (confidence > 90%; conf. > 95%, respectively).

2) T = Dietary level of *trans* fatty acids (en%).

3) LA = Dietary level of linoleic acid (en%).

4) Log transformed data are geometric means and c.v.m. of 12 animals.

5) n = 24.

TABLE 10

Activity of enzymes and concentration of some components in serum (Second experiment)[1].

Enzyme	CB	OV	PHSO	PHSOMix
SDH	0.035 ± 0.0060[b,c,d]	0.019 ± 0.0063[a]	0.014 ± 0.0028[a]	0.018 ± 0.0054[a]
AP	6.3 ± 0.25[b,c,d]	7.3 ± 0.44[a]	7.4 ± 0.38[a]	7.0 ± 0.37[a]
ALAT	0.61 ± 0.033[b,c,d]	0.48 ± 0.024[a]	0.53 ± 0.026[a]	0.55 ± 0.028[a]
ICDH	0.22 ± 0.038	0.16 ± 0.015	0.18 ± 0.017	0.018 ± 0.019
ASAT	3.14 ± 0.178	3.08 ± 0.118	3.27 ± 0.152	3.07 ± 0.118
BChE	6.0 ± 0.59	6.5 ± 0.92	7.4 ± 0.75	8.1 ± 1.03
α-HBDH	6.8 ± 0.48[b,c,d]	8.6 ± 0.49[a]	9.0 ± 0.51[a]	8.1 ± 0.51[a]
CK	51 ± 4.6	44 ± 2.2	48 ± 2.9	48 ± 3.7

Component (unit)	CB	OV	PHSO	PHSOMix
Urea (mM)	8.1 ± 0.17	8.0 ± 0.40	7.7 ± 0.19	7.4 ± 0.29
Creatinine (μM)	40.4 ± 0.98	38.8 ± 1.66	38.4 ± 0.99	37.4 ± 2.27
TC (mM)	2.60 ± 0.078[b,c]	3.02 ± 0.138[a,c,d]	1.95 ± 0.085[a,b,c]	2.44 ± 0.128[b,c]
Glucose (mM)	8.1 ± 0.11	8.2 ± 0.29	8.0 ± 0.19	8.4 ± 0.26
PI (mM)	2.30 ± 0.097	2.25 ± 0.122	2.52 ± 0.248	2.74 ± 0.175
Total protein (g/l)	70 ± 1.2[d]	74 ± 2.4	78 ± 4.5	81 ± 3.8a
Albumin (g/l)	45 ± 1.2	47 ± 2.2	48 ± 1.9	50 ± 2.2
Mg (mM)	0.924 ± 0.0125	0.995 ± 0.0206	0.936 ± 0.0184	0.942 ± 0.0160
Ca (mM)	2.46 ± 0.027[b,c,d]	2.53 ± 0.025[a]	2.59 ± 0.023[a]	2.56 ± 0.012[a]

[1]) Values indicated are means ± s.e.m. of 10 animals. Activity of enzymes in serum in μkatal/l. Values with superscript a, b, c and d are significantly different from those from CB, OV, PHSO and PHSOMix respectively, with a confidence > 90%.

DISCUSSION

In previous experiments with rats, no detrimental effects of partially hydrogenated vegetable oils were found on longevity, growth, reproduction, organ function and food intake, and on the histopathological examination of various organs (1,2,14).
The amount of *trans* fatty acids in the diets of those experiments differed widely and ranged from about 8 to 36 en%. The lowest linoleic acid level was approx. 1.7 en% and the ratio *trans* fatty acids/linoleic acid never exceeded 10. However, some adverse effects were reported, which were attributed to the feeding of *trans* fatty acids in EFA-deficient rats. The growth was somewhat more reduced in rats fed hydrogenated arachis oil than in those fed a fat-free diet (10). The animals fed hydrogenated arachis oil consumed less food than those in a group fed an adequate amount of linoleic acid. The absolute testes weight in the EFA-deficient animals fed *trans* fatty acids was somewhat lower than that in the animals fed a fat-free diet. In addition, there was an almost total degeneration of the spermatogenic tissue. In the group fed *trans* fatty acids this degradation was even more severe than that in the group fed a fat-free diet. In the animals that were fed large amounts of linoleic acid, the spermatogenesis was not affected.

In other experiments, too, slight deviations caused by the incorporation of *trans* fatty acids in the diet were incidently observed: decreased bodyweights (11,15-17), increased scaliness of the skin (11), decrease in heart weight (18), decrease in food intake and food-conversion efficiency and concomitant increase in total body-heat production (17), and alterations in myloid/erythroid ratios in bone marrow (19). All these deviations could be corrected by adding linoleic acid to the diet.

Trans fatty acids in partially hydrogenated oils and ruminant fats form a heterogeneous group of positional isomers (20,21). The metabolism of each isomer may differ significantly (7), and the biological effects of the isomers could vary accordingly. In our

experiment partially hydrogenated oil with a very high level (more than 50% and 10% respectively) of both monoenoic and dienoic isomers was used. The advantage of this approach is that most of the isomers commonly present in the diet of western societies are incorporated into the experimental rat diet. Moreover, these fatty acid isomers are present in a similar distribution as in the human diet, which enables the drawing of more realistic conclusions with regard to the nutritive value of food products containing *trans* fatty acids. The maximum level of *trans* fatty acids in our experiments was 20 en%, while the linoleic acid level varied from 0.4 to 7.1 en%. The ratios of *trans* fatty acids to linoleic acid in the diets ranged from almost 3 to 50. In our second experiment, a direct comparison has been made between the diets rich in *trans* fatty acids (20 and 10 en%) and diets rich in oleic acid or saturated fatty acids; in both cases linoleic acid was present (2 en%).

In spite of this high dose of *trans* fatty acids, no treatment-related differences in body weight and clinical appearance were found in either experiment, nor was the food consumption significantly influenced. However, in the first experiment the food conversion efficiency (defined as gram weight gain per gram of food consumed over a specific period) in all the groups fed *trans* fatty acids appeared slightly lower than that in the two reference groups. This finding is in agreement with that of Nolen (22) with two partially hydrogenated vegetable oils, viz. soybean oil and rapeseed oil.

In the second experiment we did not observe this trend, which is in line with experiments in which no effects of PHSO with adequate linoleic acid on energy metabolism (17) or food efficiency (23) were found. A mixture of *trans*-isomers of linoleic acid reduced the food conversion efficiency, even in the presence of 7 en% linoleic acid (17). An increased food intake in rats fed *trans* fatty acids, without any effect on bodyweight, has also been reported (2).

Water consumption and trans-epidermal waterloss (TEWL) are normally increased in EFA-deficient animals (13,24), which can be attributed to an increased skin permeability for water due to linoleic acid

deficiency. The group fed the lowest dose of linoleic acid in the first experiment (group 1: 0.4 en%) indeed showed the highest TEWL. However, the water consumption, compared to that in previous experiments, was very low in view of the dietary linoleic acid level. From 0.8 to 7.1 en% linoleic acid, both the TEWL and the water consumption show the same trend. The TEWL in both reference groups was higher than that in the groups fed *trans* fatty acids. From these findings we may conclude that *trans* fatty acids from PHSO do not increase the requirement of the skin for linoleic acid. Also trilinolelaidate in the presence of 1.1. en% linoleic acid did not influence the insensible water loss (total water loss via skin permeation and expired air) and water consumption, while both parameters were significantly increased in EFA-deficient rats (25).

In agreement with other studies we found no effects of *trans* fatty acids on relative organ weights (1,22,25,26), nor on hematological parameters (22,26). Pathological changes attributable to dietary *trans* fatty acids were not found in either experiment. A somewhat lower periportal fatty infiltration in the liver was noted in the *trans* fatty acids-fed groups in the first experiment. Histochemical measurements confirmed this finding by showing a slight reduction in the neutral fat content in the liver in the *trans* fatty acids fed animals compared to especially the olive oil fed animals. Cho et al. (27) reported that only in the fed state, the triacylglycerol content in the liver in rats fed olive oil was significantly higher than that in rats fed partially hydrogenated corn oil; this was not the case when the animals were fasted. Since Aaes-Jørgensen and Hølmer (10) found that partially hydrogenated arachis oil aggravated the degeneration of spermatogenic tissue in testes of EFA-deficient rats, special attention was focused on the testes. In multigeneration studies, however, no effect of *trans* fatty acids on reproduction was observed (14,28). In our studies we could not detect any evidence for adverse effects, either. The absence of histopathological findings was supported by the lack of specific effects of *trans* fatty acids on clinical chemistry parameters. The serum activities of SDH, ALAT, ASAT and AP, which are

generally regarded as very sensitive indicators for pathological changes in (mainly) the liver (29,31), were not influenced by *trans* fatty acids in the present rat studies. The influence of the linoleic acid level on SDH and AP activity in our first experiment is worth mentioning. In this experiment the reference diet with 2 en% linoleic acid, a mixture of coconut oil and olive oil, evoked a similar, though less pronounced, response in AP, ALAT and SDH activity as the CB-diet in the second experiment. This response might be ascribed to the high levels of saturated fatty acids in these diets.

The response of blood cholesterol levels to changes in dietary fat usually differ widely in man and rat. The predominant cholesterol-carrying lipoprotein in rats is high-density lipoprotein (HDL), while low-density lipoprotein (LDL) is virtually absent (32). Most changes in blood cholesterol levels induced by dietary fat in humans are observed in the LDL-fraction, with (mostly) only minor changes in HDL-cholesterol (33). Total blood cholesterol levels in man (mainly LDL-cholesterol) decrease when the intake of saturated fat is diminished, while *cis*-monounsaturated fatty acids are thought to be neutral towards blood cholesterol (34,35). The effect of *trans* fatty acids on blood cholesterol levels in humans, compared with that of either saturated or *cis*-monounsaturated fatty acids, has not been completely elucidated. The two major studies performed may indicate intermediate effects of saturated and *cis*-monounsaturated fatty acids (36,37). In our second rat study, the fat with the lowest level of saturated fatty acids (OV) induced the highest blood cholesterol level. The lowest values were obtained in the two groups that were fed *trans* fatty acids. Other workers, too, reported that *trans* fatty acids did not increase the blood cholesterol level in rats (14,26,27).

Our results confirm previous findings that dietary *trans* fatty acids from partially hydrogenated vegetable oils do not cause detrimental effects with regard to general health, pathology, clinical chemistry and hematology, when the rats are fed with an adequate level of linoleic acid.

Moreover, it is found that 2 en% linoleic acid is certainly an adequate level, even when the amount of *trans* fatty acids is as high as 20 en%. Also other parameters like mitochondrial respiration, prostaglandin synthesis and β-oxidative capacity - included in our extensive study on the biological effects of *trans* fatty acids - were not influenced by dietary *trans* fatty acids in the presence of 2 en% linoleic acid (38,39). Therefore we fully support the conclusion of the ad-hoc panel of the Federation of American Societies for Experimental Biology (40) that there is little reason for concern with respect to the safety of dietary *trans* fatty acids at the present and expected levels of consumption of linoleic acid.

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LITERATURE CITED

1. Thomasson, H.J., Gottenbos, J.J., Kloeze, J. & Vles, R.O. (1966) Nutritional evaluation of hydrogenated fats. *Proc. Nutr. Soc.* 25: 1-4.

2. Vles, R.O. & Gottenbos, J.J. (1972) Long-term effects of feeding butterfat, coconut oil and hydrogenated or non-hydrogenated soyabean oils 2. Life span experiment in rats. *Voeding* 33: 455-465.

3. Vles, R.O. & Gottenbos, J.J. (1972) Long-term effects of feeding butterfat, coconut oil and hydrogenated or non-hydrogenated soyabean oils 1. Eighteen-month experiment in mice. *Voeding* 33: 428-433.

4. Vles, R.O., Gottenbos, J.J. & Pijpen, P.L. van. (1977) Aspects nutritionnels des huiles de soja hydrogénées et de leurs acides gras insaturés isomériques. *Bibl. Nutr. Dieta* 25: 186-196.

5. Applewhite, T.H. (1981) Nutritional effects of hydrogenated soya oil. *J. Am. Oil Chem. Soc* 58: 260-269.

6. Beare-Rogers, J.L. (1983) *Trans-* and positional isomers of common fatty acids. In: *Advances in nutritional research, vol 5,* (Draper, H.H., ed.) Plenum Publishing Corporation, New York.

7. Emken, E.A. (1983) Biochemistry of unsaturated fatty acid isomers. *J. Am. Oil Chem. Soc* 60: 995-1004.

8. Gurr, M.I. (1983) *Trans* fatty acids. *Int. Dairy Fed. Doc.* 166: 5-18.

9. Gottenbos, J.J. (1983) Biological effects of *trans* fatty acids In: *Dietary fats and health* (Perkins, E.G. and Visek, W.J., eds.) pp. 375-389, Am. Oil Chemists' Soc., Campaign.

10. Aaes-Jørgensen, E. & Hølmer, G. (1969) Essential fatty acid-deficient rats: 1. Growth and testes development. *Lipids* 4: 501-506.

11. Hill, E.G., Johnson, S.B. & Holman, R.T. (1979) Intensification of essential fatty acid deficiency in the rat by dietary trans fatty acids. *J. Nutr.* 109: 1759-1765.

12. Zevenbergen, J.L., Houtsmuller, U.M.T. & Gottenbos, J.J. (1988) Linoleic acid requirement of rats fed *trans* fatty acids. *Lipids* 23: 178-186.

13. Houtsmuller, U.M.T. & van der Beek, A. (1981) Effects of topical application of fatty acids. *Prog. Lipid Res.* 20: 219-224.

14. Alfin-Slater, R.B., Wells, A.F., Aftergood, L. & Deuel, H.J. Jr. (1957) Nutritive value and safety of hydrogenated vegetable fats as evaluated by long-term feeding experiments with rats. *J. Nutr.* 63: 241-261.

15. Privett, O.S., Phillips, F., Shimasaki, H., Nozawa, M.D. & Nickell, E.C. (1977) Studies on the effects of *trans* fatty acids in the diet on lipid metabolism in essential fatty acid deficient rats. *Am. J Clin. Nutr.* 30: 1009-1017.

16. Hwang, D.H. & Kinsella, J.E. (1979) The effects of *trans,trans* methyl linoleate on the concentration of prostaglandins and their precursors in rat. *Prostaglandins* 17: 543-559.

17. De Schrijver, R. & Privett, O.S. (1984) Energetic efficiency and mitochondrial function in rats fed *trans* fatty acids. *J. Nutr* 114: 1183-1191.

18. Blomstrand R. & Svensson, L. (1983) The effects of partially hydrogenated marine oils on the mitochondrial function and membrane phospholipid fatty acids in rat heart. *Lipids* 18: 151-170.

19. Bruckner, G., Goswami, S, Trimbo, S. & Kinsella, J.E. (1982) Dietary trilinoelaidate: effects on metabolic, hematologic and biochemical responses in rats. *Am. J. Clin. Nutr.* 35: 91.

20. Dutton, H.J. (1979) Hydrogenation of fats and its significance. In: *Geometric and positional fatty acid isomers* (Emken, E.A. & Dutton, H.J., eds), pp. 1-16, Am. Oil Chemists' Soc., Campaign.

21. Ohlrogge, J.B. (1983) Distribution in human tissues of fatty acid isomers from hydrogenated oils. In: *Dietary fats and health* (Perkins, E.G. & Visek, W.J., eds.) pp. 359-374, Am. Oil Chemists' Soc., Campaign.

22. Nolen, G.A. (1981) Biological evaluation of hydrogenated rapeseed oil. *J. Am. Oil Chem. Soc.* 58: 31- 37.

23. Blomstrand, R., Diczfalusy, U., Sisfontes, L. & Svensson, L. (1985) Influence of dietary partially hydrogenated vegetable and marine oils on membrane composition and function of liver microsomes and platelets in the rat. *Lipids* 20: 283-295.

24. Houtsmuller, U.M.T. (1975) Specific biological effects of polyunsaturated fatty acids. In: *The role of fats in human nutrition* (Vergroesen, A.J., ed.) pp.331-352, Academic Press, London.

25. Bruckner, G., Shimp, J., Goswami, S, Mai, J. & Kinsella, J.E. (1982) Dietary trilinoelaidate: Effects on metabolic parameters related to EFA metabolism in rats. *J. Nutr.* 112: 126-135.

26. Nolen, G.A. , Alexander, J.G. & Artman, N.R. (1967) Long-term rat feeding study with used frying fats. *J. Nutr.* 93: 337-348.

27. Cho, Y.-J., Ide, T. & Sugano, M. (1984) Effects of overnight fasting on lipid and fatty acid profiles of rats fed *cis*- and *trans*-fat. *J. Fac. Agr. Kyushu Univ.* 29: 77-90.

28. Alfin-Slater, R.B. & Aftergood, L. (1979) Nutritional role of hydrogenated fats (in rats). In: *Geometrical and positional fatty acid isomers* (Emken, E.A. & Dutton, H.J., eds), pp. 53-74, Am. Oil Chemists' Soc., Campaign.

29. Ringler, D.H. & Dabich, L. (1979) Hematology and clinical biochemistry. In: *The laboratory rat, Vol. I.* (Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., eds.) pp. 112-121, Academic Press Inc., Orlando.

30. Korsrud, G.O., Grice, H.G., Goodman, T.K., Knipfel, J.E. & McLaughlan, J.M. (1973) Sensitivity of several serum enzymes for the detection of thioacetamide-, dimethylnitrosamine- and diethanol-amine-induced liver damage in rats. *Toxicol. Appl. Pharmacol.* 26: 299-313.

31. Reddrop, C.J., Cheeseman, K.H. & Slater, T.F. (1983) Correlations between common tests for assessment of liver damage: indices of the hepatoprotective activity of promethazine in carbon tetrachloride hepatotoxicity. *Cell Biochem. Function* 1: 55-63.

32. Chapman, M.J. (1980) Animal lipoproteins: chemistry, structure, and comparative aspects. *J. Lipid Res.* 21: 789-853.

33. Havel, R.J. (1983) Dietary regulation of plasma lipoprotein metabolism in humans. *Prog. Biochem. Pharmacol.* 19: 110-122.

34. Keys, A., Anderson, J.T. & Grande, F. (1957) Prediction of serum-cholesterol responses of man to changes in fats in the diet. *Lancet* 2: 959-966.

35. Rifkind, B.M. (1986) Diet, plasma cholesterol and coronary heart disease, *J. Nutr.* 116: 1578-1580.

36. Vergroesen, A.J. (1972) Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. *Proc. Nutr. Soc.* 31: 323-329.

37. Mattson, F.H., Hollenbach, E.J. & Kligman, A.M. (1975) Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels of man. *Am. J. Clin. Nutr.* 28: 726-731.

38. Zevenbergen, J.L. (1987) Biological effects of *trans* fatty acids. In: *Fat production and consumption.* (Galli, C. & Fedeli, E. eds.), pp. 157-167, Plenum Press, New York.

39. Veerkamp, J.H. & Zevenbergen, J.L. (1986) Effect of dietary fat on total and peroxisomal fatty acid oxidation in rat tissues. *Biochim. Biophys. Acta* 878: 102-109.

40. Senti, F.R. (1985) Health aspects of dietary *trans* fatty acids. Report of the ad hoc Committee of the Federation of American Societies for Experimental Biology, FDA223-83-2020.

Footnote 1.

For composition of vitamin and mineral mixture see:
De Deckere, E.A.M., Jansen-Verplanke, C., Blonk, C.G. &
Van Nielen, W.G.L. (1988) J. Nutr. 188: 11-18.

Chapter 3.

LINOLEIC ACID REQUIREMENT OF RATS FED *TRANS* FATTY ACIDS. STUDIES ON MITOCHONDRIAL RESPIRATION

J.L. Zevenbergen, U.M.T. Houtsmuller and J.J. Gottenbos
Linoleic acid requirement of rats fed *trans* fatty acids.
Lipids 23, 178-186, 1988.

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The amount of linoleic acid required to prevent undesirable effects of C18 *trans* fatty acids was investigated. In a first experiment, six groups of rats were fed diets with a high content of *trans* fatty acids (20% of energy (en%)), and increasing amounts of linoleic acid (0.4 to 7.1% en%). In a second experiment, four groups of rats were fed diets designed to compare *trans* fatty acids with saturated and *cis*-monounsaturated fatty acids of the same chain length at the 2 en% linoleic acid level. After 9 to 14 weeks, the oxygen uptake, lipid composition, and ATP-synthesis of heart and liver mitochondria were determined.

The phospholipid composition of the mitochondria did not change, but the fatty acid compositions of the two main mitochondrial phospholipids were influenced by the dietary fats. *Trans* fatty acids were incorporated in all phospholipids investigated. The linoleic acid level in the phospholipids, irrespective of the dietary content of linoleic acid, increased on incorporation of *trans* fatty acids. The arachidonic acid level had decreased in most phospholipids in animals fed diets containing 2 en% linoleic acid. At higher linoleic acid intakes, the effect of *trans* fatty acids on the phospholipid arachidonic acid level diminished. However, in heart mitochondrial phosphatidylethanolamine, *trans* fatty acids significantly increased the arachidonic acid level. Despite these changes in composition, neither the amount of dietary linoleic acid nor the addition of *trans* fatty acids influenced the mitochondrial function. For rats, a level of 2 en% of linoleic acid is sufficient to prevent undesirable effects of high amounts of dietary C18 *trans* fatty acids on the mitochondrial function.

Lipids 23, 178-186 (1988)

Trans fatty acids from partially hydrogenated oils and from ruminant fats form part of the human diet. Throughout the years, there has been a marked interest in the biological effects of *trans* fatty acids. It is now recognized that they are well absorbed by both man and

animals, and that they are found in most tissues (1-5). *Trans* fatty acids are incorporated into triacylglycerol and phospholipids of biological membranes (1,2,6). The effect of the incorporation of *trans* fatty acids on the biological function of membranes is an unanswered question.

Many investigators have used mitochondria to investigate the effects of dietary fatty acids on biological membranes (7-14). Mitochondrial oxygen uptake and ATP-synthesis are regarded as sensitive markers for the functionality of the mitochondria. Changes in the fatty acid composition of mitochondria were shown to cause diminished mitochondrial function in rats fed diets containing erucic acid (9,15,16). The amount of essential fatty acids in the diet also affected the mitochondrial composition and/or function (7,11,12). The amount of *trans* fatty acids incorporated into mitochondrial membranes may be as high as 17% of the total fatty acid content (17).

Trans fatty acids formed during the partial hydrogenation of vegetable oils appear not to have undesirable effects when compared to saturated or *cis*-monounsaturated fatty acids, provided sufficient linoleic acid is present in the diet (1-3,6,18). It was our aim to quantify the amount of linoleic acid necessary.

In a first experiment, we investigated the effects of diets having a constant amount of C18 *trans* fatty acids and an increasing amount of linoleic acid on the mitochondrial composition and function. In the second, we compared the effects of *trans* fatty acids with those of long chain (C16,C18) saturated and *cis*-monounsaturated fatty acids at a linoleic acid level of 2 en%.

EXPERIMENTAL

<u>Materials</u>. Partially hydrogenated soybean oil (PHSO) was specially prepared by Van den Bergh and Jurgens (Rotterdam, The Netherlands); hydrogenated coconut oil (HCNO) from Chempry BV (Raamsdonkveer, The Netherlands), sunflower seed oil (SSO) from Union (Antwerpen, Belgium) and cocoa butter (CB) from J. Schoenmaker BV (Zaandam, The

Netherlands). Olive oil (OV) was purchased from Fol Jr & Co (Krimpen aan de IJssel, The Netherlands) and the low-linoleic acid olive oil (OV-LL) was a gift from Elais (Greece). Bovine serum albumin (fraction V, essentially free from fatty acid), L-glutamate, and DL-malate were from Sigma Chemical Co. (St. Louis, MO); ADP and ATP were from Boehringer (Mannheim, FRG), Nagarose from Bacillus amyloliquefaciens was from Serva (Heidelberg, FRG), EDTA (p.a.), diethyl ether and hexane were from Baker Chemicals (Deventer, The Netherlands), sucrose was from BDH Chemicals Ltd. (Poole, England), BHT (2,6-di-tert-butyl-p-cresol) was from Fluka AG (Buchs, Switzerland). The HPTLC-plates (Silica gel F254) and all other reagents were from Merck (Darmstadt, FRG).

TABLE 1

Composition (en%) of Experimental Fats and Calculated Linoleic Acid Content (en%) in the Final Diet

Dietary oil[a]	Experimental fats							
	1	2	3	4	5	6	7[b]	8[b]
PHSO	30.0	30.0	30.0	30.0	30.0	30.0	-	-
HCNO	7.3	5.0	5.0	5.0	3.0	-	22.6	-
OV	2.7	4.8	2.7	1.0	-	-	17.4	39.3
SSO	-	0.2	2.3	4.0	7.0	10.0	-	0.7
LA[c]	0.4	0.8	2.0	3.0	5.0	7.1	2.0	5.0

[a] PHSO, partially hydrogenated soybean oil; HCNO, hydrogenated coconut oil; OV, olive oil; SSO, sunflower seed oil.

[b] References.

[c] Linoleic acid.

Animals and diets. Weanling male SPF-Wistar rats (CPB=WU, Central Breeding Station TNO Zeist, The Netherlands) were used in both experiments. The animals were housed individually in a climatized room. The mean temperature was 23.0°C, the relative humidity was 45-70%, and there was a day/night cycle of 12/12 hr. The animals had free access to water and food. This food, a semisynthetic diet with 40 en% fat, was composed of (in $g.MJ^{-1}$): casein 14.8; vitamin mixture 0.2; salt mixture 1.3; cellulose 3.8; maize starch 25.2; experimental fat 10.3 (for composition of vitamin and salt mixture see Ref. 19). The animals were weighed and examined weekly.

For the first experiment, PHSO, HCNO, OV and SSO were mixed to yield 6 diets having a graduated linoleic acid content (0.4-7.1 en%) but a constant *trans* fatty acids level of 20 en% (Table 1). Two fats served as references, one consisting of HCNO and OV (2 en% linoleic acid), another mainly of OV (5 en% linoleic acid). Table 2 shows the calculated fatty acid compositions of the experimental fats. Seven groups consisted of 12 animals each and one of 24 animals: group 3. The rats were fed the experimental diets for 13-14 weeks.

In the second experiment, 4 groups of 24 rats each (randomly selected from 24 litters of 4 rats) were fed diets containing either 20 en% *trans* fatty acid, PHSO, or mainly saturated fatty acids, CB, or *cis*-monounsaturated fatty acids, OV-LL, or a mixture of PHSO, CB and OV (PHSO mix, 10 en% *trans* fatty acids, see Table 3). All diets contained 2 en% linoleic acid and were fed for 9-11 weeks. The (*trans*) fatty acid compositions are given in Table 4.

Analytical. Fatty acid composition of dietary fats. The composition of PHSO was determined by a combination of $AgNO_3$-TLC separation and capillary GC. Fatty acid methyl esters were prepared by methylation of fatty acids with BF_3 and subsequent separation on pre-coated thin-layer SiO_2-plates (impregnated with $AgNO_3$) by elution with toluene. The fatty acid methyl ester fractions were analyzed according to Scholfield (20). The compositions of the other fats and oils were determined by GLC (5% DEGS packed column) after trans-methylation with methanolic HCl.

TABLE 2

Calculated Fatty Acid Composition (%) of the Experimental Fats (groups), First Experiment

Type of fatty acid[a]	Group							
	1	2	3	4	5	6	7[b]	8[b]
6:0	0.1	-	-	-	-	-	0.2	-
8:0	1.2	0.8	0.8	0.8	0.5	-	3.8	-
10:0	1.0	0.7	0.7	0.7	0.4	-	3.2	-
12.0	8.9	6.1	6.1	6.1	3.7	0.1	27.0	-
14:0	3.6	2.5	2.5	2.5	1.6	0.2	10.3	-
16:0	10.3	10.4	10.1	9.9	9.6	9.4	10.5	12.2
16.1	0.3	0.3	0.3	0.2	0.2	0.2	0.4	1.0
17:0	0.1	0.1	0.1	0.1	0.1	0.2	-	0.1
17:1	-	-	-	-	-	0.1	0.1	0.2
18:0	8.5	7.9	8.1	8.1	7.8	7.2	8.0	2.9
18:1 *c*	16.5	20.3	17.5	15.3	14.8	16.1	30.7	69.7
18:1 *t*	39.1	39.1	39.1	39.1	39.1	39.1	-	-
18:2 9*c*,12*c*	1.0	2.0	5.0	7.0	12.5	17.7	5.0	12.5
18:2 *ct*,*tc* 9,12	0.6	0.6	0.6	0.6	0.6	0.6	-	-
18:2 9*t*,12*t*	0.5	0.5	0.5	0.5	0.5	0.5	-	-
18:2 other	7.7	7.7	7.7	7.7	7.7	7.7	-	-
18:3	-	-	-	-	-	0.1	0.2	0.5
20:0	0.3	0.3	0.3	0.3	0.4	0.4	0.2	0.2
20:1*c*	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0.3
22:0	0.3	0.4	0.3	0.3	0.4	0.5	0.1	0.3
actual LA-level[d](%)	1.1	n.d.	5.4	n.d.	n.d.	n.d.	5.2	n.d.

[a] The notation for the fatty acid indicates chain length and number of double bonds *c* = *cis*-isomers, *t* = *trans*-isomers.

[b] References.

[c] Also contains conjugated 18:2 isomers.

[d] Determined by GLC.

n.d. not determined.

Mitochondrial oxygen uptake and ATP synthesis. At the end of the feeding period the animals were fasted overnight, weighed, and sacrificed by aorta cannulation under ether anesthesia. Heart and liver were removed immediately and washed in ice-cold buffer. The liver mitochondria were prepared essentially as described by Clouet and Bezard (21). The heart mitochondria were prepared according to Hülsmann (22). The mitochondrial respiration was measured according to Swarttouw (23) using a Clark oxygen electrode (Gilson Oxygraph, Gilson Electronics, Middleton, WI). Glutamate and malate were used as substrate. The reaction was started by adding ADP. The state-3 respiratory rate in the presence of ADP, the state-4 respiration after exhaustion of ADP, the respiratory control ratio, the ADP/O ratio, and the ATP synthesis rate were calculated as described by Estabrook (24).

Lipid composition of mitochondrial membranes. Immediately after isolation and addition of BHT, part of the mitochondria were frozen and stored at -25°C. Before analysis, 6 samples within each group were pooled. Lipid was extracted according to Bligh and Dyer (25). The phospholipid fraction of the liver mitochondria (first experiment) was isolated on a silica gel column; the phospholipid classes were separated by HPLC according to Patton et al. (26). The phospholipid fraction of the heart mitochondria (both experiments) was isolated on TLC. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), cardiolipin (CL) and sphingomyelin (S) were isolated by TLC according to Christie (27). The phospholipids were quantitated as described by Bartlett (28). The fatty acids of PC and PE were trans-methylated with methanolic HCl and analyzed by GLC using a packed (5% DEGS) column or a glass capillary column (Silar 88). The fatty acid distribution was expressed as area percentage. Because the fatty acid analyses were performed on 2 or 4 pooled samples of 6 animals each, only the means and no standard errors of the means are given.

Statistical. The mitochondrial respiration data were subjected to an analysis of variance according to the randomized complete blocks design. Days of sacrifice served as blocks in the first experiment, litters in

TABLE 3

Composition (en%) of Experimental Fats (groups), Second Experiment

Dietary oils[a]	Experimental fats			
	CB	OV	PHSO	PHSOMix
PHSO	-	-	30.0	15.0
OV-LL	-	40.0	-	-
OV	-	-	2.7	15.0
CB	39.4	-	-	10.0
SSO	0.6	-	2.3	-
HCNO	-	-	5.0	-

[a] PHSO, partially hydrogenated soybean oil; OV-LL, low-linoleic acid olive oil; OV, olive oil; CB, cocoa butter; SSO, sunflower seed oil; HCNO, hydrogenated coconut oil.

the second. In the first experiment, Dunnett's procedure was applied to find out which groups differed systematically from the standard group (group 3, 20 en% *trans* fatty acids and 2 en% linoleic acid). In the second experiment, the Student-Newman-Keuls multiple-range test was applied to locate possible systematic differences between the dietary treatments.

RESULTS

General condition of the animals. All animals were in good health in both experiments, showing no overt signs of EFA-deficiency. Macroscopic examination of the animals did not reveal treatment-related differences. Systematic differences in food consumption or body weight related to dietary treatment did not occur in either experiment. At the time of sacrifice the weight of the animals ranged from 370-400 g in the first experiment, and from 350-400 g in the second.

TABLE 4

Fatty Acid Compositions (%) of the Experimental Fats (groups), Second Experiment

Type of fatty acid[a]	Group			
	CB	OV	PHSO	PHSOMix
6:0	-	-	-	-
8:0	-	-	0.8	-
10:0	-	-	0.7	-
12:0	-	0.1	5.6	-
14:0	0.2	-	2.4	0.1
16:0	23.5	11.9	10.0	15.3
16:1	-	-	0.3	-
17:0	0.3	-	0.1	0.1
17:1	-	0.1	0.1	0.1
18:0	31.9	2.0	8.0	12.1
18:1 *c*	36.9	78.7	17.5	39.9
18:1 *t*	-	-	39.1	19.5
18:2 9*c*,12*c*	4.5	5.5	5.3	4.7
18:2 *ct*,*tc* 9,12	-	-	0.6	0.3
18:2 9*t*,12*t*	-	-	0.5	0.2
18:2 other	-	-	7.7	3.9
18:3	0.2	0.6	-	-
20:0	1.3	0.4	0.5	0.6
20:1	-	0.4	0.5[b]	0.4[b]
22:0	0.3	0.2	0.4	0.3

[a] The notation indicates chain length and number of double bonds. *c* = *cis*-isomer, *t* = *trans*-isomer.

[b] Also contains conjugated 18:2 isomers.

Oxygen uptake and ATP-synthesis in mitochondria. The data on the heart mitochondria, i.e. oxygen consumption (QO_2), number of ADP moles converted into ATP per mol O (ADP/O), rate of ATP-synthesis (rATP) and respiratory control ratio (RCR), have been depicted in Table 5 (first experiment). No significant differences, induced by the dietary treatments, could be detected. Even at a very low linoleic acid intake (0.4 en%), the functionality of the mitochondria was not impaired. The respiratory capacity of the liver mitochondria was also analyzed (Table 5). In the liver mitochondria of group 2 (0.8 en% linoleic acid), the QO_2, rATP, ADP/O and RCR decreased significantly compared to group 3 (2.0 en% linoleic acid). There were no significant differences between group 3 (20 en% *trans* fatty acids and 2 en% linoleic acid) and the reference groups 7 and 8. Data on the respiratory capacity of the heart mitochondria (second experiment) are given in Table 6. No significant differences were detected between any of the groups.

Phospholipid composition of mitochondria. Heart. There was some variation between the groups in the phospholipid composition of the heart mitochondria of the first experiment (Table 7), although no trends in phospholipid levels induced by the dietary linoleic acid could be detected. No effect of *trans* fatty acids on phospholipid distribution was observed. In the second experiment, a similar distribution of phospholipids in the mitochondria was found (Table 8). The differences in the phospholipid composition (mainly that of the minor phospholipids) in both experiments were probably caused by slight differences in the preparation procedure of the mitochondria and by differences in the phospholipid analysis. Since reliable values for phospholipids other than PC cannot be given, the phospholipid composition of the CB-group is incomplete. Only minor differences between the groups can be detected. The differences between the groups fed diets containing *trans* fatty acids (PHSO and PHSOMix) were in the same order of magnitude or even greater than those between groups fed *trans* fatty acids and those fed other diets, indicating that no changes related to *trans* fatty acid were induced.

TABLE 5

Oxygen Uptake and Rate of ATP-Synthesis of Rat Heart and Liver Mitochondria, First Experiment[a]

		Heart				Liver			
Group (en%)	TFA[c]	QO_2[d]	rATP[e]	ADP/O[f]	RCR[g]	QO_2[d]	rATP[e]	ADP/O[f]	RCR[g]
1	20	371±10	2038±50	2.75±0.025	6.4±0.44	68±2.9	287±17	2.10±0.059	2.2±0.13
2	20	365±18	1993±93	2.74±0.025	6.2±0.45	64±3.2[h]	263±18[h]	2.04±0.055[i]	2.1±0.20[i]
3	20	347±14	1901±79	2.73±0.027	6.5±0.43	76±4.1	338±24	2.21±0.052	2.5±0.15
4	20	373±16	2032±82	2.73±0.032	6.7±0.50	77±3.1	344±16	2.22±0.048	2.6±0.16
5	20	369±19	2027±101	2.75±0.026	6.5±0.56	75±4.9	336±23	2.24±0.028	2.6±0.13
6	20	378±17	2071±94	2.74±0.030	6.6±0.46	68±3.4	300±18	2.20±0.043	2.5±0.12
7[b]	0	366±17	2006±88	2.75±0.023	6.4±0.47	71±3.3	310±17	2.17±0.034	2.5±0.11
8[b]	0	354±19	1933±103	2.73±0.033	6.3±0.49	72±3.5	301±20	2.20±0.050	2.7±0.16

[a] Each value represents the mean value ± s.e.m. of 12 animals.
[b] References.
[c] TFA = *trans* fatty acids.
[d] QO_2 = oxygen consumption ($nmol.min^{-1}.mg^{-1}$protein).
[e] rATP = rate of ATP-synthesis ($nmol.min^{-1}.mg^{-1}$protein).
[f] ADP/O = mole ADP converted into ATP per mole O.
[g] RCR = respiratory control ratio.
[h], [i] = significantly different from values of group 3: $P < 0.1$ and $P < 0.05$, respectively.

Liver. The phospholipid composition of the liver mitochondria was somewhat different from that of the heart mitochondria (Table 7): their PC-level was higher whereas their cardiolipin level was lower. Increasing linoleic acid intake increased the fraction of PC of the phospholipids at the expense of PE. No effects of *trans* fatty acids were observed.

Fatty acid composition of phosphatidylcholine. Heart. Saturated and monounsaturated fatty acids were hardly influenced by the amount of linoleic acid in the diet except for a slight decrease in the 18:1 level with increased linoleic acid intake (first experiment; Table 9). Surprisingly, the 18:2 level (mainly linoleic acid) itself remained rather constant, despite its wide range of dietary levels (0.4-7.1 en%). With an increasing linoleic acid intake, the level of arachidonic acid increased to about 34%, then it remained constant; this level was reached with 2-3 en% linoleic acid. The 22:5n-6 level doubled as the linoleic acid intake rose from 0.4 to 7.1 en%. With 22:6n-3, the reverse was observed. A significant incorporation of 20:3n-9, an indicator of essential fatty acid deficiency, could not be detected, not even at the lowest intake of linoleic acid (0.4 en%).

The effects of dietary *trans* fatty acids on the fatty acid composition of PC were more pronounced than those of linoleic acid in the range 0.4-7.1 en%. Compared to the two reference diets (groups 7 and 8), the diets containing *trans* fatty acids decreased the amount of saturated fatty acids (mainly 18:0) irrespective of the linoleic acid level. The 18:1 levels were doubled, probably because of the incorporation of *trans*-18:1 (in this experiment, no differentation was made between *cis* and *trans* fatty acids). The 18:2 level had increased significantly, which can be explained only partially by the incorporation of the *trans* isomers of linoleic acid. See below.

The arachidonic acid level of PC in the reference groups is only slightly higher than that in the corresponding *trans* fatty acid groups. The fatty acids, 22:5n-6 and 22:6n-3, however, were more

TABLE 6

Oxygen Uptake and Rate of ATP-Synthesis of Rat Heart Mitochondria, Second Experiment[a]

Group	Parameter			
	QO_2[b]	rATP[c]	ADP/O[d]	RCR[e]
CB	50±2.9	261±16	2.63±0.067	4.8±0.37
OV	50±4.1	256±23	2.63±0.088	5.5±0.44
PHSO	53±3.7	283±23	2.69±0.103	4.9±0.35
PHSOMix	49±3.4	268±22	2.80±0.113	5.3±0.40

[a] Each value represents the mean value ± s.e.m. of 24 animals.
[b] QO_2 = oxygen consumption ($nmol.min^{-1}.mg^{-1}$ protein).
[c] rATP = rate of ATP-synthesis.
[d] ADP/O = mole ADP converted into ATP per mole O.
[e] RCR = respiratory control ratio.

affected by the change in dietary fatty acids. The amount of total n-6 fatty acids in the groups containing *trans* fatty acid is similar to that of the corresponding reference group, despite all changes in the levels of n-6 fatty acids (including 18:2).

In the second experiment, heart mitochondrial PC contained 6.0% *trans* 18:1 in the group fed PHSO (about 40% dietary monoenoic *trans* fatty acids) (Table 10). A decrease of 50% in dietary *trans* fatty acids (PHSOMix) more than halved the *trans* 18:1 content. The incorporation of *trans* isomers of linoleic acid was low: maximally 0.5% of *trans,trans*-isomers and 0.6% *trans,cis* or *cis,trans* isomers in PC.

TABLE 7

Phospholipid Composition (%) of Rat Heart and Liver Mitochondria, First Experiment[a]

Organ	group	Phospholipids					
		PC[b]	PE[c]	PI[d]	PS[e]	CL[f]	S[g]
Heart	1	33.2	25.4	11.0	8.3	12.6	4.3
	2	34.4	27.3	12.8	10.2	10.5	4.1
	3[h]	34.6	29.6	10.0	7.0	14.3	4.0
	4	31.8	28.6	9.8	4.5	15.8	4.2
	5	33.8	28.2	10.0	7.8	14.1	6.2
	6	32.6	28.2	11.8	8.5	14.3	4.4
	7[i]	34.5	31.2	7.5	4.2	15.8	3.8
	8[i]	32.3	27.6	12.9	7.8	15.6	4.5
				PI + PS			
Liver	1	42	38	14		6	-
	2	48	35	12		5	-
	3[h]	49	34	12		5	-
	4	48	33	13		6	-
	5	58	29	9		3	-
	6	49	34	13		4	-
	7[i]	45	37	12		7	-
	8[i]	50	33	13		4	-

[a] Each value represents the mean of 2 pools of 6 animals.

[b] PC = phosphatidylcholine.

[c] PE = phosphatidylethanolamine.

[d] PI = phosphatidylinositol.

[e] PS = phosphatidylserine.

[f] CL = cardiolipin.

[g] S = sphingomyelin.

[h] = Mean of 4 pools of 6 animals.

[i] = References.

\- = Not determined.

TABLE 8
Phospholipid Composition (%) of Rat Heart Mitochondria,
Second Experiment[a]

group	Phospholipid					
	PC[b]	PE[c]	PI[d]	PS[e]	CL[f]	S[g]
CB	36.0±1.2	-	-	-	-	-
OV	36.8±1.0	33.2±0.2	4.8±0.2	2.1±0.8	20.0±0.5	3.0±0.4
PHSO	35.2±0.4	30.6±1.6	5.2±0.6	2.1±0.5	23.5±0.4	3.3±0.8
PHSOMix	34.9±0.8	35.0±0.9	5.7±0.5	2.4±0.6	19.4±0.7	2.5±0.5

[a] Each value represents the mean value ± s.e.m. of 3 pools of 4 animals.
[b] PC = phosphatidylcholine.
[c] PE = phosphatidylethanolamine.
[d] PI = phosphatidylinositol.
[e] PS = phosphatidylserine.
[f] CL = cardiolipin.
[g] S = sphingomyelin.
\- = Not determined.

The diets containing *trans* fatty acids (PHSO and PHSOMix) increased the 18:1-level and decreased the 18:0-level in PC compared to the other diets. The amount of total saturated fatty acids and *trans*-18:1 together is fairly constant over all groups, except for the CB-group, in which this sum is considerably higher. The 18:2 level in the groups fed *trans* fatty acids was significantly higher than that in the other two groups (18:2 levels in the pooled samples ranged from 7.5 to 8.0 in the PHSO-group, and from 3.0 to 3.6 in the OV-group), despite the same content of linoleic acid in the diets. The level of

TABLE 9
Fatty Acid Composition (%) of Phosphatidylcholine from Rat Heart and Liver Mitochondria, First Experiment[a]

Organ	Type of fatty acid[b]	Groups							
		1	2	3[c]	4	5	6	7[d]	8[d]
Heart	16:0	10.6	10.8	10.5	10.2	10.8	11.0	12.4	12.3
	16:1	1.2	2.5	1.2	1.1	1.1	1.4	0.6	0.6
	18:0	17.0	17.0	17.3	17.9	17.2	16.5	28.6	26.6
	18:1[e]	25.6	23.4	22.3	21.6	21.2	20.7	10.2	11.3
	18:2[e]	9.6	7.6	7.9	8.2	9.2	9.0	3.7	3.1
	20:4 n-6	26.8	29.4	34.0	34.9	33.1	33.5	35.6	36.4
	22:5 n-6	1.0	1.0	1.2	1.4	1.8	2.1	2.1	2.2
	22:6 n-3	2.6	3.1	1.8	1.5	1.5	1.3	3.0	3.4
	sum	94.4	94.8	96.2	96.8	95.9	95.5	96.2	95.9
	total n-6[f]	37.4	38.0	43.1	44.5	44.1	44.6	41.4	41.7
	total sat[f]	27.6	27.8	27.8	28.1	28.0	27.5	41.0	38.9
Liver	16:0	12.7	12.5	11.7	10:0	10.4	10.2	14.8	16.0
	16:1	2.4	1.9	2.5	2.1	1.5	1.6	1.4	1.2
	18:0	18.0	16.9	15.7	17.8	15.3	15.5	26.2	22.8
	18:1[e]	25.1	23.8	22.6	21.5	22.2	21.6	9.9	11.4
	18:2[e]	9.1	9.6	9.5	8.2	9.7	9.2	3.8	3.1
	20:3 n-9	4.0	2.8	1.0	0.8	-	-	0.9	-
	20:3 n-6	1.6	1.6	1.1	0.8	0.8	-	0.3	-
	20:4 n-6	16.8	19.5	25.9	29.7	32.6	33.5	30.5	33.3
	22:5 n-6	3.2	4.1	3.5	2.7	2.7	3.1	3.1	2.6
	22:6 n-3	4.2	4.0	2.5	1.9	1.6	1.4	3.6	4.3
	sum	97.1	96.7	96.0	95.5	96.7	96.1	94.5	94.7
	total n-6[f]	30.7	34.8	40.0	41.4	45.7	45.8	37.7	39.0
	total sat[f]	30.7	29.4	27.4	27.8	25.7	25.7	41.0	38.8

[a] Each value represents the mean of 2 pools of 6 animals.
[b] The notation indicates chain length and number of double bonds.
[c] Mean of 4 pools of 6 animals.
[d] References.
[e] Both *cis* and *trans*-isomers.
[f] Total n-6 and total sat = the sum of the relative amounts of n-6 polyunsaturated fatty acids and 18:2, and saturated fatty acids, respectively.

TABLE 10
Fatty Acid Compositions (%) of Phosphatidylcholine from Rat Heart Mitochondria as Found by Packed Column GLC (A) and by Capillary Column GLC (B), Second Experiment[a]

	Type of fatty acid[b]	Group			
		CB	OV	PHSO	PHSOMix
A	14:0	0.1	0.3	0.2	0.3
	15:0	2.2	2.2	4.0	2.6
	15:1	0.5	0.3	0.4	0.5
	16:0	13.0	14.0	13.3	14.4
	16:1	0.3	0.5	1.0	0.7
	18:0	39.8	30.3	19.8	30.8
	18:1[c]	3.2	10.9	21.4	13.0
	18:2[c]	3.8	3.3	7.9	4.0
	20:4 n-6	30.6	28.2	24.0	23.8
	20:1 n-9	0.2	0.5	0.2	0.3
	20:2 n-9	-	-	0.1	-
	22:1 n-9	0.4	0.1	0.1	0.4
	22:5 n-6	2.2	2.2	2.6	3.6
	22:6 n-3	1.7	2.7	1.2	1.8
	unknown	1.2	1.3	1.3	1.7
	sum	98.2	96.8	97.5	97.9
	total n-6[d]	35.6	33.7	34.5	31.4
	total sat[d]	55.1	46.8	37.3	48.1
B	18:1 *t*			6.0	2.5
	18:1 *c*			9.6	7.5
	18:1 total			15.6	10.0
	18:2 *t,t*			0.5	-
	18:2 *c,t, 5,c*			0.6	0.5
	18:2 *c,c*			4.4	2.5
	18:2 total			5.5	3.0

[a] Each value represents the mean value of 4 pools of 6 animals.
[b] The notation indicates chain length and number of double bonds; *c* = *cis*-isomer, *t* = *trans*-isomer.
[c] Both *cis* and *trans*-isomers.
[d] Total n-6 and total sat = the sum of the relative amount of n-6 fatty acids and 18:2 and saturated fatty acids, respectively.

9-*cis*,12-*cis*-18:2 (linoleic acid) as found by capillary GLC was clearly lower than that found in the fatty acid analyses by packed column GLC. This difference could not be ascribed to *trans* isomers of linoleic acid, because of the low level of these components in the phospholipids investigated. *Cis*,*cis* isomers of linoleic acid were only present in trace amounts. So, since both capillary GLC and packed-column GLC indicated the difference in the linoleic acid level of PC in the PHSO and PHSOMix groups (20 and 10 en% *trans* fatty acids, respectively), it must be concluded that *trans* fatty acids increased the level of linoleic acid in this phospholipid. The arachidonic acid level in the two groups fed *trans* fatty acids diminished compared to that of the other two groups (levels in the PHSO-group ranging from 22.2 to 26.2, in the OV-group from 27.2 to 29.1). There is, however, no apparent relation with the amount of dietary *trans* fatty acids.

Liver. Liver mitochondrial PC (Table 9) has a fatty acid pattern similar to that of heart mitochondrial PC. Mitochondrial PC from the liver was more susceptible to changes in linoleic acid intake than that from the heart. In PC, 20:3n-9 was present at low levels of linoleic acid but not at higher levels.

Trans fatty acids decreased the arachidonic acid level in liver mitochondrial PC only at the linoleic acid level of 2 en%; at the 5 en% level, this effect had disappeared. The 18:2 level increased, irrespective of its dietary level. *Trans* fatty acids had no effect on the level of 20:3n-9.

Fatty acid composition of phosphatidylethanolamine. Heart. The effects of increasing amounts of dietary linoleic acid (first experiment) on both saturated and monounsaturated fatty acids, and 18:2 content of heart mitochondrial PE were similar to those in heart mitochondrial PC (Table 11). In this phospholipid, however, the arachidonic acid level was much less affected by an increasing linoleic acid intake than it was in PC; it even tended to decrease slightly as the dietary level of its precursor increased. The effects of dietary *trans* fatty acids on the fatty acid composition of PE and PC did not differ very much either

TABLE 11
Fatty Acid Composition (%) of Phosphatidylethanolamine from Rat Heart and Liver Mitochondria, First Experiment[a]

Organ	Type of fatty acid[b]	Group							
		1	2	3[c]	4	5	6	7[d]	8[d]
Heart	16:0	4.4	5.4	4.9	4.3	4.6	4.7	7.6	7.0
	16:1	1.2	1.2	1.2	1.0	1.3	1.7	0.8	0.8
	18:0	18.3	18.9	18.5	19.0	18.4	17.9	27.6	25.1
	18:1[e]	19.8	18.5	18.8	18.5	18.1	17.9	8.0	9.8
	18:2[e]	4.9	6.1	5.8	6.2	6.8	6.9	3.1	2.9
	20:4 n-6	29.9	29.8	30.2	30.7	28.2	26.7	22.9	21.6
	22:5 n-6	4.5	4.7	6.6	7.4	8.9	10.7	10.6	10.5
	22:6 n-3	10.2	9.4	7.8	6.3	6.0	5.4	12.4	14.0
	sum	93.2	94.0	93.8	93.4	92.3	91.9	93.0	91.7
	total n-6[f]	39.3	40.6	42.6	44.3	43.9	44.3	36.6	35.0
	total sat[f]	22.7	24.3	23.4	23.3	23.0	22.6	35.2	32.1
Liver	16:0	10.1	10.0	8.6	9.8	7.4	9.2	12.5	12.2
	16:1	2.5	2.2	1.6	2.1	1.5	1.7	1.4	0.9
	18:0	16.8	16.3	16.4	15.6	15.4	14.1	25.2	21.6
	18:1[e]	19.5	19.1	19.3	19.5	19.9	17.7	9.4	12.2
	18:2[e]	10.3	9.3	11.2	14.6	14.5	12.7	5.5	5.4
	20:3 n-9	2.2	1.5	0.7	0.5	0.4	0.2	0.6	0.3
	20:3 n-6	1.2	1.1	1.0	0.8	0.7	0.6	0.7	0.4
	20:4 n-6	21.1	22.4	27.1	27.3	28.8	25.9	28.0	28.8
	22:5 n-6	4.1	5.7	4.8	4.3	4.1	4.9	4.5	4.5
	22:6 n-3	8.2	7.9	4.8	3.7	2.9	2.8	7.4	8.5
	sum	96.0	95.5	95.5	98.2	95.6	89.8	95.2	94.8
	total n-6[f]	36.7	38.5	44.1	47.0	48.1	44.1	38.7	39.1
	total sat[f]	26.9	26.3	25.0	25.4	22.8	23.3	37.7	33.8

[a] Each value represents the mean of 2 pools of 6 animals.
[b] The notation indicates chain lenght and number of double bonds.
[c] Mean of 4 pools of 6 animals.
[d] References.
[e] Both *cis* and *trans*-isomers.
[f] Total n-6 and total sat = the sum of the relative amounts of n-6 polyunsaturated fatty acids and 18:2, and saturated fatty acids, respectively.

TABLE 12
Fatty Acid Composition (%) of Phosphatidylethanolamine from Rat Heart Mitochondria as Found by Packed Column GLC (A), and by Capillary Column GLC (B), Second Experiment[a]

	Type of fatty acid[b]	Group		
		OV	PHSO	PHSOMix
A	14:0	0.3	0.3	0.3
	15:0	2.3	2.9	2.1
	15:1	1.3	0.7	1.0
	16:0	9.7	6.8	8.1
	16:1	0.2	0.4	0.8
	18:0	29.1	19.7	26.7
	18:1	10.3	19.1	12.3
	18:2	3.1	5.9	3.5
	20:4 n-6	18.3	23.5	21.1
	20:1 n-9	1.1	0.4	0.2
	20:2 n-9	0.2	0.2	0.7
	22:1 n-9	0.4	-	0.1
	22:5 n-6	4.7	7.6	7.2
	22:6 n-3	13.3	6.7	9.1
	unknown	1.5	1.3	1.8
	sum	95.8	95.4	94.9
	total n-6[c]	26.1	37.0	31.8
	total sat[c]	41.4	29.6	37.1
B	18:1 *t*		7.1	2.7
	18:1 *c*		6.9	6.5
	18:1 total		14.0	9.2
	18:2 *t,t*		0.2	0.2
	18:2 *ct, tc*		0.7	0.5
	18:2 *c,c*		3.6	2.3
	18:2 total		4.5	3.7

[a] Each value represents the mean value of 4 pools of 6 animals.
[b] The notation used for the fatty acids indicates chain length and number of double bonds, *c* = *cis*-isomer, *t* = *trans*-isomer.
[c] Total n-6 and total sat = the sum of the relative amounts of n-6 fatty acids and 18:2 and saturated fatty acids, respectively.

except for a striking increase in arachidonic acid levels in PE caused by *trans* fatty acids. This increase was compensated for by a reduction in 22:5n-6 and 22:6n-3 fatty acids.

In the second experiment (Table 12), similar changes occurred. *Trans* fatty acids caused a significant increase in the sum of the n-6 polyunsaturated fatty acids; these fatty acids were incorporated partially at the expense of 22:6n-3. Unfortunately, determination of the fatty acid composition of PE of the CB-group was impossible. In PE, *trans* monoenoic and dienoic acids were incorporated to an extent similar to that in PC (*trans*-18:1 max. 7.1%; *trans*-18:2 isomers max. 0.9%). The sum of all saturated fatty acids and *trans* 18:1 in the PHSO-group was slightly lower than that in the other two groups.

Liver. The influence of dietary linoleic acid on the fatty acid composition of PE in the liver mitochondria (Table 11) was similar to that of PC (Table 9) with respect to the levels of arachidonic acid, 22:5n-6 and 22:6n-3. There was far less similarity in this respect between PE from liver on the one hand and heart on the other. Compared to the reference groups, the 22:6n-3 level was significantly lower in the *trans* fatty acid groups. Moreover, it decreased with an increasing level of linoleic acid. Arachidonic acid was not influenced by *trans* fatty acids.

DISCUSSION

We observed that *trans* fatty acids could significantly alter the fatty acid composition of mitochondrial membranes, irrespective of the linoleic acid level. However, with 2 and 5 en% linoleic acid in the diet, no influence of *trans* fatty acids on the function of the mitochondria was found. Also in the second experiment no differences in mitochondrial function were detected between the two groups that were fed *trans* fatty acids and the two reference groups.

The effect of *trans* fatty acids on the mitochondrial respiration has been the subject of many publications (29-34). It is generally

agreed that specific effects of *trans* fatty acids on the mitochondrial respiration cannot be detected, provided an adequate level of linoleic acid is present in the diet (31,32,34). We have demonstrated that 2 en% linoleic acid is enough to prevent effects of *trans* fatty acids on the mitochondrial respiration.

The high RCR and ADP/O-values in the first experiment show that the mitochondria were tightly coupled. The QO_2 and ATP-values were also high compared to the results of other investigators (30,31,34). In the second experiment, the values for QO_2 (and, consequently, the ATP-synthesis) were much lower than those in the first experiment, and even lower than those found by other investigators (30,31,34). However, the RCR and ADP/O values demonstrate the good quality of these mitochondria. This low QO_2 value can probably be ascribed to the use of a different batch of nagarose in the second experiment, which may have resulted in a mitochondrial fraction contaminated with another protein-rich fraction.

Many adverse effects have been attributed to linolelaidic acid (9-*trans*,12-*trans*-18:2), on the basis of both in vitro experiments and feeding studies (36-39). However, as has been pointed out in some reviews, the amount of this fatty acid in commercial partially hydrogenated oils is very low (1-3,35). As a consequence, the total dietary intake of 9-*trans*,12-*trans*-18:2 by humans can be neglected.

Monoenoic *trans* fatty acids in partially hydrogenated oils and ruminant fats form a heterogenous group of positional isomers (4,40). The metabolism of the isomers may differ significantly (3); the biological effects of the isomers could therefore vary accordingly. In most experiments dealing with *trans* fatty acids, use is made of PHSO, the advantage being that both monoenoic and polyenoic *trans* fatty acids are found in the experimental diet. Moreover, in PHSO, the distribution of these fatty acid isomers matches that of the human diet. The conclusion drawn with regard to the nutritive value of food products containing *trans* fatty acids is therefore more realistic.

As can be concluded from the capillary GC analysis of the fatty acid composition (second experiment), the incorporation of *trans* isomers of linoleic acid is low in heart mitochondrial phospholipids. *Trans*

monoenoic fatty acids are readily incorporated in these phospholipids, their level depending on the diet. The changes in the fatty acid composition of the heart mitochondrial phospholipids in both experiments corresponded well, except for a slight difference in the relative levels of the main fatty acids probably caused by contamination of the mitochondrial fraction in the isolation of the mitochondria in the second experiment.

The fatty acid composition of heart mitochondrial PC in the HCNO/OV-group (group 7) of the first experiment corresponds well with that of the CB-group of the second experiment. The influence of the medium-chain saturated fatty acids, as present in the HCNO/OV-diet, seemed to be similar to that of long-chain saturated fatty acids. Although the polyunsaturated fatty acid compositions of heart mitochondrial phospholipids were considerably affected by a decrease of the linoleic acid intake, even at very low linoleic acid levels (0.4 en%), no 20:3n-9 appeared. This is in contrast to the liver phospholipids and may indicate a highly specific uptake or incorporation of polyunsaturated fatty acids by the heart.

Trans fatty acids (both 10 and 20 en%), when compared to saturated fatty acids and *cis*-monounsaturated fatty acids, decreased the arachidonic acid level in heart mitochondrial PC. In PE, however, the arachidonic acid level increased and was partially compensated for by a decrease in 22:6n-3. These may be effects of dietary *trans* fatty acids on PE in heart tissue (30,31,41). The regulation of polyunsaturated fatty acids of heart mitochondrial PC is probably different from that of PE (31).

In liver mitochondrial PC, the *trans* fatty acids decreased the arachidonic acid level only at a linoleic acid level of 2 en% or lower. The arachidonic acid content of PE in liver mitochondria did not change upon the incorporation of *trans* fatty acids in the diet. The linoleic acid level, however, had increased upon incorporation of *trans* fatty acids in both PE and PC.

The effect of the *trans* fatty acids in PHSO on the fatty acid composition of PC is different from that on PE. The mechanism

responsible for the effects is not yet clear. However, *trans* fatty acids change the fatty acid composition in both phospholipids, compared to saturated or *cis*-monounsaturated fatty acids. Despite these changes, the C18 *trans* fatty acids do not influence the mitochondrial function in the presence of 2 en% or more linoleic acid. This supports the view of Royce and Holmes (34) that, in non-EFA-deficient animals, the mitochondrial membranes adapt to a changing fatty acid composition in a way that leaves their functional properties unaffected.

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REFERENCES

1. Beare-Rogers, J.L. (1983) in Advances in Nutritional Research (Draper, H.H., ed.) Vol. 5, pp. 171-200, Plenum Press, New York.
2. Gurr, M.I. (1983) Bull. Int. Dairy Fed. Doc. 166, 5-18.
3. Emken, E.A. (1983) J. Am. Oil Chem. Soc. 60, 995-1004.
4. Ohlrogge, J.B. (1983) in Dietary Fats and Health (Perkins, E.G. and Visek, W.J., eds.) pp. 359-374, American Oil Chemists' Society, Champaign, IL.
5. Wood, R. (1979) in Geometrical and Positional Fatty Acid Isomers (Emken, E.A., and Dutton, H.J., eds.) pp. 213-281,American Oil Chemists' Society, Champaign, IL.
6. Gottenbos, J.J. (1983) in Dietary Fats and Health (Perkins, E.G. and Visek, W.J., eds.) pp. 375-390, American Oil Chemists' Society, Champaign, IL.

7. Stancliff, R.C., Williams, M.A., Utsumi, K. and Packer, L. (1969) Arch. Biochem. Biophys. 131, 629-642.
8. Divakaran, P., and Venkataraman, A. (1977) J. Nutr. 107, 1621-1631.
9. Clandinin, M.T. (1978) J. Nutr. 108, 273-281.
10. Divakaran, P. (1978) Experientia 34, 1540-1541.
11. Clandinin, M.T. (1979) FEBS Lett. 102, 173-176.
12. Abuirmeileh, N.M., and Elson, C.E. (1980) Lipids 15, 918-924.
13. Tyzbir, R.S., Kunin, A.S., Sims, N.M., and Danforth Jr, E (1981) J. Nutr. 111, 252-259.
14. McMurchie, E.J., Abeywardena, M.Y., Charnock, J.S., and Gibson, R.A. (1983) Biochim. Biophys. Acta 734, 114-124.
15. Houtsmuller, U.M.T., Stuijk, C.B., and Van der Beek, A. (1970) Biochim. Biophys. Acta 218, 564-566.
16. Renner, R., Innis, S.M., and Clandinin, M.T. (1979) J. Nutr. 109, 378-387.
17. Høy, C.E., and Hølmer, G. (1979) Lipids 14, 727-733.
18. Applewhite, T.H. (1983) in Dietary Fats and Health (Perkins, E.G. and Visek, W.J., eds.) pp. 414-424, American Oil Chemists' Society, Champaign, IL.
19. De Deckere, E.A.M., Jansen-Verplanke, C., Blonk, C.G., and Van Nielen, W.G.L. (1988) J. Nutr. In press.
20. Scholfield, C.R. (1981) J. Am. Oil Chem. Soc. 58, 662-662.
21. Clouet, P., and Bezard, J. (1979) Lipids 14, 268-273.
22. Hülsmann, W.C. (1970) Biochem. J. 166, 32-33.
23. Swarttouw, M.A. (1974) Biochim. Biophys. Acta 337, 13-21.
24. Estabrook, R.W. (1967) in Methods in Enzymology (Estabrook, R.W., and Pullman, M.E., eds.) Vol. 10, pp. 41-47, Academic Press, New York and London.
25. Bligh, E.G., and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911-917.
26. Patton, G.M., Fasulo, J.M., and Robins, S.J. (1982) J. Lipid Res. 23, 190-196.

27. Christie, W.W. (1973) in Lipid Analysis (Christie, W.W., ed.) p. 191, Pergamon Press, Oxford, England.
28. Bartlett, G.R. (1959) J. Biol. Chem. 234, 466-470.
29. Hsu, C.M.L., and Kummerow, F.A. (1977) Lipids 12, 486-494.
30. Combe, N., Rietsch, J., Wolff, R. and Entressangles, B. (1980) Ann. Nutr. Aliment. 34, 305-316.
31. Blomstrand, R., and Svensson, L. (1983) Lipids 18, 151-170.
32. Entressangles, B., Combe, N., and Wolff, R. (1984) J. Am. Oil Chem. Soc. 61, 679.
33. De Schrijver, R., and Privett, O.S. (1984) J. Nutr. 114, 1183-1191.
34. Royce, S.M. and Holmes, R.P. (1984) Biochim. Biophys. Acta 792, 371-375.
35. Houtsmuller, U.M.T. (1978) Fette Seifen Anstrichm. 80, 162-169.
36. Privett, O.S., Phillips, F., Shimasaki, H., Nozawa, T., and Nickell, E.C. (1977) Am. J. Clin. Nutr. 30, 1009-1017.
37. Kinsella, J.E., Bruckner, G., Mai, J., and Shimp, J., (1981) Am. J. Clin. Nutr. 34, 2307-2318.
38. Srivastava, K.C., and Awasthi, K.K. (1982) Prostagland. Leuk. Med. 9, 669-684.
39. Goswami, S.K., Bruckner, G., and Kinsella, J.E. (1983) Prostagland. Leuk. Med. 10, 19-26.
40. Dutton, H.J. (1979) in Geometric and Positional Fatty Acid Isomers (Emken, E.A., and Dutton, J.H., eds.) pp. 1-6, American Oil Chemists' Society, Champaign, IL.
41. Wolff, R.L., Combe, N., and Entressangles, B. (1984) Rev. Fr. Corps Gras 11, 161-170.

Chapter 4.

LINOLEIC ACID REQUIREMENT OF RATS FED *TRANS* FATTY ACIDS. STUDIES ON EICOSANOID SYNTHESIS

J.L. Zevenbergen and E. Haddeman
Assessment of the minimum requirement of linoleic acid to prevent effects of *trans* fatty acids on eicosanoid biosynthesis.
Submitted to Lipids.

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The minimum requirement of linoleic acid to prevent effects of dietary C18 *trans* fatty acids on eicosanoid biosynthesis in rats was assessed. In a first experiment, six groups of animals were fed diets with a high content of *trans* fatty acids (20% of energy (en%)), and increasing amounts of linoleic acid (0.4 to 7.1 en%). In a second experiment, four groups of rats were fed diets designed to compare *trans* fatty acids with saturated and *cis*-monounsaturated fatty acids of the same chain length at the 2 en% linoleic acid level. After 9-14 weeks the biosynthesis of prostacyclin by pieces of aorta and the biosynthesis of hydroxy-heptadecatrienoic acid (HHT) and 12-hydroxy-eicosatetraenoic acid (12-HETE) by platelets were measured. The fatty acid compositions of aorta phospholipid and platelet lipid were also determined.

Both the prostacyclin-production by aorta pieces and the production of HHT and 12-HETE by platelets appeared to be a lineair function of the arachidonic acid level in aorta phospholipid and platelet lipid, irrespective of the *trans* fatty acid content in the diet. This indicates that *trans* fatty acids do not directly influence enzymes involved in eicosanoid biosynthesis. In a direct comparison with *cis*-monounsaturated or saturated fatty acids with 2 en% linoleic acid in the diet, only a moderate reduction in arachidonic acid level in aorta phospholipids in the group fed *trans* fatty acids was observed. The geometry of the double bond did not influence the arachidonic acid level in platelet lipid, although the diet rich in saturated fatty acids increased arachidonic acid levels significantly compared to all other diets.

Prostacyclin-production nor HHT- or 12-HETE-production were significantly affected by *trans* fatty acids when 2 en% linoleic acid was present in the diet. Our study indicates that in rats 2 en% linoleic acid is sufficient to prevent effects of dietary *trans* fatty acids on eicosanoid synthesis.

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Trans fatty acids from partially hydrogenated oils and ruminant fats form part of the human diet. Throughout the years there has been a marked interest in the biological effects of *trans* fatty acids. It is now recognized that they are well absorbed by both man and experimental animals and can be found in most tissues after ingestion (1-5). *Trans* fatty acids are incorporated into phospholipids of biological membranes, altering their fatty acid composition (1,3,6,7). They also might influence the linoleic acid metabolism, resulting in a decreased formation of eicosanoid precursors (7,8). Either by this decrease in precursor level or by direct effects on the eicosanoid-synthesizing enzymes, *trans* fatty acids could possibly diminish the production of prostaglandins, thromboxanes and hydroxy fatty acids.

During the last few years there has been a growing interest in the effect of dietary *trans* fatty acids on the eicosanoid metabolism. Kinsella (8) reported the inhibition of prostaglandin production in rats fed *trans,trans* linolelaidic acid (9*t*,12*t*-18:2). This inhibition, deduced from decreased serum prostaglandin E_1 (PGE_1), PGE_2 and $PGF_{2\alpha}$ concentrations, could be partly attributed to a decreased amount of prostaglandin precursors in the tissues, e.g. di-homo-γ-linolenic acid and arachidonic acid. But also a direct inhibitory effect of 9*t*,12*t*-18:2 on prostaglandin synthesis was considered. Hwang et al. (9) found that at a dietary dose equal to or higher than that of linoleic acid, *t*,*t*-18:2 decreased the arachidonic acid level in platelets and diminished (although not significantly) the production by platelets of thromboxane B_2 (TXB_2), $PGF_{2\alpha}$ and 12-hydroxy-eicosatetraenoic acid (12-HETE). The serum concentration of TXB_2 and $PGF_{2\alpha}$ also decreased. *trans,trans*-18:2 however, is not a substantial component of the human diet (2,4). *trans* Monoenoic acids (*t*-18:1) are quantitatively much more important components in our diet.

Presently the generally accepted view is that *trans* fatty acids formed during the partial hydrogenation of vegetable oils (mainly monoenoic isomers) do not exert undesirable effects when compared to saturated or *cis*-monounsaturated fatty acids, provided sufficient linoleic acid is present in the diet (2-4,6,10). Recently, it has been

reported that high dietary levels of *trans* isomers of monoenoic acids do not interfere with platelet cyclooxygenase or lipoxygenase, provided sufficient amounts of linoleic acid are available (11).

It was our aim to investigate the relationship between dietary *trans* fatty acids and linoleic acid and the eicosanoid synthesis in the rat in a quantitative way by determining the amount of linoleic acid necessary to prevent any effect of *trans* fatty acids on the eicosanoid synthesis. To investigate possible effects of *trans* fatty acids on the eicosanoid metabolism, two tissues that are known to be actively involved in eicosanoid production, aorta and platelet, were chosen for our studies. In a first experiment we investigated the effects of diets having a constant amount of C18 *trans* fatty acids and an increasing amount of linoleic acid on the prostacyclin (PGI_2) synthesis by pieces of aorta, and on the syntheses of hydroxy-heptadecatrienoic acid (HHT), a cyclooxygenase product, and 12-HETE, a lipoxygenase product, by blood platelets of the rat. In the second we compared the effects of *trans* fatty acids with those of long-chain (C18) saturated or *cis*-monounsaturated fatty acids at a linoleic acid level of 2 en%, on the same parameters.

EXPERIMENTAL

Materials. The fats used in the two experiments were: a special, partially hydrogenated soybean oil (PHSO), hydrogenated coconut oil (HCNO), sunflower seed oil (SSO), cocoabutter (CB), olive oil (OV) and a low-linoleic acid olive oil (OV-LL). The origin of the experimental fats is described by Zevenbergen et al. (7).

Diethylether and hexane were obtained from Baker Chemicals (Deventer, The Netherlands). BHT (2,6-di-tert-butyl-p-cresol) was obtained from Fluka AG (Buchs, Switzerland). 15-Hydroxy-11,13-eicosadienoic acid (C20:2-15OH) was prepared as described by Claeys et al. (12). Collagen, HPTLC-plates (Silicagel F254), Uvasol solvents and all other reagents and chemicals were obtained from Merck (Darmstadt, FRG).

Animals and diets. Weanling male SPF-Wistar rats (CPB/WU, Central Breeding Station TNO Zeist, The Netherlands) were used in both experiments. The animals were housed individually in a climatized room. The mean temperature was 23.0 ± 1.0°C, the relative humidity 45-70%, and there was a day/night cycle of 12/12 h. The animals had free access to water and food. This food, a semi-synthetic diet with 40 en% fat was composed of (in $g.MJ^{-1}$ of the total diet): casein 14.8; vitamin mixture 0.2; salt mixture 1.3; cellulose 3.8; maize starch 25.2; experimental fat 10.3 (for compositions of vitamin and salt mixture, see Ref. 13). The animals were weighed and examined weekly.

In the first experiment, 8 groups (7 of 12 animals each and one of 24 animals: group 3) were fed diets on the basis of the PHSO, HCNO, OV and SSO. The oils were mixed in such a way as to yield 6 diets having an increasing linoleic acid content (0.4-7.1 en%) but a constant *trans* fatty acid level of 20 en% (for detailed composition of experimental fats see Ref. 7). Two fats served as references, one consisting of HCNO and OV (2 en% linoleic acid), another mainly of OV (5 en% linoleic acid). Table 1 shows the calculated fatty acid compositions of the experimental fats. The feeding period lasted 13-14 weeks.

In the second experiment 4 groups of 24 rats each (randomly selected from 24 litters of 4 rats) were fed diets containing either 20 en% *trans* fatty acids (PHSO), or mainly saturated fatty acids (CB), or *cis*-monounsaturated fatty acids (OV-LL), or a mixture of PHSO, CB and OV (PHSOMix, 10 en% *trans* fatty acids; see Ref. 7 for composition). All diets contained 2 en% linoleic acid. The fatty acid compositions are given in Table 2. These diets were fed for 9-11 weeks.

Fatty acid composition of dietary fats. The composition of PHSO was determined by a combination of $AgNO_3$-TLC separation and capillary GC as described before (7). The compositions of the other fats and oils were determined by GLC (5% DEGS packed column) after trans-methylation with methanolic HCl.

Aggregation experiments with platelets. At the end of the feeding period the animals were fasted overnight, weighed and then sacrificed by aorta cannulation under ether anesthesia. Blood was collected and mixed

TABLE 1 Calculated Fatty Acid Composition (%) of the Experimental Fats (Groups), First Experiment

Type of fatty acid[a]	Group 1	2	3	4	5	6	7[b]	8[b]
6:0	0.1	-	-	-	-	-	0.2	-
8:0	1.2	0.8	0.8	0.8	0.5	-	3.8	-
10:0	1.0	0.7	0.7	0.7	0.4	-	3.2	-
12:0	8.9	6.1	6.1	6.1	3.7	0.1	27.0	-
14:0	3.6	2.5	2.5	2.5	1.6	0.2	10.3	-
16:0	10.3	10.4	10.1	9.9	9.6	9.4	10.5	12.2
16:1	0.3	0.3	0.3	0.2	0.2	0.2	0.4	1.0
17:0	0.1	0.1	0.1	0.1	0.1	0.2	-	0.1
17:1	-	-	-	-	-	0.1	0.1	0.2
18:0	8.5	7.9	8.1	8.1	7.8	7.2	8.0	2.9
18:1 *c*	16.5	20.3	17.5	15.3	14.8	16.1	30.7	69.7
18:1 *t*	39.1	39.1	39.1	39.1	39.1	39.1	-	-
18:2 9*c*,12*c*	1.0	2.0	5.0	7.5	12.5	17.7	5.0	12.5
18:2 *ct*,*tc*9,12	0.6	0.6	0.6	0.6	0.6	0.6	-	-
18:2 9*t*,12*t*	0.5	0.5	0.5	0.5	0.5	0.5	-	-
18:2 other	7.7	7.7	7.7	7.7	7.7	7.7	-	-
18:3	-	-	-	-	-	0.1	0.2	0.5
20:0	0.3	0.3	0.3	0.3	0.4	0.4	0.2	0.2
20:1 c	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0.3
22:0	0.3	0.4	0.3	0.3	0.4	0.5	0.1	0.3
actual LA-level[d] (%)	1.1	n.d.	5.4	n.d.	n.d.	n.d.	5.2	n.d.

a The notation for the fatty acid indicates chain length and number of double bonds *c* = *cis*-isomers, *t* = *trans*-isomers, other = non-9,12-18:2 isomers (non-conjugatable).

b Reference-fats.

c Also contains conjugated 18:2 isomers.

d Linoleic acid level determined by GLC, n.d. = not determined.

TABLE 2 Fatty Acid Compositions (%) of the Experimental Fats (Groups), Second Experiment

Type of fatty acids[a]	Group			
	CB	OV	PHSO	PHSOMix
6:0	-	-	-	-
8:0	-	-	0.8	-
10:0	-	-	0.7	-
12:0		0.1	5.6	-
14:0	0.2	-	2.4	0.1
16:0	23.5	11.9	10.0	15.3
16:1	-	-	0.3	-
17:0	0.3	-	0.1	0.1
17:1	-	0.1	0.1	0.1
18:0	31.9	2.0	8.0	12.1
18:1 *c*	36.9	78.7	17.5	39.9
18:1 *t*	-	-	39.1	19.5
18:2 9*c*,12*c*	4.5	5.5	5.3	4.7
18:2 *ct*,*tc* 9,12	-	-	0.6	0.3
18:2 9*t*,12*t*	-	-	0.5	0.2
18:2 other	-	-	7.7	3.9
18:3	0.2	0.6	-	-
20:0	1.3	0.4	0.5	0.6
20:1	-	0.4	0.5[b]	0.4[b]
22:0	0.3	0.2	0.4	0.3

a The notation for the fatty acid indicates chain length and number of double bonds *c* = *cis*-isomers, *t* = *trans*-isomers, other = n 18:2 isomers (non-conjugatable).

b Also contains conjugated 18:2 isomers.

gently with Na-citrate (3.8%; v/v 9:1). Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 140xG at room temperature for 20 min. After taking off the PRP, the blood was centrifuged a second time (600xG; 10 min) to prepare platelet-poor plasma (PPP). The platelet count of the PRP was adjusted to $1*10^6$ platelets/μl with autologous PPP. Aggregation of the platelets was induced in 0.5 ml PRP in a Chronolog aggregometer with four different dosages of collagen (18, 30, 50 and 150 μl; concentration 0.29 mg collagen/ml on nitrogen-basis). The delay and the slope (tg α) of the aggregation curves were monitored and 5 min. after the addition of the collagen, the reaction was stopped by adding 0.5 ml of Uvasol methanol. To these samples and samples of non-stimulated PRP, a drop of butyrylhydroxytoluene-solution (BHT in methanol; 4 g/l) was added and the samples were frozen and stored at -25°C for lipid and hydroxy fatty acid analysis.

Bioassay of PGI_2-production by aorta pieces. Immediately after the collection of blood, the aorta was removed and pieces (ø 3 mm) were punched out of the thoracic part of the aorta. These pieces were incubated in 0.2 ml of buffered saline (17 g NaCl; 0.4 g KCl; 0.4 g KH_2PO_4; 2.9 g $Na_2HPO_4.H_2O$) during 4 minutes. The 50 μl of the incubate was then added to PRP of control rats and the effect of the incubate on the ADP-induced aggregation of this PRP was measured according to Hornstra et al. (14). The prostacyclin-like activity was quantitated by using a calibration curve of purified prostacyclin (PGI_2). The abdominal part of the aorta was brought in 0.2 ml of buffered saline with one drop of BHT and stored at -25°C for lipid analysis.

Determination of hydroxy fatty acids with HPLC. To the aggregated PRP-samples 15-hydroxy-11*cis*,13*trans*-eicosadienoic acid (C20:2-15OH) was added as internal standard. The lipids were extracted according to Folch et al. (15), with Uvasol-quality solvents. Hydroxy fatty acids were separated from prostaglandins and other lipids on small silica gel columns (16). The hydroxy fatty acids were separated on a Varian HPLC with a Lichrosorb RP-18 column (5 μm, 250x4.6 mm)(detection by U.V. at 234 nm) and quantitated.

Fatty acid analysis. Platelets and aorta pieces were extracted according to Bligh and Dyer (17). The phospholipid fraction of the aorta was isolated by preparative TLC, essentially according to Christie (18). This fraction and the total lipids of the platelets were trans-esterified with methanolic HCl. The methyl esters were analyzed by GLC on packed columns (5% DEGS or Apiezon) or glass capillary columns (Silar 88) by standard methods.

Statistics. All data except for the fatty acid compositions were subjected to an analysis of variance according to the randomized complete blocks design. Days of sacrifice served as blocks in the first experiment, litters in the second. In the first experiment, Dunnett's procedure was applied to find out which groups differed systematically from the standard group (group 3, 20 en% *trans* fatty acids and 2 en% linoleic acid). In the second experiment, the Student-Newman-Keuls multiple-range test was applied to locate possible systematic differences between the dietary treatments.

RESULTS

General condition of the animals. All animals seemed to be in good health in both experiments, showing no overt signs of EFA-deficiency. In neither experiment systematic differences in food consumption or body weight related to dietary treatment occurred. At the time of sacrifice the weight of the animals ranged from 370-400 g in the first experiment and from 350-400 g in the second. The results of histo-pathological examination, haematology and clinical chemistry will be reported separately (Zevenbergen and Verschuren, submitted for publication).

Fatty acid composition of the aorta phospholipids. The dietary linoleic acid level had hardly any influence on the 18:2 level of aorta phospholipid in the first experiment, while the arachidonic acid incorporation was increased by increasing linoleic acid intake (Table 3). Other prostaglandin precursors were present in low amount (20:3 n-6) or were not detected (20:5 n-3). The total sum of saturated fatty acids remained rather constant, whereas the sum of all n-6 fatty acids, including 18:2, increased.

TABLE 3
Fatty Acid Composition (%) of Aorta Phospholipids, First Experiment[a]

Type of fatty acid[b]	Groups							
	1	2	3[c]	4	5	6	7[d]	8[d]
12:0	0.1	0.1	-	-	0.1	0.1	-	-
14:0	0.5	0.5	0.4	0.4	0.3	0.3	0.9	0.5
16 al.	1.7	1.3	1.4	1.6	1.6	1.4	2.2	1.4
16:0	18.2	18.9	18.1	18.0	18.1	18.4	19.0	18.9
16:1 n-7	2.7	2.6	2.3	2.2	2.2	2.0	1.3	1.4
18 al.	2.1	2.2	2.1	2.1	2.3	2.5	2.6	1.9
18:0	14.7	14.9	15.3	15.8	16.1	15.8	18.6	16.4
18:1[e]	18.6	18.0	16.2	15.3	14.0	14.0	11.9	14.0
18:2[e]	7.0	6.1	6.9	7.1	7.0	7.4	3.5	4.7
18:3 n-6	0.2	0.1	0.2	-	0.1	0.2	0.1	0.1
20:0[f]	0.7	0.9	0.9	0.7	0.8	0.9	0.9	0.9
20:1 n-9	0.6	0.6	0.6	0.6	0.4	0.4	0.4	0.4
20.9[g]	0.5	0.4	0.3	0.3	0.3	0.2	0.1	-
20:3 n-9	0.4	0.3	0.5	0.5	0.6	0.6	0.3	0.4
21.5[g]	1.8	1.4	0.6	0.5	0.3	0.3	0.6	0.5
20:3 n-6	1.1	1.1	1.0	0.9	0.8	0.7	0.9	0.7
22:0[f]	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.9
20:4 n-6	17.5	18.5	21.2	22.0	22.4	22.5	23.5	22.5
23.4[g]	0.8	0.6	0.2	-	-	0.1	0.5	0.3
22:4 n-6	4.1	4.3	5.2	5.4	6.0	6.1	5.0	4.9
22:5 n-6	3.7	3.9	4.1	3.8	3.9	3.9	4.1	4.2
22:5 n-3	0.3	0.3	0.1	-	0.2	0.2	0.1	0.4
22:6 n-3	1.4	1.7	1.1	0.9	0.8	0.7	1.7	1.9
24:0[f]	1.3	1.1	1.2	1.2	1.1	1.4	1.4	1.4
sum	97.5	97.4	97.5	97.4	97.3	97.4	97.5	95.0
total n-6[h]	33.6	34.0	38.6	39.3	40.3	40.7	37.1	37.0
total sat[h]	36.0	36.7	36.4	36.8	37.2	37.5	41.1	38.5

a Each value represents the mean of two pools of 6 animals. Analysis is performed on packed column (5% DEGS). - = Not detected.
b The notation indicates chain lenght and number of double bonds al. = dimethylacetal.
c Mean of 4 pools of 6 animals.
d Reference-groups.
e Both *cis* and *trans* isomers.
f Quantitated with apiezon-column.
g Carbon-number of un-identified fatty acids.
h Total n-6 and total sat = the sum of the relative amount of n-6 fatty acids and 18:2 and saturated fatty acids, respectively.

Dietary *trans* fatty acids clearly increased the level of linoleic acid. The arachidonic acid level was highest in reference group 7 (with 2 en% linoleic acid). The other reference diet did not induce an arachidonic acid level different from that induced by the corresponding *trans* fatty acid-containing diet (compare groups 5 and 8). Other polyunsaturated fatty acids were hardly influenced by *trans* fatty acids, except for 22:6 n-3, of which the level in the *trans* fatty acids-fed groups was lower than that in the groups fed reference diets. This could be due to the somewhat higher level of 18:3 n-3 in the diets of the latter groups. *Trans* fatty acids did not reduce the total amount of n-6 fatty acids. Also in the second experiment (Table 4) *trans* fatty acids increased the linoleic acid level. Compared to that in the groups fed either of the other two diets, the arachidonic acid level in the groups fed *trans* fatty acids was diminished in a dose-dependent manner (difference between the PHSO-group (20 en% *trans* fatty acids) and the PHSOMix-group (10 en% *trans* fatty acids)). The total amount of n-6 fatty acids of all groups was similar. Both monoenoic and dienoic *trans* fatty acids were incorporated into aorta phospholipids at low levels (respectively, 3.2 and 1.2% with a dietary level of total *trans* fatty acids of 20 en%), as determined by capillary GLC. *Trans* fatty acids seemed to be incorporated mainly at the expense of stearic acid. The total saturated fatty acids level was therefore somewhat reduced in the groups fed *trans* fatty acids. The capillary GLC technique also revealed that the increase in 18:2 level caused by *trans* fatty acids was largely the result of an increase in the amount of 9*cis*,12*cis*-18:2 (linoleic acid), as the amount of other dienoic C18 fatty acids was low.

<u>PGI_2-production of aorta pieces</u>. The PGI_2-production by aorta pieces shows a strong linear relationship (confidence > 99%) with the log-dose of linoleic acid in the diet (first experiment; Fig. 1). The reference group fed 2 en% linoleic acid (diet consisting of HCNO and OV) displayed a remarkably high PGI_2-production: not only higher than the corresponding *trans* group (group 3), but also higher than the reference group fed 5 en% linoleic acid. With 5 en% linoleic acid in

TABLE 4 Fatty Acid Composition of Aorta Phospholipids as Found by Packed Column GLC (A) and by Capillary Column GLC (B). Second experiment[a]

	Type of fatty acod[b]	Group			
		CB	OV	PHSO	PHSOMix
A	14:0	0.2	0.3	0.5	0.3
	16:0	18.4	18.5	17.3	18.0
	16:1	0.8	1.0	2.1	1.7
	18:0	20.5	18.0	15.0	17.5
	18:1[c]	12.3	16.2	17.5	16.1
	18:2[c]	3.2	2.9	6.5	4.5
	18:3 n-6	0.3	0.1	-	0.1
	20:0[d]	1.0	0.9	0.8	0.9
	20:1	0.4	0.6	0.5	0.5
	20:3 n-9	1.0	0.9	0.9	1.0
	20:3 n-6	0.7	0.7	0.9	0.8
	20:4 n-6	22.9	21.1	20.3	21.4
	22:0[d]	1.0	0.7	0.8	0.9
	22:3	0.7	0.7	0.3	0.5
	22:4 n-6	3.5	3.1	3.6	3.5
	24:0[d]	1.3	0.9	0.9	1.0
	22:5 n-6	3.5	3.4	3.5	3.4
	22:5 n-3	-	0.4	0.2	0.3
	22:6 n-3	1.8	2.5	1.3	1.8
	24:1[d]	1.4	2.1	1.6	1.6
	sum	94.8	95.0	94.3	95.4
	total n-6[e]	34.1	31.3	34.8	33.7
	total sat[e]	42.4	39.3	35.3	38.6
B	18:1 *t*			3.2	2.0
	18:1 *c*			13.1	13.4
	18:1 total			16.1	15.4
	18:2 *t*			1.2	0.1
	18:2 9*c*,12*c*			5.0	3.7
	18:2 total			6.2	3.8

a Each value represents the mean value of 4 pools of 6 animals.
b The notation indicates chain lenght and number of double bonds; *c* = *cis* isomer, *t* = *trans* isomer, - = not detected.
c Both *cis* and *trans* isomers.
d Quantitated with apiezon-column.
e Total n-6 and total sat = the sum of the relative amount of n-6 fatty acids and 18:2 and saturated fatty acids, respectively.

the diet, no difference could be found between the *trans* fatty acids-fed group and the reference group. Also when the PGI_2-production of aorta pieces of the *trans* groups was plotted against the arachidonic acid level in aorta phospholipids, a strong linear relationship appeared (Fig. 2). When extrapolated to higher arachidonic acid levels, the two reference groups did not deviate significantly from this relationship. The PGI_2-production of aorta pieces in the second experiment did not reveal significant differences between the groups (mean values $\pm$ s.e.m. of 24 animals for the CB, OV, PHSO and PHSOMix-group respectively: 1.20 $\pm$ 0.051, 1.19 $\pm$ 0.049, 1.15 $\pm$ 0.066 and 1.15 $\pm$ 0.049 ng/ml).

Fatty acid composition of platelets. The fatty acid composition of platelets was influenced by the dietary level of linoleic acid in a similar way as that of aorta phospholipids (Table 5). *Trans* fatty acids clearly increased the amount of linoleic acid incorporated in platelet lipids. The arachidonic acid level in the reference group fed 2 en% linoleic acid was higher than that in all other groups. There was no difference between the reference group fed 5 en% linoleic acid and the corresponding *trans* group. The levels of other prostaglandin precursors (20:3 n-6 and 20:5 n-3) were low; they were not influenced by *trans* fatty acids. Capillary GLC showed a slight decrease in the amount of *trans*-18:2 isomers with increasing dietary linoleic acid; the level of *trans* monoenes was not decreased. Again a clear effect of *trans* fatty acids on the levels of 9*cis*,12*cis*-18:2 was demonstrated. In the second experiment a significant difference in arachidonic acid incorporation between the OV-group and the CB-group was evident (Table 6). The *trans* fatty acids-containing diets caused arachidonic acid levels in between those of the OV-and CB-groups. As in aorta phospholipid, platelet lipid in the OV-group contained the lowest level of total n-6 fatty acids. The incorporation of *trans* fatty acids in platelet lipids was somewhat higher than that in aorta phospholipids.

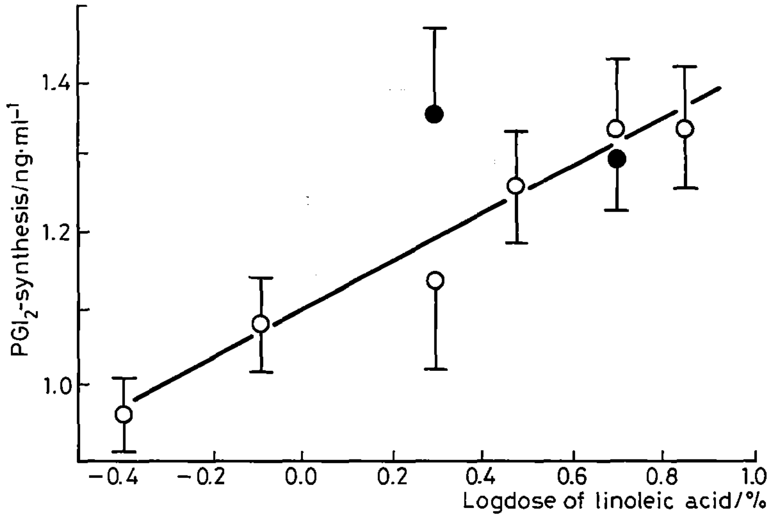

FIG. 1. PGI_2-synthesis (ng/ml) of aorta pieces as a function of the logdose of dietary linoleic acid (in en%) in the first experiment. ● represents the two reference groups (groups 7 and 8); ○ represents the *trans* fatty acids-fed groups. Data given are means ± s.e.m. of 11 animals. The regression line was calculated by the least square method, excluding the two reference groups.

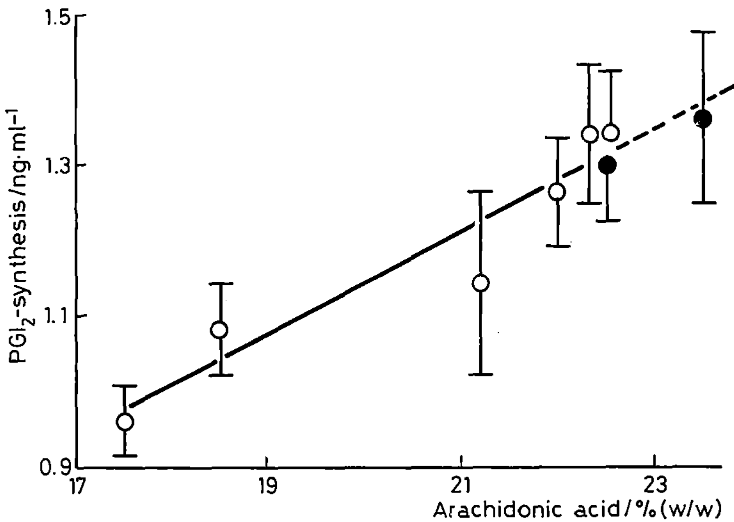

FIG. 2. PGI_2-synthesis (ng/ml) of aorta pieces as a function of the arachidonic acid level (wt%) in aorta phospholipid in the first experiment. ● represents the two reference groups (groups 7 and 8); ○ represents the *trans* fatty acids-fed groups. Data given are means ± s.e.m. of 11 animals. The regression line was calculated by the least square method, excluding the two reference groups.

TABLE 5 Fatty Acid Composition (%) of Platelet Lipids as Found by Packed Column GLC (A) and by Capillary Column GLC (B), First Experiment[a]

	Type of fatty acid[b]	Groups							
		1	2	3[c]	4	5	6	7[d]	8[d]
A	12:0	-	-	-	0.2	0.1	-	-	-
	14:0	0.7	0.7	0.7	0.7	0.5	0.4	1.8	0.4
	16 al.	1.1	1.1	1.0	0.9	0.9	1.0	1.7	1.4
	16:0	22.3	23.0	23.7	23.6	23.3	23.3	26.4	29.2
	16:1 n-7	4.5	4.1	4.3	4.4	4.1	4.4	1.4	1.6
	18 al.	2.0	2.4	2.4	2.4	2.3	2.1	2.5	1.8
	18:0	12.2	12.8	12.5	12.7	12.7	12.8	16.4	15.4
	18:1[e]	19.5	18.1	16.8	16.3	15.6	15.4	10.2	13.3
	18:2[e]	5.6	5.1	5.4	5.9	6.5	7.0	2.8	3.0
	18:3 n-6	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1
	20:0[f]	0.6	0.8	0.8	0.6	0.7	0.6	0.6	0.6
	20:1 n-9	1.3	1.3	1.2	0.9	1.1	1.3	0.9	1.2
	20:3 n-9	3.3	2.1	1.4	1.1	1.1	1.0	0.9	0.7
	20:3 n-6	1.2	0.9	0.7	0.5	0.5	0.4	0.5	0.3
	22:0[f]	1.1	1.1	1.3	1.2	1.2	1.2	1.2	1.0
	20:4 n-6	14.8	16.4	17.3	18.2	18.6	18.3	22.2	18.4
	22:1 n-9	1.3	1.3	1.3	1.1	1.3	1.3	0.5	0.8
	20:5 n-3	0.3	0.1	0.1	-	-	-	-	-
	22:4 n-6	1.6	2.6	3.7	4.5	4.8	5.0	4.1	4.6
	22:5 n-6	3.6	3.3	3.1	2.9	2.9	2.7	4.2	4.7
	22:5 n-3	-	-	0.1	0.7	0.8	1.1	0.3	-
	22:6 n-3	-	-	-	-	-	-	-	-
	24:0[f]	1.2	0.8	1.2	1.1	1.6	1.2	1.4	1.1
	sum	95.5	95.4	95.8	97.1	97.3	97.6	96.7	96.9
	total n-6[g]	25.8	27.5	29.6	31.6	33.0	33.1	33.4	30.8
	total sat[g]	38.1	39.2	40.2	40.1	40.1	39.5	47.8	47.7
B	18:1 *t*	4.9	5.4	5.7	7.2	7.7	5.6	0.1	0.3
	18:1 *c*	14.4	13.0	10.7	9.3	8.5	9.8	9.5	12.9
	18:1 total	19.3	18.4	16.4	16.5	16.2	16.4	9.6	13.2
	18:2 *t*	1.1	0.9	0.9	0.8	0.5	0.7	-	-
	18:2 9*c*,12*c*	4.1	3.7	4.2	4.9	5.5	5.4	2.9	3.2
	18:2 total	5.2	4.6	5.1	5.7	6.0	6.1	2.9	3.2

a Each value represents the mean of two pools of 6 animals. Analysis is performed on packed column (5% DEGS).
b The notation indicates chain lenght and number of double bonds al. = dimethylacetal, - = not detected, *c* = *cis*-isomers, *t* = *trans*-isomers.
c Mean of 4 pools of 6 animals.
d Reference-groups.
e Both *cis* and *trans* isomers.
f Quantitated with apiezon-column.
g Total n-6 and total sat = the sum of the relative amount of n-6 fatty acids and 18:2 and saturated fatty acids, respectively.

TABLE 6 Fatty Acid Composition (%) of Platelet Lipids as Found by Packed Column GLC (A) and by Capillary Column GLC (B), Second experiment[a].

	Type of fatty acid[b]	Group			
		CB	OV	PHSO	PHSOMix
	14:0	0.3	0.5	0.7	0.3
	16:0	26.2	29.7	24.0	25.4
	16:1	0.9	1.6	4.0	2.3
	18:0	17.4	14.0	10.9	14.1
	18:1[c]	9.4	15.6	17.2	14.5
	18:2[c]	2.7	2.2	6.0	4.0
	18:3 n-6	0.3	0.2	-	0.2
A	20:0[d]	0.7	0.6	0.6	0.7
	20:1	0.7	1.4	1.1	1.2
	20:3 n-9	1.2	1.0	0.3	1.2
	20:3 n-6	0.4	0.4	0.7	0.6
	20:4 n-6	23.6	18.3	18.4	20.0
	22:0[d]	1.2	0.6	0.9	0.9
	22:3	0.8	0.7	0.3	0.6
	22:4 n-6	3.9	3.4	3.3	3.6
	24:0[d]	0.7	0.5	0.4	0.5
	22:5 n-6	2.5	3.0	2.0	2.4
	22:6 n-3	0.4	0.5	0.2	0.4
	24:1[d]	1.6	2.6	1.6	1.8
	sum	94.9	96.5	92.6	94.7
	total n-6[e]	33.4	27.5	30.4	30.8
	total sat[e]	46.5	45.9	37.5	41.9
	18:1 *t*			5.9	2.6
	18:1 *c*			11.5	11.8
	18:1 total			17.4	14.4
B	18:2 *t*			1.2	0.6
	18:2 9*c*,12*c*			4.5	3.2
	18:2 total			5.7	3.8

a Each value represents the mean value of 4 pools of 6 animals.
b The notation indicates chain lenght and number of double bonds; *c* = *cis* isomer, *t* = *trans* isomer, - = not detected.
c Both *cis* and *trans* isomers.
d Quantitated with apiezon-column.
e Total n-6 and total sat = the sum of the relative amount of n-6 fatty acids and 18:2 and saturated fatty acids, respectively.

Platelet aggregation experiments. In the first experiment platelet aggregation was induced with 4 doses of collagen. The lowest dose (5.2 μg) failed in triggering all PRP-samples and was therefore not included in the statistical analysis. The delays (in s) of the aggregation curves of PRP induced by 8.7, 14.5 or 43.5 μg collagen are presented in Fig. 3. With the low dose of collagen (8.7 μg) there seems to be a dose-response relationship between the dietary linoleic acid level and the delay. No significant differences could be found between group 3 and the other groups for each dose of collagen. In the second experiment some systematic differences were detected in the delay as well as the tangent α of the aggregation curves (Table 7). The CB-diet induced the highest delay and the lowest tg α compared to the other diets. The *trans* fatty acids-fed groups had values between those of the CB-group and the OV-group when 11.6 μg collagen was applied. When 29 μg collagen was used, the PHSO-diet caused the same delay as the CB-diet but a significantly higher tg α.

Hydroxy fatty acid production of platelets. Hydroxy fatty acid-production was measured after stimulation of PRP. Both maximal (43.5 μg collagen) and submaximal (14.5 μg collagen) triggering were used. The concentrations of the monohydroxy fatty acids 12-hydroxy-5*cis*, 8*cis*,10*trans*-heptadecatrienoic acid (HHT) and 12-hydroxy-5*cis*,8*cis*,10*trans*,14*cis*-eicosatetraenoic acid (12-HETE) in the supernatans of the stimulated platelets, after maximal triggering, as determined by HPLC are given in Fig. 4 as a function of the dietary linoleic acid level. Both HHT and 12-HETE values of group 1 (0.4 en% linoleic acid; 20 en% *trans* fatty acids) were significantly lower than those of group 3 (2 en % linoleic acid) (conf. > 99%). The HETE-concentration of group 6 (7.1 en% linoleic acid) was significantly higher than that of group 3 (conf. > 95%). The reference groups (7 and 8) with 2 and 5 en% linoleic acid respectively, were not significantly different from the standard *trans* group (group 3). Sub-maximal triggering of the platelets produced less 12-HETE and HHT, but the effects of the dietary fats were very similar as with maximal

TABLE 7 Aggregation of PRP Induced with Two Doses of Collagen, Second Experiment[a]

	11.6 μg collagen		29 μg collagen	
group	delay	tg α	delay	tg α
CB	163 ± 9.2[2]	4.30 ± 0.233[234]	78 ± 1.9[2]	5.91 ± 0.122[3]
OV	133 ± 5.2[1]	4.99 ± 0.155[1]	70 ± 1.9[13]	6.13 ± 0.097
PHSO	149 ± 7.2	4.84 ± 0.185[1]	78 ± 2.6[2]	6.23 ± 0.111[1]
PHSOMix	147 ± 6.7	4.84 ± 0.168[1]	73 ± 2.4	6.16 ± 0.111

a Each values represent the mean value and s.e.m of the delay (in s) and tg α of the aggregation curves for the four groups (n=24). 1,2,3,4. Values with superscript 1,2,3 or 4 are significantly different from those of group CB, OV, PHSO or PHSOMix respectively for $p < 0.05$.

triggering, i.e. a significantly decreased HHT and 12-HETE in group 1 compared to group 3 (conf. > 99%)(data not shown). No significant differences in HHT-production between the groups were found in the second experiment (Table 8). The 12-HETE-production in the CB-group was significantly higher than that in the other groups.

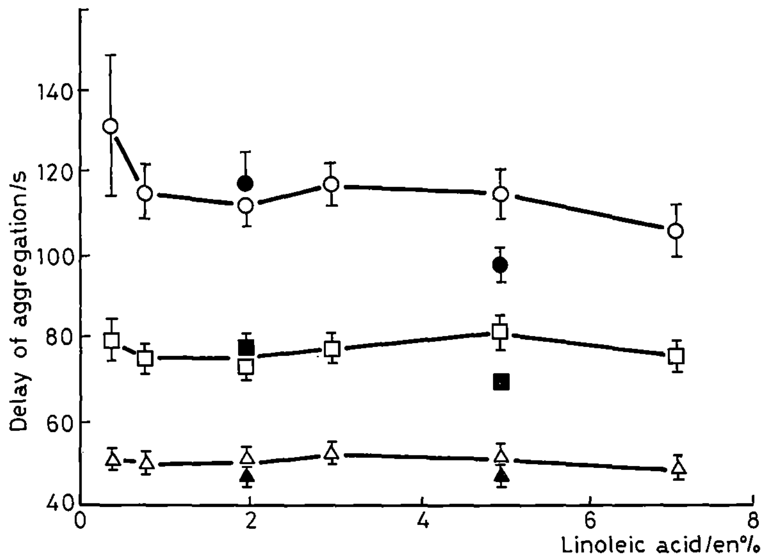

FIG. 3. Delay of aggregation (s) of PRP, stimulated with three doses of collagen as a function of the level of dietary linoleic acid (en%) in the first experiment. ● ○ - 8.7μg; □ ■ - 14.5 μg and △▲ - 43.5 μg collagen.

● ■ ▲ represent the two reference groups (groups 7 and 8); ○ □ △ represent the *trans* fatty acids-fed groups. Data given are means ± s.e.m. of 11 animals.

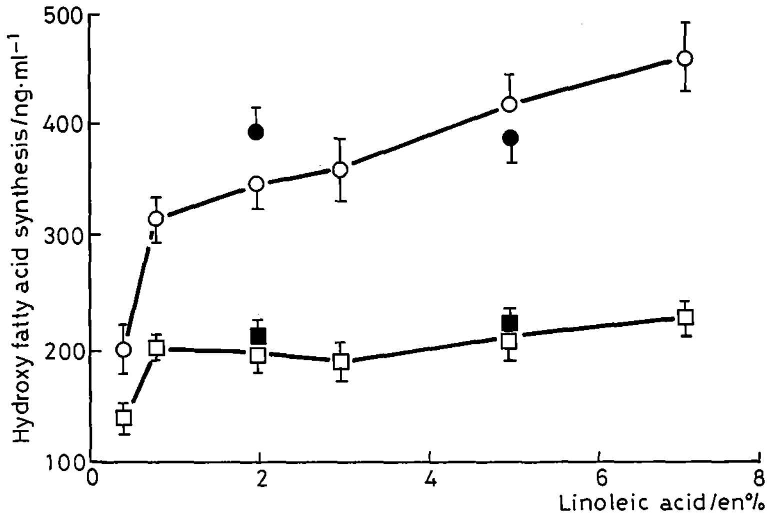

FIG. 4. Hydroxy fatty acid synthesis (ng/ml) of platelets after stimulation with 43.5 μg collagen (maximal triggering), as a function of the level of dietary linoleic acid (en%) in the first experiment. ○ ● - HETE; □ ■ - HHT.

● ■ represent the two reference groups (groups 7 and 8); ○ □ represent the *trans* fatty acids-fed groups. Data given are means ± s.e.m. of 11 animals.

TABLE 8

Production of Hydroxy Fatty Acids (in ng) by 10^9 Platelets Stimulated with 29 μg Collagen, Second Experiment[a].

group	HHT	HETE
CB	323 ± 13.0	631 ± 26[234]
OV	337 ± 15.8	573 ± 23[1]
PHSO	306 ± 11.4	537 ± 15[1]
PHSOMix	321 ± 12.0	568 ± 28[1]

a The values represent the mean ± s.e.m. of 24 animals per group. 1,2,3,4. Values with superscript 1,2,3 or 4 are significantly different from those of group CB, OV, PHSO or PHSOMix respectively for $p < 0.05$.

DISCUSSION

The influence of dietary *trans* fatty acids on tissue fatty acid composition in rats has been the subject of many investigations. All tissues investigated have been reported to incorporate (monoenoic) *trans* fatty acids, be it to a different degree (1,19). In this report emphasis is laid on the changes in the tissue levels of polyunsaturated fatty acids brought about by *trans* fatty acids, as polyunsaturated fatty acids are precursors of the biosynthesis of eicosanoids.

HHT and malondialdehyde are formed during the biosynthesis of the potent pro-aggregatory compound thromboxane A_2 (TXA_2) (20). TXA_2 is a product of the cyclooxygenase pathway. This biologically very active

compound is rapidly converted into thromboxane B_2 (TXB_2), a stable, inactive product. Concomittantly with the synthesis of every TXA_2 molecule, approximately one molecule of HHT and one of MDA are formed. The synthesis of TXA_2 can thus be followed via the analysis of the hydroxy fatty acid HHT, which can be quantitated via HPLC (16). PGI_2, also a product of the cyclooxygenase pathway, formed by the vascular tissue (21), and TXA_2, formed by platelets, are thought to play an important role in platelet aggregation, and hence in thrombotic processes (22). So both the anti-aggregatory PGI2 and the pro-aggregatory TXA_2 originate from the same precursor arachidonic acid, and have the same endoperoxide as intermediate. In a second pathway in platelets, arachidonic acid is converted by a lipoxygenase into a hydroperoxyde fatty acid, which in turn is reduced to the hydroxy fatty acid 12-HETE. The role of hydroxy fatty acids in thrombotic processes is not clear; it has been suggested that they support the clotting process (23) and promote adhesion of leucocytes to the vascular wall (24).

Arachidonic acid is formed from linoleic acid by enzymatic desaturation and elongation pathways (25,26). The first step in the synthesis of arachidonic acid, the Δ6-desaturation step, is generally considered to be the rate-limiting step. It has frequently been suggested that *trans* fatty acids inhibit this Δ6-desaturation, resulting in an increased level of linoleic acid and a decreased arachidonic acid level in tissue lipids (27-30). However, the fatty acid composition of tissue lipids is the result of many competing metabolic pathways which are controlled by many enzyme systems, including acyltransferases, oxidation enzymes and fatty acid-synthesizing enzymes. This means that changes in the composition of tissue fatty acid brought about by dietary fatty acids cannot a priori be ascribed to effects on one enzyme system only (31, and Zevenbergen and Houtsmuller, submitted for publication). However, irrespective of the mechanism by which *trans* fatty acids influence tissue fatty acid composition, a reduction in arachidonic acid level is often observed (32).

In this experiment *trans* fatty acids, as present in PHSO, increased the linoleic acid content in both platelets and aorta, compared to either a fat rich in saturated fatty acids (mixture of HCNO and OV: group 7 in the first experiment or CB in the second experiment) or a fat rich in *cis*-monounsaturated fatty acids (OV: group 8 or OV in the first and second experiment, respectively). The arachidonic acid level in the groups fed *trans* fatty acids is diminished when compared to that in groups fed diets high in saturated fatty acids (group 7 and CB). However, when effects of dietary *trans* fatty acids and *cis*-monounsaturated fatty acids (group 8 and OV) are compared, the situation is less clear. In aorta phospholipids the level of arachidonic acid in the animals fed OV is somewhat higher than that in the animals fed *trans* fatty acids in the second experiment, while there is no difference between those groups with respect to the platelet arachidonic acid content. For both linoleic and arachidonic acid the effects of *trans* fatty acids seem to be dose-dependent as is evident from a comparison of the PHSOMix group (10 en% *trans* fatty acids), the PHSO group (20 en% *trans* fatty acids) and the other two groups (no *trans* fatty acids). The relative effect of these isomeric fatty acids on tissue arachidonic acid level is certainly not universal and may depend on tissue and phospholipid class (7) as well as on the diet used for comparison. In both our experiments, the diets rich in saturated fatty acids (the HCNO/OV-diet (group 7) and the CB-diet) induced (much) higher arachidonic acid levels than the OV-diets (group 8 and OV-diet). As is evident from our experiments and those of others (11) the lowering of arachidonic acid induced by *trans* fatty acids compared to *cis*-monounsaturated fatty acids is small or non-existing in platelets.

Houtsmuller (33) emphasizes that a distinction should be made between monoenoic and polyenoic *trans* fatty acids. Some adverse effects, e.g. on prostaglandin synthesis, are attributed to the best known representative of the latter group, linolelaidic acid (9*t*,12*t*-18:2), which was concluded from both in vitro experiments and from feeding studies (8,9,34). However, as pointed out in some recent reviews, the amount of this fatty acid in commercial

partially hydrogenated oils is very low (2,4). As a consequence, the total dietary intake of 9*t*,12*t*-18:2 by humans can be neglected.

Monoenoic *trans* fatty acids in partially hydrogenated oils and in ruminant fats form a heterogenous group of positional isomers (5,35). The metabolism of each isomer may differ significantly (32), and the biological effects of the isomers could vary accordingly. In our experiment we used PHSO. This is a special, partially hydrogenated soybean oil, containing a high amount of various monoenoic *trans* isomers next to a relatively high amount of dienoic *trans* fatty acids: isomers of linoleic acid. The diets were composed in such a way as to contain 40% *trans* monoenes and almost 8% *trans* dienes (ca. 0.5% 9*t*,12*t*-18:2 and 0.6% other 9,12-18:2 isomers), thus by far exceeding the *trans* content of human diets (36,37). The advantage of this approach is that the distribution of positional isomers of both monoenoic and polyenoic *trans* fatty acids in the experimental diet is similar to that in the human diet. In this way, more realistic conclusions can be reached with regard to the nutritive value of *trans* fatty acids-containing food products.

Many investigators have found that significant changes in eicosanoid synthesis can be brought about by dietary fat (38-41). These changes are, at least partly, caused by reduced eicosanoid precursor levels in tissues. A linear relationship between PGI_2-synthesis of aorta pieces and phospholipid arachidonic acid level in rabbits has been demonstrated (39). Similarly, HHT-and HETE-formation by rat platelets is well correlated with platelet arachidonic acid content (11,39). In our first experiment, where a wide range of dietary linoleic acid levels was applied, these relationships were confirmed. PGI_2-synthesis showed a strong linear relationship with the arachidonic acid level in aorta phospholipid. The two reference groups, devoid of *trans* fatty acids, fitted the regression line calculated from the data of the *trans* fatty acids fed-groups perfectly. For the HHT-and HETE-production by platelets similar relationships were observed (data not shown). We therefore conclude that *trans* fatty acids do not directly influence the enzymes involved in eicosanoid synthesis, but exert their effect on eicosanoid production by influencing the tissue precursor level i.e.

arachidonic acid. This is in line with findings of Nugteren (42), who indicated that the cyclooxygenase activity of sheep seminal vesicles was not inhibited substantially by isomeric fatty acids, unless a *cis,trans* monoconjungated system was present. Levels of *cis, trans* conjugated fatty acids are negligible in our experimental diets.

In the second experiment no significant differences in PGI_2- and HHT-synthesis were found between the groups. This is in accordance with a study where the 6-keto-$PGF_{1\alpha}$ production (the stable PGI_2-metabolite) from aorta pieces did not differ between rats fed olive oil or a partially hydrogenated low-erucic-acid-rapeseed oil (both with 5 en% linoleic acid) (11). Royce et al. found that in swine, too, a diet rich in *trans* fatty acids and a diet containing lard (both with 5 en% linoleic acid) caused a similar release of PGI_2, measured as 6-keto-$PGF_{1\alpha}$, from coronary arteries. (43).

Neither Blomstrand et al. (11) nor Royce et al. (43) found any effects of *trans* fatty acids on TXA_2- production of platelets, either measured via a radio-immuno assay for TXB_2 (43) or via HHT-release (11), which is in agreement with our experiment. Blomstrand et al. (11) also reported that *trans* fatty acids-rich diets, unlike oleic acid-rich diets, do not influence the HETE-synthesis by platelets. This again agrees with our study. We thus confirm the conclusions of others that high amounts of *trans* fatty acids in hydrogenated vegetable oils do not interfere with the cyclooxygenase or lipoxygenase pathways if sufficient linoleic acid is present in the diet. Our study indicates that in rats 2 en% linoleic acid is sufficient to prevent effects of dietary *trans* fatty acids on eicosanoid production.

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REFERENCES

1. Wood, R. (1979) in *Geometrical and Positional Fatty Acid Isomers* (Emken, E.A., and Dutton, H.J., eds.) pp. 213-281, American Oil Chemists' Society, Champaign, IL.

2. Beare-Rogers, J.L. (1983) in *Advances in Nutritional Research* (Draper, H.H. ed.) Vol 5, pp. 171-200, Plenum Press, New York.

3. Gurr, M.I. (1983) *Bull. Int. Dairy Fed. Doc. 166*, 5-18.

4. Emken, E.A. (1983) *J. Am. Oil Chem. Soc. 60*, 995-1004.

5. Ohlrogge, J.B. (1983) in *Dietary Fats and Health* (Perkins, E.G. and Visek, W.J. eds.) pp. 359-374, American Oil Chemists' Society, Champaign, IL.

6. Gottenbos, J.J. (1983) in *Dietary Fats and Health* (Perkins, E.G. and Visek, W.J. eds.) pp. 375-390, American Oil Chemists' Society, Champaign, IL.

7. Zevenbergen, J.L., Houtsmuller, U.M.T. and Gottenbos, J.J. (1988) *Lipids 23*, 178-186.

8. Kinsella, J.E., Bruckner, G., Mai, J. and Shimp, J. (1981) *Am. J. Clin. Nutr. 34*, 2307-2318.

9. Hwang, D.H., Chanmugam, P. and Anding, R. (1982) *Lipids 17*, 307-313.

10. Applewhite, T.H. (1983) in *Dietary Fats and Health* (Perkins, E.G. and Visek, W.J. eds.) pp. 414-424, American Oil Chemists' Society, Champaign, IL.

11. Blomstrand, R., Diczfalusy, U., Sisfontes, L. and Svensson, L. (1985) *Lipids 20*, 283-295.

12. Claeys, M., Kivits, G.A.A., Christ-Hazelhof, E. and Nugteren, D.H. (1985) *Biochim. Biophys. Acta 837*, 35-51.

13. De Deckere, E.A.M., Jansen Verplanke, C., Blonk, C.G. and Van Nielen, W.G.L. (1988) *J. Nutr. 118*, 11-18.

14. Hornstra, G., Haddeman, E. and Don, J.A. (1979) *Nature 279*, 66-68.

15. Folch, J., Lees, M., and Stanley, G.H.S. (1957) *J. Biol. Chem. 226*, 497-509.

16. Nugteren, D.H. and Kivits, G.A.A. (1987) *Biochim. Biophys. Acta 921*, 135-141.

17. Bligh, E.G. and Dyer, W.J. (1959) *Can. J. Biochem. Physiol. 37*, 911-917.

18. Christie, W.W. (1973) in *Lipid Analysis* p. 152, Pergamon Press, Oxford.

19. Reichwald-Hacker, I., Ilsemann, K. and Mukherjee, K.D. (1979) *J. Nutr. 109*, 1051-1056.

20. Hamberg, M. and Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. USA 71*, 3400-3404.

21. Johnson, R.A., Morton, D.R., Kinner, J.H., Gorman, R.R., McGuire, J.C., Sun, F.F., Whittaker, N., Bunting, S., Salmon, J.A., Moncada, S. and Vane, J.R. (1976) *Prostaglandins 12*, 915-929.

22. Moncada, S. and Vane, J.R. (1978) *Br. Med. Bull. 34*, 129-135.

23. Dutilh, C.E., Haddeman, E., Don, J.A. and Ten Hoor, F. (1981) *Prost. and Med. 6*, 111-126.

24. Turk, J., Rand, T.H. and Oates, J. (1983) *Biochim. Biophys. Acta 750*, 78-90.

25. Brenner, R.R. (1970) *Lipids 6*, 567-575.

26. Sprecher, H. (1977) In *Polyunsaturated Fatty Acids* (Kunau, W.-H and Holman, R.T., eds.) pp. 1-18, American Oil Chemsist' Society, Champaign, Il.

27. De Schrijver, R. and Privett, O.S. (1982) *Lipids 17*, 27-34.

28. Lawson, L.D., Hill, E.G. and Holman, R.T. (1983) *J. Nutr. 113*, 1827-1835.

29. Mahfouz, M.M., Smith, T.L. and Kummerow, F.A. (1984) *Lipids 19*, 214-222.

30. Thomassen, M.S., Rörtveit, T., Christiansen, E.N. and Norum, K.R. (1984) *British J. Nutr. 51*, 315-322.

31. Zevenbergen, J.L. (1986) In *Enzymes of Lipid Metabolism II.* (Freysz, L., Dreyfus, H., Massarelli, R. and Gatt, S., eds.) pp. 647-649, Plenum Publishing Corporation, New York.

32. Emken, E.A. (1984) *Ann. Rev. Nutr. 4*, 339-376.

33. Houtsmuller, U.M.T. (1978) *Fette Seifen Anstrichm. 80*, 162-169.

34. Privett, O.S., Stearns, E.M. Jr and Nickell, E.C. (1967) *J. Nutr. 192*, 303-310.

35. Dutton, H.J. (1979) in *Geometric and Positional Fatty Acid Isomers* (Emken, E.A. and Dutton, H.J. eds.) pp. 1-16, American Oil Chemists' Society, Champaign, Illinois.

36. FASEB Report of the ad-hoc Committee of the Federation of American Societies for Experimental Biology (1985) Health Aspects of Dietary *Trans* Fatty Acids (Senti, F.R., ed.), FDA 223-83-2020, Washington.

37. Zevenbergen, J.L. (1987) In *Fat Production and Consumption* (Galli, C. and Fedeli, E., eds.) pp. 157-168, Plenum Press, New York.

38. Ten Hoor, F., De Deckere, E.A.M., Haddeman, E., Hornstra, G. and Quadt, J.F.A. (1980) *Adv. Prostaglandin Thromboxane Leukotriene Res. 8*, 1771-1778.

39. Hornstra, G., Haddeman, E., Kloeze, J. and Verschuren, P.M. (1983) Adv. *Prostaglandin Thromboxane Leukotriene Res. 12*, 193-202.

40. Croft, K.D., Beilin, L.J., Vandongen, R. and Mathews, E. (1984) *Biochim. Biophys. Acta 795*, 196-207.

41. Blankenship, J.W. and Miller, T. (1986) *Prostaglandins, Leukotrienes Med. 23*, 45-52.

42. Nugteren, D.H. (1970) *Biochim. Biophys. Acta 210*, 171-176.

43. Royce, S.M., Holmes, R.P., Takagi, T. and Kummerow, F.A. (1984) *Am. J. Clin. Nutr. 39*, 215-222.

Chapter 5.

EFFECT OF DIETARY FAT ON TOTAL AND PEROXISOMAL FATTY ACID OXIDATION IN RAT TISSUES

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Effect of dietary fat on total and peroxisomal fatty acid oxidation in rat tissues
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SUMMARY

In this study the effect of dietary *trans* fatty acids on the peroxisomal and mitochondrial β-oxidation is compared with that of saturated or *cis*-monounsaturated fatty acids. Oxidation of [1-^{14}C]- and [16-^{14}C]palmitate was assayed in the absence as well as in the presence of antimycin plus rotenone in homogenates of liver, heart and skeletal muscle of four groups of rats, fed diets containing 40 energy% fat of different fatty acid composition. Three groups were given fat blends rich in C_{16}, C_{18} saturated (cocoabutter), *cis*-monounsaturated (low-linoleic-acid olive oil) or *trans* fatty acids (partially hydrogenated soybean oil), respectively. The fourth group received a mixture of these fats with half the amount of *trans* fatty acids of the third group.

Total oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate in the absence of antimycin were not significantly influenced by the type of dietary fat in the investigated tissues. The antimycin-insensitive [1-^{14}C]palmitate oxidation rate and the proportion of peroxisomal oxidation of the total oxidation were lower in all tissues of animals fed the mixed dietary fat than in those of the rats fed the other diets; both parameters were higher in the liver of cocoabutter-fed rats than in those of the other groups.

Comparison of the results with literature data and with previous results obtained with a low-fat diet (Veerkamp and Van Moerkerk (1986), Biochim. Biophys. Acta 875, 301-310) indicates that high-fat diets only induce peroxisomal β-oxidation activity if they also contain C_{20}, C_{22} fatty acids. High dietary concentrations of *trans* C_{18} fatty acids do not result in a higher peroxisomal activity than that observed for other fatty acids with the same length.

INTRODUCTION

Peroxisomes contribute, in addition to mitochondria, to the oxidation of fatty acids in various tissues (1-6). The properties of both oxidation systems differ largely (2, 7, 8) and these differences can be used for comparative assays (2, 5, 6, 9). Several conditions affect the activity of the peroxisomal β-oxidation and its relative contribution to the total fatty acid oxidation but such conditions may influence the mitochondrial capacity as well. Treatment with hypolipidemic drugs (e.g. clofibrate) increases peroxisomal oxidation in liver, heart and kidney (1, 2, 6, 10, 11). Such treatments increase the oxidation rate of erucic acid ($C_{22:1}$, n-9 *cis*) in hepatocytes (12) and perfused heart (13) of rats. Peroxisomes of liver show a higher rate of oxidation of this fatty acid than mitochondria (14). The influence of the diet on peroxisomal and mitochondrial β-oxidation has been the subject of many studies. The relationship between dietary fatty acids and peroxisomal oxidation activity appears to be complex. High-fat diets and especially diets rich in $C_{22:1}$ fatty acids such as hydrogenated fish oils or high erucic acid-rapeseed oil, increase the capacity of peroxisomal fatty acid oxidation in rat liver (15-21) and heart (22). These diets cause also a marked increase in chain-shortening of erucic acid in rat hepatocytes (23) and in perfused rat heart (24). Peroxisomes could therefore play an important role in the oxidation of those fatty acids that are poorly degraded by mitochondria (17). Induction of peroxisomes and/or their β-oxidative capacity may form an adaptation mechanism to the diet (20).
It is still not clear if fatty acids are the primary agents which stimulate peroxisomal activity and if certain fatty acids are more specific stimulators than others. The increase in heart (micro-) peroxisomal β-oxidation is directly correlated to the content of erucic acid in the diet of the rat (22). In liver, however, no direct relationship exists, between these parameters (19); erucic acid even has a negative modulating effect on peroxisomal β-oxidation and biogenesis (21).

Peroxisomes from liver of rats fed various diets oxidize *cis*- and *trans*- mono-unsaturated fatty acids with comparable rates (14, 17) in contrast to mitochondria from both liver and heart, which show a lower oxidation rate for *trans* isomers (17, 25-27). Feeding of partially hydrogenated marine-, rapeseed- or soybean-oil causes a higher peroxisomal β-oxidation activity in rat liver than feeding the corresponding unhydrogenated oils (19). These observations led to the suggestion that *trans* isomers (formed by hydrogenation) are more potent inducers of peroxisomal β-oxidation than *cis* isomers (19). However, the presence of *trans* isomers in the dietary oil has quite a different effect on the (micro-)peroxisomal β-oxidation activity of the rat heart (22): hydrogenation of rapeseed- or soybean-oil caused a lower activity; with marine oil no significant differences were observed in peroxisomal activity of the hearts from animals fed the partially hydrogenated or the unhydrogenated oil (22).

Since the relationship between the dietary fatty acids and peroxisomal activity appears to be complex and possibly tissue-dependent, we intended to compare the effect of dietary *trans* fatty acids with those of saturated and *cis* mono-unsaturated fatty acids on mitochondrial and peroxisomal oxidation in different tissues. Rats were fed 4 different dietary fat blends. A partially hydrogenated soybean oil was used for preparation of a diet with a high content of (C_{18}) *trans* fatty acids (20 energy %). Furthermore we used a special olive oil (low linoleic acid) and cocoabutter to prepare diets containing high amounts of *cis*-mono-unsaturated or saturated (C_{16}, C_{18}) fatty acids, respectively. Finally, the effect of a lower content of *trans* fatty acids (10 energy %) was investigated using a mixture of the partially hydrogenated soybean oil, cocoabutter, olive oil and fully hydrogenated coconut oil. By assaying total and antimycin-insensitive oxidation of [1-^{14}C]- and [16-^{14}C]palmitate we were able to discriminate effects on peroxisomal and mitochondrial oxidation (6) in homogenates of liver, heart and quadriceps muscle.

MATERIALS AND METHODS

Materials

Partially hydrogenated soybean oil was obtained from van den Bergh and Jurgens (Rotterdam, The Netherlands), hydrogenated coconut oil from Chenpry BV (Raamsdonkveer, The Netherlands), sunflower seed oil from Union (Antwerpen, Belgium) and cocoabutter from J. Schoenmaker BV (Zaandam, The Netherlands). Low-linoleic-acid (5.5%) olive oil was a gift from Elais (Athens, Greece) and "normal" olive oil (more than 10% linoleic acid) was purchased from Fol Jr. and Co (Krimpen and de IJssel, The Netherlands). Biochemicals and ^{14}C-labelled palmitic acids were obtained as mentioned previously (28).

TABLE I Composition of the experimental fat blends

Values represent energy% of the diet. All experimental fat blends comprised 40% of the total energy of the diets and contained approximately 2 energy% linoleic acid.

	Experimental fat blend			
	CB	OV	PHSO	PHSOMix
Partially hydrogenated soybean oil	-	-	30.0	15.0
Olive oil (low linoleic acid)	-	40.0	-	-
Olive oil	-	-	2.7	15.0
Cocoabutter	39.4	-	-	10.0
Sunflower seed oil	0.6	-	2.3	-
Hydrogenated coconut oil	-	-	5.0	-

Animals and diets

Male weanling SPF-Wistar rats were obtained from the Central Breeding Station TNO (Zeist, The Netherlands). The animals were housed individually in a climatised room (mean temperature 23±1°C and relative humidity 45-70%) with a day/night cycle of 12 h, and had free access to water and food. Pups of 6 litters were divided over 4 experimental groups with one littermate per litter per group (n=6). The animals received semi-synthetic diets with 40 energy % (18.6%, w/w) as fat for 9 weeks. The diet contained per 1000 kJ: 14.8 g casein, 3.6 g cellulose, 25.2 g corn starch, 0.24 g vitamin mixture, 1.3 g salt mixture and 10.3 g experimental fat blend. Three experimental fat blends were composed in such a way to give diets characterized by high amounts of *trans* fatty acids (approx. 20 energy %, originating from partially hydrogenated soybean oil), of saturated fatty acids (cocoabutter) or of *cis*-mono-unsaturated fatty acids (low-linoleic-acid olive oil) (Table I). Furthermore, one experimental fat blend was composed, containing half the amount of *trans* fatty acids (10 energy %) of the first blend. All experimental diets contained approx. 2 energy % linoleic acid to prevent essential fatty acid deficiency. The fatty acid composition of the experimental fat blends is given in Table II. The animals were weighed and examined weekly.

Experimental procedures

After 9 weeks rats were killed by cervical dislocation. Tissues were removed and immediately cooled in ice-cold buffer, consisting of 0.25 M sucrose, 2 mM EDTA and 10 mM Tris-HCl (pH 7.4). Whole homogenates (5% w/v) were prepared in the same buffer by hand homogenization (28).

Palmitate oxidation rates were measured in a total volume of 0.5 ml medium containing 25 μl (heart and liver) or 100 μl (muscle) homogenate. The complete composition was described previously (28). It contained 0.5 mM malate, 0.1 mM coenzyme A, 5 mM ATP, 0.5 mM L-carnitine and 120 μM ^{14}C-labelled palmitate (specific activity 3500 dpm/nmol), bound to albumin in 5:1 molar ratio. Where indicated inhibitors of mitochondrial oxidation (36 μM antimycin A plus 10 μM rotenone) were added 5 min

TABLE II Fatty acid composition of the experimental fat blends

The composition is given in % (w/w). Methyl esters were separated by $AgNO_3$ thin-layer chromatography and analyzed by capillary gas-liquid chromatography on Silar 10C essentially according to Ref. 41. The shorthand notation used for the fatty acids indicates chain length: number of double bonds. c=*cis* isomer, t=*trans* isomer, ot=other isomers (*cis* and *trans*)

	Experimental fat blend			
Fatty acid[a]	CB	OV	PHSO	PHSOMix
12:0		0.1	5.6	
14:0	0.2		2.4	0.1
16:0	23.5	11.9	10.0	15.3
16:1			0.3	
17:0	0.3		0.1	0.1
17:1		0.1	0.1	0.1
18:0	31.9	2.0	8.0	12.1
18:1 *c*	36.9	78.7	17.5	39.9
18:1 *t*			39.1	19.5
18:2 *c,c*	4.5	5.5	5.3	4.7
18:2 *c,t*; *t,c*			0.6	0.3
18:2 *t,t*			0.5	0.2
18:2 ot			7.7	3.9
18:3	0.2	0.6		
20:0	1.3	0.4	0.5	0.6
20:1		0.4	0.5[a]	0.4[a]
22:0	0.3	0.2	0.4	0.3
other	0.9		1.5	2.5

[a] contains also conjugated 18:2 isomers

before addition of the substrate. Incubations were carried out for 30 min at 37°C with shaking and stopped by addition of 0.2 ml of 3 M perchloric acid. Radio-activity of trapped $^{14}CO_2$ and of ^{14}C-labelled acid-soluble products was assayed as described (28). The proportion of peroxisomal of total palmitate oxidation was calculated by dividing the difference of the oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate in the presence of antimycin by the [1-^{14}C]palmitate oxidation rate in the absence of antimycin (total palmitate oxidation rate). The oxidation rate of [16-^{14}C]palmitate in the presence of antimycin was substracted to correct for possible uninhibited mitochondrial oxidation. This correction, however, tends to be overestimated and therefore the thus obtained proportion values are minimal values (see Ref. 6 for details).
Citrate synthase (EC 4.1.3.7) activity was assayed according to the procedure of Shepherd and Garland (29) in sonicated homogenates. The assay of cytochrome c oxidase (EC 1.9.3.1) activity was described previously (30).
The Student-Newman-Keuls multiple range test was applied to localize the systematic differences between the dietary treatments, if present.

RESULTS

No symptoms of essential fatty acid deficiency were observed during the experimental period. All animals seemed to be in good health. There were no differences in the food or water consumption between the groups. Body and liver weights did not differ between the groups at the time of sacrifice (Table III). The assay conditions and the procedures of total and antimycin-insensitive palmitate oxidation were previously evaluated (6, 28). All data are for animals sacrificed in the fed state.
The total oxidation rates of [1-^{14}C]palmitate in liver homogenates do not differ between the 4 experimental groups (Table III). The ratios of the oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate do also not differ, but showed large individual variations. Addition of antimycin plus rotenone did not change the ratios. The antimycin-insensitive oxidation rates of [1-^{14}C]palmitate are significantly higher and lower

in the cocoabutter and the mixed-fat group respectively, than in the other groups. The proportion of peroxisomal of total palmitate oxidation (calculated as given in Materials and Methods) is higher in the cocaobutter group than in the other groups. The rate of antimycin-sensitive (or mitochondrial) oxidation (= total - antimycin-insensitive) of [1-^{14}C]palmitate is significantly higher in the mixed-fat group than in the cocaobutter- and olive-oil groups. Mitochondrial marker enzymes do not show large differences, except a significantly lower cytochrome c oxidase activity in liver homogenates of the rats fed the partially hydrogenated soybean oil.

The total oxidation rates of [1-^{14}C]palmitate are much higher in heart homogenates (Table IV) than the liver homogenates in the fed state (6), but they do not differ between the four experimental groups. Neither did the type of the diet influence the ratio of oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate both in the absence and presence of antimycin in the heart. The only significant dietary effects are a decrease of the antimycin-insensitive oxidation and of the proportion of peroxisomal oxidation after feeding the mixed-fat diet.

In quadriceps muscle the oxidation rates of [1-^{14}C]palmitate and the ratios of the oxidation rates do not show differences between the groups (Table V) when assayed in the absence of antimycin. The mixed-fat diet appears to influence significantly the antimycin-insensitive oxidation rate and the proportion of peroxisomal oxidation. The ratio of the oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate in the presence of antimycin is also lower after this diet. Some differences are present in the activities of citrate synthase and cytochrome c oxidase between the four groups, but they show a somewhat different pattern than the mitochondrial oxidation rates of [1-^{14}C]palmitate.

DISCUSSION

Since we applied a higher fat content of the diets (18.6%, w/w) than in a previous study (4.8%, w/w; Ref. 6), we will first discuss the effects of the fat content on the parameters studied. All data and comparisons

TABLE III Effect of type of dietary fat on body- and liver-weight and on total or antimycin-insensitive palmitate oxidation and mitochondrial enzyme activities in liver homogenates

Values represent mean ± S.D. of 6 animals. Values with superscript 1, 2, 3 or 4 are significantly different from those of group CB, OV, PHSO and PHSOMix, respectively for $P < 0.05$. Weights are given in g, oxidation rates in nmol/min per g tissue, proportion of peroxisomal oxidation in % and mitochondrial enzyme activities in μmol/min per g tissue.

Parameter	EXPERIMENTAL GROUP			
	CB	OV	PHSO	PHSOMix
Body weight	317 ±32	312 ±24	338 ±33	320 ±35
Liver weight	13.2± 2.3	12.1± 1.1	13.7± 1.6	12.5± 2.0
[1-^{14}C]Palmitate oxidation rate	427 ±65	414 ±32	469 ±49	467 ±52
Ratio of oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate	13.4± 3.0	12.1± 3.5	9.0± 4.4	17.0± 9.0
[1-^{14}C]Palmitate oxidation rate with antimycin and rotenone	199 ±18[2,3,4]	143 ± 7[1,3,4]	160 ±11[1,2,4]	113 ±13[1,2,3]
Ratio of oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate with antimycin and rotenone	12.3± 1.3	10.2± 2.0	10.3± 2.2	10.3± 1.0
Proportion of peroxisomal oxidation	44.1± 7.2[2,3,4]	31.2± 3.0[1]	31.1± 5.6[1]	22.1± 3.2[1]
Antimycin-sensitive [1-^{14}C]palmitate oxidation rate (mitochondrial oxidation)	227 ±60[4]	271 ±30[4]	309 ±56	354 ±51[1,2]
Citrate synthase activity	13.2± 1.0	12.8± 0.6	13.4± 1.0	13.9± 1.5
Cytochrome c oxidase activity	229 ±26[3]	228 ±29[3]	198 ±10[1,2]	203 ±10

TABLE IV Effect of type of dietary fat on total or antimycin-insensitive palmitate oxidation and mitochondrial enzyme activities in heart homogenates

Values represent mean ± S.D. of 6 animals. Values with superscript 1, 2, 3 or 4 are significantly different from those of group CB, OV, PHSO and PHSOMix, respectively for $P < 0.05$. Other details are given in Table III.

Parameter	EXPERIMENTAL GROUP			
	CB	OV	PHSO	PHSOMix
[1-^{14}C]Palmitate oxidation rate	872 ±98	805 ±71	833 ±71	782 ±07
Ratio of oxidation rates of [1-^{14}C]- and [16-^{14}C]-palmitate	1.54± 0.40	1.40± 0.19	1.43± 0.16	1.28± 0.19
[1-^{14}C]Palmitate oxidation rate with antimycin and rotenone	184 ±16^{4}	168 ±23^{4}	155 ±19	132 ±15^{1,2}
Ratio of oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate with antimycin and rotenone	4.08± 0.35^{3,4}	3.73± 0.30^{3,4}	3.27± 0.32^{1,2}	3.13± 0.39^{1,2}
Proportion of peroxisomal oxidation	15.7 ± 1.8^{3,4}	15.3 ± 2.9^{3,4}	12.9 ± 1.6^{1,2}	11.3 ± 0.5^{1,2}
Antimycin-sensitive [1-^{14}C]palmitate oxidation rate (mitochondrial oxidation)	701 ±82	638 ±76	668 ±66	653 ±95
Citrate synthase activity	96 ±15	91 ±15	88 ± 6	87 ± 9
Cytochrome c oxidase activity	390 ±38	375 ±35	349 ±29	365 ±17

TABLE V Effect of type of dietary fat on total or antimycin-insensitive palmitate oxidation and mitochondrial enzyme activities in quadriceps homogenates

Values represent mean ± S.D. of 6 animals. Values with superscript 1, 2, 3 or 4 are significantly different from those of group CB, OV, PHSO and PHSOMix, respectively for $P < 0.05$. Other details are given in Table III.

Parameter	EXPERIMENTAL GROUP			
	CB	OV	PHSO	PHSOMix
[1-^{14}C]Palmitate oxidation rate	84 ±14	102 ±15	94 ±18	94 ± 8
Ratio of oxidation rates of [1-^{14}C]- and [16-^{14}C]-palmitate	1.74± 0.25	2.04± 0.88	1.75± 0.34	2.52± 0.74
[1-^{14}C]Palmitate oxidation rate with antimycin and rotenone	19.1 ± 4.1[2]	24.6 ± 3.1[1,4]	21.6 ± 6.2[4]	16.2 ± 0.9 [2,3]
Ratio of oxidation rates of [1-^{14}C]- and [16-^{14}C]palmitate with antimycin and rotenone	3.65± 0.76[4]	3.83± 0.29[4]	3.17± 0.27	2.65± 0.51[1,2]
Proportion of peroxisomal oxidation	16.6 ± 4.2[4]	18.0 ± 2.8[4]	15.6 ± 2.7[4]	10.6 ± 1.4[1,2,3]
Antimycin-sensitive [1-^{14}C]palmitate oxidation rate (mitochondrial oxidation)	66.5 ± 9.5	77.4 ±13.2	77.2 ±12.2	79.5 ± 7.2
Citrate synthase activity	11.5 ± 0.9[2]	14.5 ± 1.8[1,3,4]	11.2 ± 1.8[2]	10.6 ± 1.6[2]
Cytochrome c oxidase activity	52.9 ± 3.9[2,3,4]	69.0 ±11.2[1]	65.0 ± 6.4[1]	68.5 ± 6.4[1]

are for animals sacrificed in the fed state. The animals in the previous study were, however, sacrificed at a younger age (about 70 days) and had lower body and organ weights.

In our present study (with high-fat diets) total oxidation rates of [1-^{14}C]palmitate in liver homogenates were somewhat higher than with the low-fat diet (6), but the antimycin-insensitive oxidation rates were similar or lower. So the peroxisomal contribution to total oxidation was lower in the high-fat-fed animals than in those fed the low-fat diet except for the cocoabutter group in this experiment.

Usually effects of the fat content of diets on peroxisomal β-oxidation activity are assayed with cyanide-insensitive palmitoyl-CoA-dependent NAD^+ reduction in isolated peroxisomal fractions (16-19, 21) or homogenates (15). Total liver capacity for peroxisomal β-oxidation was slightly higher in rats fed a diet containing 15% (w/w) soybean oil than in animals fed a diet containing 5% for 17 days (16, 17), but not after 3 weeks on the diet (19). With partially hydrogenated marine oil the capacity remained similar at 5-15% (w/w) fat but rose at 20-23% with a sigmoidal dose-response relationship (19, 31). A moderate fat diet (30 energy %) of marine oil or rapeseed oil caused a small increase that was transitory with time compared to a standard laboratory pellet (low-fat) diet (21). An eightfold increase was observed in liver homogenates of rats fed 30% (w/w) linol salad oil in comparison to animals fed 6% of this oil (15). Liver acyl-CoA oxidase activity did not differ in rats fed high-fat diets (48 energy %) of beef tallow or corn oil from animals fed diets with 8 energy % fat (32). Therefore a rise in the fat content of the diet was not always found to increase liver peroxisomal β-oxidation capacity; the composition of the fat seems to play an important role as well, as will be discussed later.

Comparison of the results of the present and our previous investigation (6) shows that the capacity of mitrochondrial oxidation (antimycin-sensitive oxidation) in liver homogenates is increased by the higher fat content of the diet (except with cocoabutter). This is due to a higher specific activity of the mitochondria, rather than to an increase of

their number, since mitochondrial marker enzymes did not show a parallel change. This is in accordance with results of Osmundsen (18) who found a higher oxidation rate of palmitoyl-CoA in isolated liver mitochondria of rats fed 15% (w/w) partially hydrogenated marine oil compared to a standard pelleted diet.

A common remarkable effect of the diets in this study was that the ratio of the oxidation rates of $[1\text{-}^{14}C]$- and $[16\text{-}^{14}]$palmitate in liver homogenates was very high compared to that observed with the low-fat diet (> 9 versus 2.5) (6). The ratio is always higher than 1.0 in cell-free systems, which may be explained by an incomplete mitochondrial degradation of the products of peroxisomal chain-shortening (28). Peroxisomal oxidation proceeds only during 2-3 cycles in homogenates (6, 28) and in peroxisomal fractions (18, 33, 34). The marked increase of the ratio by the high-fat diets is caused rather more by the strong decrease of the oxidation rates measured with $[16\text{-}^{14}C]$palmitate than by the increase of the oxidation rate of $[1\text{-}^{14}C]$palmitate. Addition of antimycin and rotenone to the homogenate did not increase the ratio in the present study, in contrast to the ratio found in the previous study with the low-fat diet. Therefore increase of the dietary fat content appears to increase the mitochondrial oxidation of palmitate molecules but also gives rise to an additional incomplete oxidation of palmitate. Possibly intermediary products of β-oxidation either accumulate as acid-insoluble carnitine esters or are used for biosynthesis of lipids.

In heart homogenates we presently obtained similar total oxidation rates of $[1\text{-}^{14}C]$palmitate as with the low-fat diet (6). The increase of the fat content resulted in a comparable or lower antimycin-insensitive oxidation rate. The proportion of peroxisomal oxidation of the total oxidation was not influenced by the fat content of the diet. Norseth et al. (22) found an increased (micro-)peroxisomal β-oxidation activity by raising the content of partially hydrogenated marine oil (> 30% C_{20}, C_{22} fatty acids) in the diet, as assayed by cyanide-insensitive palmitoyl-CoA-dependent NAD^+ reduction. This technique gave markedly lower values for peroxisomal oxidation activity than ours.

Mitochondrial oxidative capacity did not change in heart homogenates when increasing the fat content of the diet as is apparent from comparison of the results of this study with those of the previous one (6). The same applies for the mitochondrial oxidation capacity divided by the activity of the mitochondrial marker enzymes (citrate synthase and cytochrome c oxidase). No literature data are available on the influence of the fat content of the diet on mitochondrial β-oxidative capacity in the heart.

The lower total and mitochondrial oxidation rates in muscle homogenates of the animals fed the high-fat diets compared to the low-fat diet (6) may relate to their age at sacrifice and not to the fat content of the diet, since palmitate oxidation capacity per gram muscle has a tendency to decrease with age in adolescent rats (35, 36) in contrast to the capacity in heart (6, 35) and liver (6).

In conclusion it is apparent that in our experiments the fat content of the diet only influences the mitochondrial β-oxidation activity in liver. The differences in results between our experiments and those described in the literature may be explained by the fatty acid composition of the diets, as will be discussed later. In the heart and muscle the effect of a rise in fat content is absent or small.

To investigate effects of dietary fatty acids on the peroxisomal and mitochondrial fatty acid oxidation, the present investigation was performed with four different fat blends. The oils and fats used to compose these fat blends were all of vegetable origin and did not contain significant amounts of C_{20}, C_{22} fatty acids.

The total [1-^{14}C]palmitate oxidation rate in the liver did not differ among the four groups. The antimycin-insensitive oxidation rate and the proportion of peroxisomal of total oxidation were only significantly higher in the cocoabutter group. The mixed-fat and the partially hydrogenated soybean oil diets (containing 10 and 20 energy % *trans* fatty acids, respectively) did not lead to an increase of peroxisomal oxidation capacity in liver when compared to the other diets. The former was even found to have the lowest capacity. In contrast, other authors found that feeding partially hydrogenated marine oil or rapeseed oil

induced a higher peroxisomal activity in rat liver than feeding the corresponding unhydrogenated oils (19). Unhydrogenated marine oil and rapeseed oil caused a higher activity than soybean oil. Hydrogenation of soybean oil increased the peroxisomal activity per mg peroxisomal protein, but not when expressed per gram liver (19). The mitochondrial palmitate oxidation relative to the total oxidation and its specific activity were only significantly increased by the mixed-fat diet compared to the other diets. Oxidative activities of rat liver mitochondria with glutamate or pyruvate as substrates are generally not affected by changes in dietary fat (37). Liver mitochondria from rats fed 15% partially hydrogenated marine oil however showed a higher oxygen consumption with palmitoyl-CoA than from rats fed 5% soybean oil (17).
In heart and muscle homogenates the parameters investigated were influenced by the dietary fats in a similar way as in the liver homogenates. Again the most striking effects were observed with the mixed-fat diet (PHSOMix), the contribution of peroxisomal oxidation to total oxidation having the lowest value. The partially hydrogenated soybean oil diet caused also lower values for this parameter than the olive oil and cocoabutter diets (significantly only in the heart). Norseth and Thomassen (22) found that rapeseed oil or marine oil induced considerable (micro-)peroxisomal β-oxidation activity in hearts of rats, compared to soybean oil. Feeding the hydrogenated forms of these oils resulted in a lower activity than feeding the corresponding unhydrogenated oils. We did not find a significant difference in mitochondrial oxidative capacity in heart and muscle between the groups. However, dietary fats may influence the activity of rat heart mitochondria (25, 38, 39). The respiratory rate with acyl-carnitine esters as substrate was lower after feeding of rapeseed oil or partially hydrogenated marine oil than after corn or peanut oil (25, 39). The observed changes were related to changes in mitochondrial lipid composition.
From literature data it can be concluded that all $C_{22:1}$ fatty acid-containing diets (marine or rapeseed oil; partially hydrogenated or not) increase peroxisomal β-oxidation activity in liver (16-21, 23), heart

(22, 24) and intestine (40), in comparison to soybean oil or peanut oil. The effect of hydrogenation of these oils is different on liver and heart peroxisomal activity (19, 22). Combination of the above mentioned literature data with our results on other oils suggests that high-fat diets only induce peroxisomal β-oxidation activity if they contain *cis* or *trans* C_{20}, C_{22} fatty acids. If only *trans* fatty acids with 18 C-atoms (as present in partially hydrogenated soybean oil) occur in the diet, this does not result in a higher peroxisomal activity compared to diets containing saturated or *cis*-mono-unsaturated long-chain fatty acids.

ACKNOWLEDGEMENTS

The authors wish to thank Mr H.T.B. van Moerkerk for the experimental assistance and Mr R.N. Lussenburg for the statistical analyses. Animal care was supervised by Mr J.S.W. Kleinekoort.

REFERENCES

1. Lazarow, P.B. and De Duve, C. (1976) Proc. Natl. Acad. Sci. USA 73, 2043-2046.

2. Mannaerts, G.P., Debeer, L., Thomas, J. and De Schepper, P.J. (1979) J. Biol. Chem. 254, 4585-4595.

3. Walusimbi-Kisitu, M. and Harrison, E.H. (1983) J. Lipid Res. 24, 1077-1084.

4. Masters, C. and Crane, D. (1985) Mol. Cell. Biochem. 65, 23-35.

5. Van Veldhoven, P. and Mannaerts, G.P. (1985) Biochem. J. 227, 737-741.

6. Veerkamp, J.H. and Van Moerkerk, H.T.B. (1986) Biochem. Biophys. Acta, in press.

7. Lazarow, P.B. (1978) in Metabolic Compartmentation (Sies, H., ed.) pp. 317-329, Academic Press, New York.

8. Hashimoto, T. (1982) Ann. N.Y. Acad. Sci. 386, 5-12.

9. Lazarow, P.B. (1981) Methods Enzymol. 72, 315-319.

10. Lazarow, P.B. (1982) J. Biol. Chem. 23, 317-326.

11. Bremer, J., Osmundsen, H., Christiansen, R.Z. and Borrebaek, B. (1981) Methods Enzymol. 72, 506-519.

12. Christiansen, R.Z. (1978) Biochim. Biophys. Acta 530, 314-324.

13. Norseth, J. (1980) Biochim. Biophys. Acta 617, 183-191.

14. Osmundsen, H., Neat, C.E. and Norum, K.R. (1979) FEBS Lett. 99, 292-296.

15. Ishii, H., Fukumori, N., Horie, S. and Suga, T. (1980) Biochim. Biophys. Acta 617, 1-11.

16. Neat, C.E., Thomassen, M.S. and Osmundsen, H. (1980) Biochim. J. 186, 369-371.

17. Neat, C.E., Thomassen, M.S. and Osmundsen, H. (1981) Biochim. J. 196, 149-159.

18. Osmundsen, H. (1982) Ann. N.Y. Acad. Sci. 386, 13-27.

19. Thomassen, M.S., Christiansen, E.N. and Norum, K.R. (1982) Biochem. J. 206, 195-202.

20. Bremer, J. and Norum, K.R. (1982) J. Lipid Res. 23, 243-256.

21. Flatmark, T., Christiansen, E.N. and Kryvi, H. (1983) Biochim. Biophys. Acta 753, 460-466.

22. Norseth, J. and Thomassen, M.S. (1983) Biochim. Biophys. Acta 751, 312-320.

23. Christiansen, R.Z., Christiansen, E.N. and Bremer, J. (1979) Biochim. Biophys. Acta 573, 417-429.

24. Norseth, J. (1979) Biochim. Biophys. Acta 575, 1-9.

25. Hsu, C.M.L. and Kummerow, F.A. (1977) Lipids 12, 486-494.

26. Lawson, L.D. and Kummerow, F.A. (1979) Biochim. Biophys. Acta 573, 245-254.

27. Lawson, L.D. and Holman, R.T. (1981) Biochim. Biophys. Acta 665, 60-65.

28. Veerkamp, J.H., Van Moerkerk, H.T.B., Glatz, J.F.C. and Van Hinsbergh, V.W.M. (1983) Biochim. Biophys. Acta 753, 399-410.

29. Shepherd, D. and Garland, P.B. (1969) Methods Enzymol. 13, 11-16.

30. Van Hinsbergen, V.W.M., Veerkamp, J.H. and Van Moerkerk, H.T.B. (1978) Arch. Biochem. Biophys. 190, 762-771.

31. Berge, R.K. and Thomassen, M.S. (1980) Lipids 20, 49-52.

32. Lawson, N., Jennings, R.J., Pollard, A.D., Sturton, R.G., Ralph, S.J., Marsden, C.A., Fears, R. and Brindley, D.N. (1981) Biochem. J. 200, 265-273.

33. Thomas, J., Debeer, L.J., De Schepper, P.J. and Mannaerts, G.P. (1980) Biochem. J. 190, 485-494.

34. Osmundsen, H., Neat, C.E. and Borrebaek, B. (1980) Int. J. Biochem. 12, 625-630.

35. Glatz, J.F.C. and Veerkamp, J.H. (1982) Biochim. Biophys. Acta 711, 327-335.

36. Zuurveld, J.G.E.M., Wirtz, P., Loermans, H.M.T. and Veerkamp, J.H. (1985) Cell Tissue Res. 241, 183-192.

37. De Schrijver, R. and Privett, O.S. (1984) J. Nutr. 114, 1183-1191.

38. Houtsmuller, U.M.T., Struijk, C.B. and Van Der Beek, A. (1970) Biochim. Biophys. Acta 218, 564-566.

39. Blomstrand, R. and Svensson, L. (1983) Lipids 18, 151-170.

40. Thomassen, M.S., Helgerud, P. and Norum, K.R. (1985) Biochem. J. 255, 301-306.

41. Lanza, E. and Slover, H.T. (1981) Lipids 16, 260-267.

Chapter 6.

EFFECT OF DIETARY FATS ON LINOLEIC ACID METABOLISM. RADIOLABEL STUDIES IN RATS

a) J.L. Zevenbergen
Relationship between arachidonic acid biosynthesis and its level in rat tissues
In: Enzymes of Lipid Metabolism II. (L. Freysz, H. Dreyfus, R. Massarelli and S. Gatt, eds.), pp. 647-649, Plenum Publishing Corporation, 1986.

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b) J.L. Zevenbergen and U.M.T. Houtsmuller
Effect of dietary fats on linoleic acid metabolism.
A radio-label study in rats
Accepted for publication by Biochimica Biophysica Acta.

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a. RELATIONSHIP BETWEEN ARACHIDONIC ACID BIOSYNTHESIS AND ITS LEVEL IN RAT TISSUES

INTRODUCTION

Arachidonic acid is the precursor of many hormone-like substances such as prostaglandins and leukotrienes and therefore plays an important role in many physiological processes. Arachidonic acid is synthesized from linoleic acid by the action of desaturase and elongation enzyme systems. The influence of dietary fats on the biosynthesis of arachidonic acid and its levels in rat tissues is studied.

METHODS

Three groups of four, weanling, male Wistar rats were fed semi-synthetic diets containing 40% of energy as (en%) fat with 2en% linoleic acid. The dietary fats comprised a mixture of hydrogenated coconut-oil and olive oil, (HCNO/OV), a pure olive oil (OV) or a partially hydrogenated soybean oil (PHSO). The fatty acid composition of the experimental fats is depicted in Fig. 1. After 7 weeks the rats were

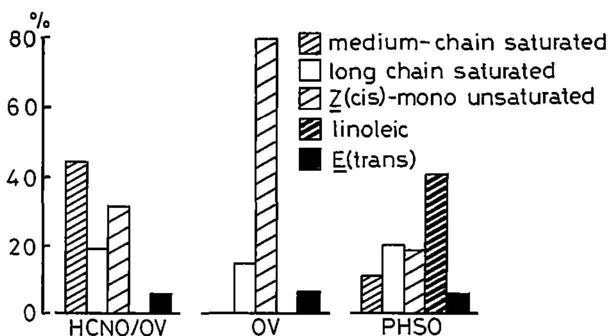

Fig. 1 Fatty acid composition (w/w%) of the experimental fats

fasted overnight and 100 μg [1-^{14}C] linoleic acid (20 μC), dissolved in 0.2 ml of the dietary oil, was administered to the rats via stomach tube. The rats were left with food and water ad-libitum and were bled under light ether anaesthesia 24 h after the administration of the labelled linoleic acid. The total lipids of liver and heart were extracted and transesterified. The methylesters were separated by $AgNO_3$-TLC and the fractions were counted for radioactivity. The fatty acid composition of the total lipids of the liver and heart was determined by GLC.

RESULTS

The type of dietary fat induced appreciable differences in the <u>ratio</u> of labelled arachidonic- and linoleic acid in both liver and heart (Table 1 and 2), indicating an effect of the dietary fats on the rate of synthesis of arachidonic acid.

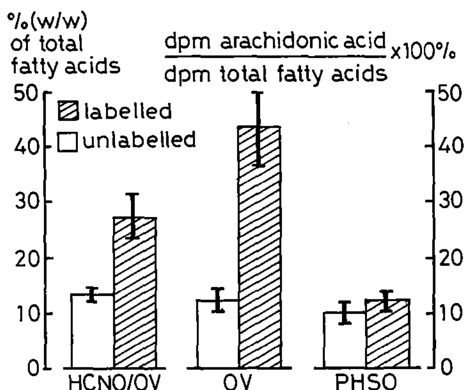

Fig. 2 Distribution of arachidonic acid in the liver (n=4)

Table 1 Distribution of labelled fatty acids in the total lipids of the liver

Each value represents the percentage (mean ± SD; n = 4) of the radioactivity recovered in the fatty acids from total lipids of the liver.

fatty acid	experimental groups HCNO/OV	OV	PHSO
saturated + 18:1	1.1±0.3	2.4±0.6	1.3±0.5
18:2	59.0±4.1	42.6±7.6	75.0±1.5
18:3 + 20:3	9.6±0.6	8.7±1.5	9.6±1.1
20:4	27.4±4.3	43.4±7.4	12.0±1.8
20:5 + 22:5 + 22:6	1.3±0.1	1.8±1.1	0.4±0.1

Table 2 Distribution of labelled fatty acids in the total lipids of the heart

Each value represents the percentage (mean ± SD; n=4) of the radioactivity recovered in the fatty acids from total lipids of the heart.

fatty acid	experimental groups HCNO/OV	OV	PHSO
saturated + 18:1	0.5±0.2	1.3±0.6	0.3±0.3
18:2	86.2±3.4	82.4±3.2	94.6±2.1
18:3 + 20:3	2.1±0.4	2.2±0.2	1.3±0.2
20:4	8.7±2.2	12.8±3.1	2.2±0.5
20:5 + 22:5 + 22:6	1.4±0.7	0.6±0.1	not detect.

However, the level (w/w%) of arachidonic acid in total lipids of heart and liver, as determined by GLC, varied much less and, moreover, these variations did not parallel the differences in the ratio of the radioactivity of the fatty acids (Fig. 2). For linoleic acid the specific activity in liver and heart is equal for the three groups (Fig. 3). Thus differences in biosynthetic rate of arachidonic acid are not reflected in the level of this fatty acid in the tissue lipids.

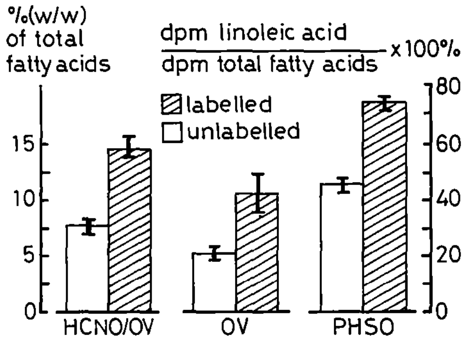

Fig. 3 Distribution of linoleic acid in the liver (n=4)

DISCUSSION

Although it is repeatedly assumed that inhibition of the enzymic conversions of polyunsaturated fatty acids - desaturation as well as chain elongation - show up more clearly in changes of the fatty acid pattern of phospholipids than in in vitro assays of desaturases, the present experiment does not support this assumption. It appears that the fatty acid compositions of membrane lipids is regulated by the selective incorporation of particular fatty acids rather than by the availability of substrates generated by desaturation and chain elongation. It is concluded that effects of dietary fats on the rate of metabolism of essential fatty acids cannot be deduced from changes in fatty acid patterns of tissue lipids alone.

b) EFFECT OF DIETARY FATS ON LINOLEIC ACID METABOLISM. A RADIO-LABEL STUDY IN RATS

Abstract

Effects on the linoleic acid metabolism *in vivo* of three dietary fats, rich in either oleic acid, *trans* fatty acids or α-linolenic acid, and all with the same linoleic acid content, were investigated in male Wistar rats. After 6 weeks of feeding, the rats were intubated with [1-^{14}C]linoleic acid and [^{3}H]oleic acid. The incorporation of these radio-labels into liver, heart and serum was investigated 2, 4, 8, 24, and 48 h after intubation. The amount of [^{14}C]labelled arachidonic acid incorporated into the liver phospholipid of the group fed the oleic acid-rich diet was significantly higher than that of the other groups. However, compared to the *trans* fatty acids-containing diet, the oleic acid-rich diet induced only a slightly higher arachidonic acid level in the phospholipid fraction of the tissues as determined by GLC. Dietary α-linolenic acid more than halved the arachidonic acid levels. The specific activity of arachidonic acid was much higher in the OV-fed group, whereas that of linoleic acid or γ-18:3 + 20:3 was hardly influenced by the dietary fats. This suggests that these dietary fats do not affect the arachidonic acid synthesis during the first steps of the linoleic acid conversion (Δ6-desaturation/elongation), but during events taking place after the formation of 20:3. The influence of dietary fats on linoleic acid metabolism cannot be simply deduced from fatty acid patterns of organ lipids.

Introduction

It is well established that the type of dietary fat can influence linoleic acid metabolism. This is evident from changes in the fatty acid composition of tissues, but it can also be shown *in vitro* by measuring the direct conversion of linoleic acid into other n-6 polyunsaturated fatty acids (PUFAs). Both methods, however, do not give reliable information on the way linoleic acid metabolism is regulated in the live animal. With the aid of radiolabelled linoleic acid, insight can be obtained into the fluxes of linoleic acid and its metabolites in the live animal under various dietary or other circumstances.

Most investigations on the effects of dietary fats on linoleic acid metabolism focused on polyunsaturated fatty acids: the effect of the amount of dietary linoleic acid itself or the influence of other PUFAs (e.g. α-linolenic acid). All PUFAs are alleged to inhibit the conversion of linoleic acid into other polyunsaturated fatty acids, as measured *in vitro* [1-3] or in liver microsomes after feeding the animals PUFAs [4-7]. As a result, the fatty acid composition of tissues of animals fed α-linolenic acid or other PUFAs will be altered [7,8-13].

In a previous experiment we used [^{14}C]labelled dietary linoleic acid to analyze effects of three types of fat, all with the same amount of PUFA (2 en% linoleic acid) [14]. Surprisingly, it was found that the type of fat could drastically influence the amount of [^{14}C]labelled arachidonic acid in the tissues. Although no irrefutable proof was found, these differences in the labelling of arachidonic acid were very likely to reflect differences in rate of synthesis of arachidonic acid. In this experiment, the three dietary fats consisted mainly of olive oil, a mixture of hydrogenated coconut oil and olive oil, and a partially hydrogenated soyabean oil. Of these fats, olive oil stimulated the arachidonic acid labelling almost threefold compared to partially hydrogenated soyabean oil. Despite this considerable difference in apparent synthesis, the level of arachidonic acid in the liver and heart did not show a comparable change.

The above experiment raised a few important questions. As we analyzed the distribution of [^{14}C]labelled arachidonic acid over the fatty acids of total liver lipids at only one point in time (24 h), no information could be gained on the changes in arachidonic acid synthesis in the course of time. Moreover, separate analysis of the various lipid classes would yield more precise information. Finally the use of a radio-labelled reference fatty acid (e.g. [^{3}H]oleic acid) would give the opportunity to compare linoleic acid metabolism with that of a non-essential unsaturated fatty acid.

Basically the same methodology as above was chosen for the present experiment. The set-up was more elaborate, however. Firstly, an oil that contained α-linolenic acid - a potent inhibitor of arachidonic acid biosynthesis - was included next to olive oil and the partially hydrogenated soyabean oil. Secondly, [^{3}H]oleic acid was intubated together with [^{14}C]linoleic acid. Thirdly, 2, 4, 8, 24 and 48 h after intubation a number of animals of each group were sacrificed.
Lipids of liver, serum and heart were isolated and the incorporation of ^{14}C and ^{3}H was analyzed. The fatty acids of the phospholipid fraction were analyzed for radioactivity. Finally, the fatty acid composition of liver, heart and serum phospholipid was determined by GLC.

Materials and Methods

Materials

[1-^{14}C]labelled linoleic acid (specific activity 56 Ci/mol) and [9,10-^{3}H]labelled oleic acid (s.a. 5.7 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, England. Methylesters for GLC standards were obtained from Supelco Inc., Bellefonte, PA, U.S.A. and methylesters for $AgNO_3$-TLC were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. The TLC plates were pre-fabricated plates from Merck, Darmstadt, FRG. All solvents and other chemicals were reagent grade.

Feeding study

Male, newly weaned Wistar rats, obtained from the Central Breeding Station, T.N.O., Zeist, The Netherlands, were divided in three groups of 25 animals and randomly allocated to one of the three dietary treatments. The rats were housed individually and had free access to food and water. The animals were fed semi-synthetic diets, with 40% of energy as fat and maize starch as the main carbohydrate. Composition of the total diet ($g.MJ^{-1}$): casein 14.8; vitamin mixture 0.2; salt mixture 1.3; cellulose 3.8; maize starch 25.2; experimental fat 10.3. The experimental fats were mixtures of commercially available fats and oils (basically a low-linoleic acid olive oil, and a mixture of linseed oil with cocoabutter and fully hydrogenated coconut oil) or a specially prepared partially hydrogenated soyabean oil. They were composed in such a way that all diets contained approx. 2% of energy as linoleic acid, but differed considerably in fatty acid composition. The fatty acid composition of the dietary fats is given in Table I.

After six weeks the animals were transferred to the radiochemical laboratory and put in plastic Macrolon boxes. Their weight was ca. 310 g at that time. They were left for 48 h to acclimatise, after which the food was removed for 18 h in order to empty the stomach of the animals. In the morning around 10 am. 0.2 ml of the dietary oils (slightly heated to prevent solidification) containing 20 μCi [1-^{14}C]linoleic acid and 50 μCi [9,10-^{3}H]oleic acid was administered to the rats via stomach intubation. The rats to be sacrificed later than 4 h after the intubation were provided again with food. Water was given to all rats. After 2, 4, 8, 24 and 48 h four rats of each group were sacrificed each time by aorta cannulation under ether anaesthesia.

Blood was collected and allowed to clot for serum preparation. The liver and heart were weighed and rapidly frozen in liquid nitrogen. The organs were kept at -20°C under nitrogen until analysis.

TABLE I

FATTY ACID COMPOSITION OF THE EXPERIMENTAL FATS

The composition is given in % (w/w). The fatty acid composition was determined by GLC and $AgNO_3$-TLC. The dietary fats were composed of a low linoleic acid olive oil (OV), a mixture of a partially hydrogenated soybean oil, coconut oil and sunflowerseed oil (PHSO) and a mixture of linseed oil, cocoabutter and coconut oil (LN/CB/CN) and fed at 40% of energy.

Type of fatty acid[a]	Experimental fats		
	OV	PHSO	LN/CB/CN
6:0	-	-	0.1
8:0	-	0.8	2.4
10:0	-	0.7	2.1
12:0	0.1	5.6	17.2
14:0	-	2.4	6.7
16:0	11.9	10.0	14.3
16:1	-	0.3	-
17:0	-	0.1	0.1
17:1	0.1	0.1	-
18:0	2.0	8.0	18.4
18:1 *c*	78.7	17.5	19.2
18:1 *t*	-	39.1	-
18:2 9*c*,12*c*	5.5	5.3	5.4
18:2 9,12*ct*,*tc*	-	0.6	-
18:2 9*t*,12*t*	-	0.5	-
18:2 ot	-	7.7	-
18:3	0.6	-	12.9
20:0	0.4	0.5	0.5
20:1	0.4	0.5[b]	0.1
22:0	0.2	0.4	0.1
sum	99.9	100.1	99.5

a The shorthand notation used for the fatty acids indicates chainlength: number of double bonds.
c=*cis* isomers, *t*=*trans* isomers, ot=other (non-9,12) isomers

b Also contains conjugated 18:2 isomers

\- not detected

Lipid analyses

Lipids were extracted from about 0.8 g liver and 1.5 g heart, according to Bligh and Dyer [15]. Butyrylhydroxytoluene (BHT) was added as an anti-oxidant. The lipid classes were separated on TLC, essentially according to Christie [16]. After transmethylation by methanolic HCl during 2 h at 65°C, the methylesters were extracted with heptane/ether and purified on TLC.

To determine the conversion of the labelled fatty acids into other fatty acids, the methylesters of the lipid classes were separated on $AgNO_3$-TLC. Merck DC Fertigplatten Kieselgel 60 were dipped in 12% $AgNO_3$ in methanol/ water (2/1) for 30 s. The plates were dried for 2 h at 70°C in the dark and stored in an aluminium box. Fatty acid methylesters were applied and the plates were developed in the dark as follows: a) for 10 min. in toluene and a 5:1 ethylacetate/acetic acid-mixture (40:3); b) about 30 min in the same solvent but with a liner of 5 cm; c) about 75 min in toluene and ethylacetate (40:3) with a liner of 10 cm. The methylesters were visualised after spraying with 2',7'-dichlorofluores-ceine (0.5% in methanol) under ultra-violet light (350 nm). The bands were scraped off into counting vials and 0.5 ml 2.5% oxalic acid and 10 ml emulsifier/scintillator 299TM (United Technologies Packard, Downers Grove, IL, U.S.A.) were added. The samples were counted for ^{14}C and ^{3}H in a liquid scintillation counter (United Technologies Packard A460C). Fatty acid composition was determined by GLC on a Varian 3700 GLC with a DEGS-packed column under standard conditions. All fatty acid compositions given represent the mean of four animals per group.

Determination of incorporation of ^{3}H and ^{14}C into whole organs

Immediately after sacrifice, pieces of liver and heart (ca. 300 mg) were incinerated in a Packard Oxidiser 306 (United Technologies Packard, Downers Grove, IL, U.S.A.). CO_2 and H_2O were automatically collected and radioactivity was counted in a liquid scintillation counter as described above.

Results

Liver weights

The liver weights of the animals after sacrifice are depicted in Fig.1. Fasting of the animals clearly decreased liver weight, as is evident from the low liver weights at 2 and 4 h (after fasting for 18 h). The liver weights of the animals that were left after the intubation for more than 4 h rapidly increased due to re-feeding. The liver weights reached their maximum 24 h after the intubation and were higher than in the steady state reached after 48 h (over-compensation). There is no systematic influence of the diet on liver weight.

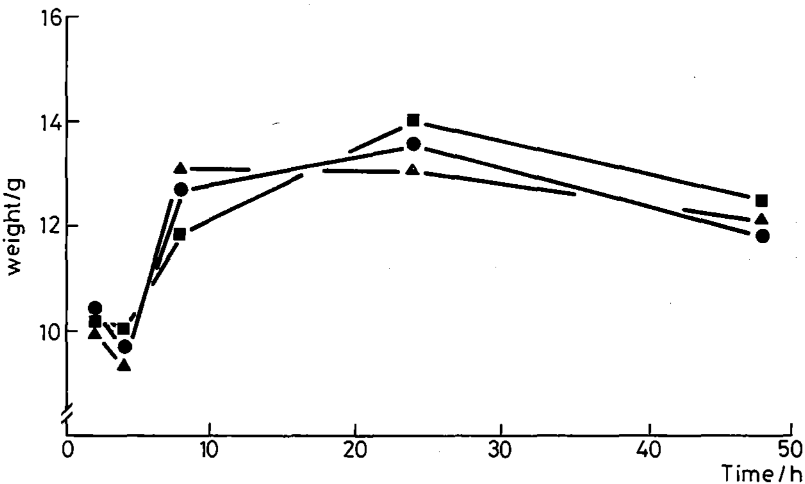

Fig. 1. Liver weights at 2, 4, 8, 24 and 48 h after intubation of [^{14}C] and [^{3}H]labelled fatty acids. After 3 months of feeding different dietary fats (■-OV, ●-PHSO,▲- LN/CB/CN), 18 h fasted male rats were intubated with a small amount of fat (t=0 h). The animals sacrificed more than 4 h after the intubation were provided again with food ad libitum. At the times indicated the animals were sacrificed, then the liver was quickly removed and weighed. The values represent the mean of four animals per group

Incorporation of ^{3}H and ^{14}C in total serum and serum lipid classes

The labelled fatty acids, [^{14}C]linoleic acid and [^{3}H]oleic acid, rapidly appear in the serum of all groups (Fig. 2). A maximum level of these fatty acids in serum is already reached within 2 h after the intubation. In both cases the olive oil (OV)-diet gave the highest incorporation, and the linseed/cocoabutter/coconut oil (LN/CB/CN)-diet the lowest. This could be an indication for a better uptake of labelled fatty acids from the gastro-intestinal tract in the first group, but also for a decreased removal from the serum. This decrease proceeds rapidly and without much difference in all groups.

Although we did not quantitate the amount of ^{3}H and ^{14}C in water, water-soluble or lipid like material, substantial amounts may have been present in a non-lipid-phase after 24 and 48 h. The ratio of $^{3}H/^{14}C$ is at first very similar to that in the original diet (2.5), but it increases in time.

24 h after the intubation, ^{14}C in serum was preferentially incorporated into the phospholipids (Fig. 3), with cholesterolesters as the second important pool. Compared to both other groups, the LN/CB/CN-group shows a slightly lower incorporation into most lipid classes, except for the cholesterolester fraction. ^{3}H, however, is almost equally divided over the phospholipids and free fatty acids. The total incorporation of ^{3}H into serum was highest in the OV-group and lowest in the LN/CB/CN-group; this is also evident in the phospholipid and free-fatty-acid fraction.

The ratio of $^{3}H/^{14}C$ clearly shows that in the free-fatty-acid fraction, [^{3}H]labelled fatty acids dominated over [^{14}C]labelled fatty acids, while the phospholipid fraction and the cholesterolester fraction predominantly carried [1-^{14}C] linoleic acid or its metabolites.

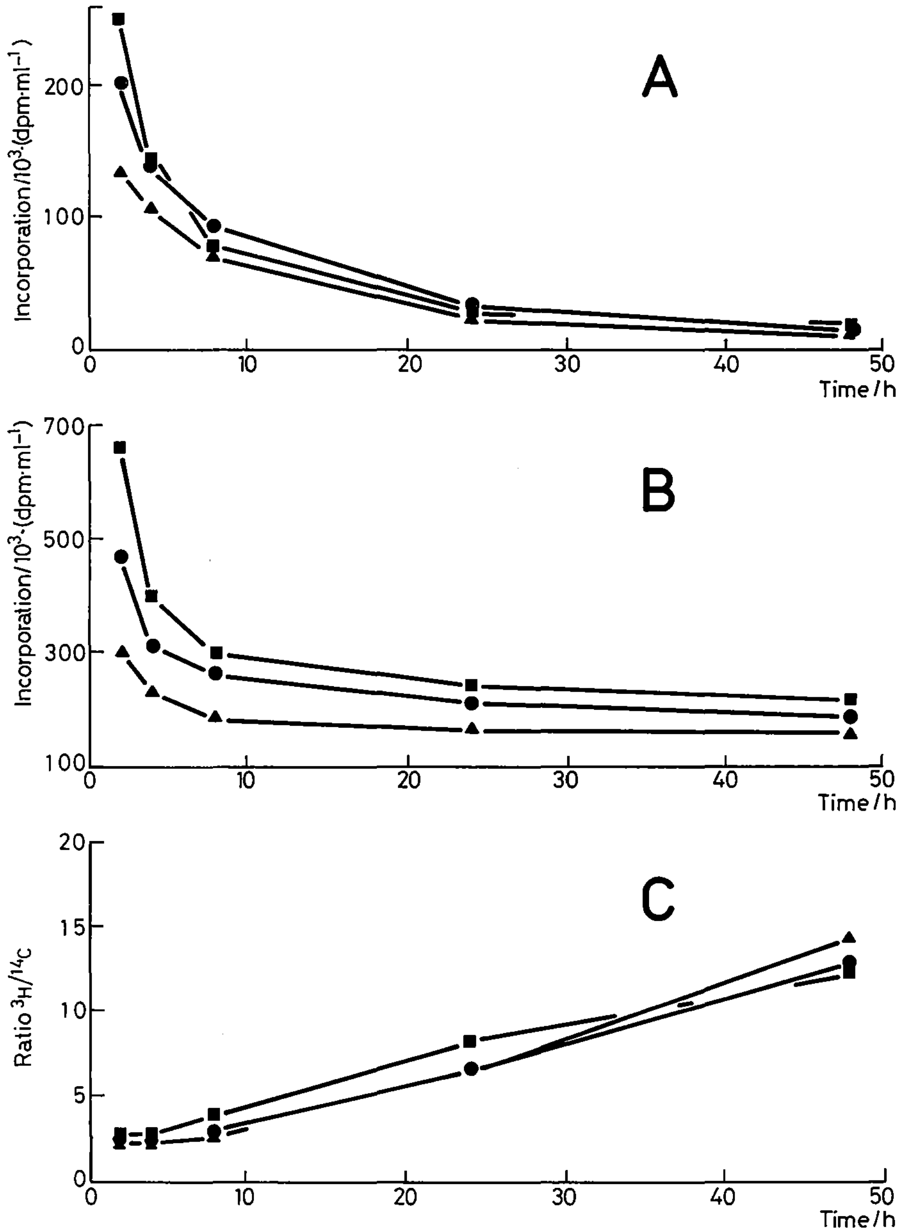

Fig. 2. Incorporation of ^{14}C (A) and ^{3}H (B) in total serum (dpm/ml) and the ratio of $^{3}H/^{14}C$ (C). Male rats were fed diets with three different dietary fats(■-OV,●-PHSO,▲-LN/CB/CN) for 3 months. 2,4,8,24 and 48 h after intubation of 20 μCi [1-^{14}C]linoleic acid and 50 μCi [^{3}H]oleic acid in a small dose of dietary fat, the animals were bled via aorta cannulation and serum was prepared. The radioactivity in serum was counted with a liquid scintillation counter. All values are means of four animals per group

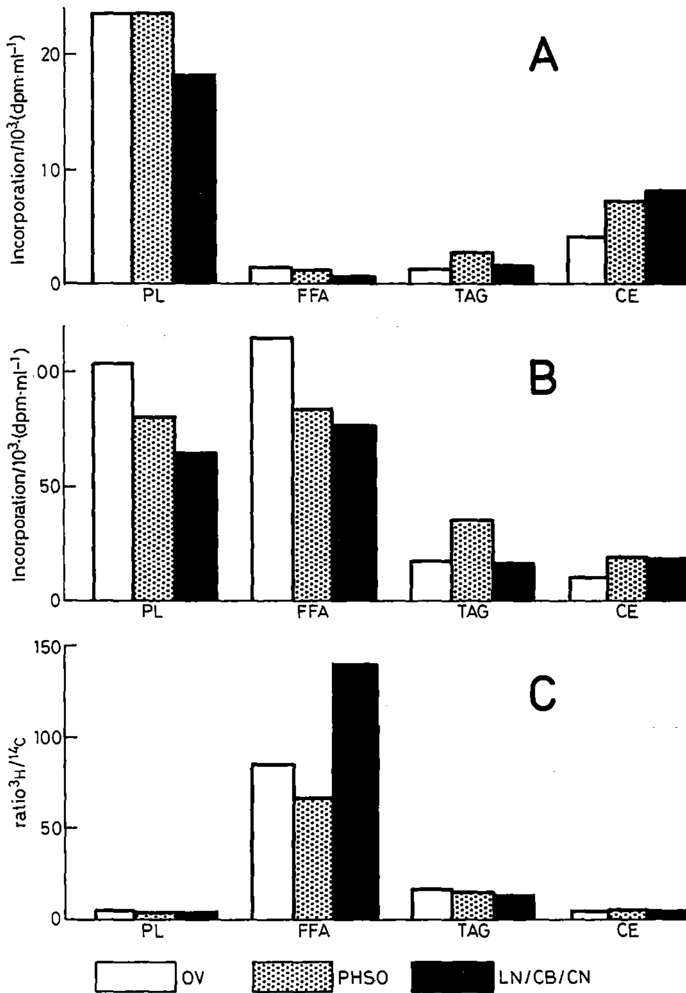

Fig. 3. Distribution of ^{14}C (A) and ^{3}H (B) over serum lipid classes (dpm/ml) and the ratio of $^{3}H/^{14}C$ (C) in the lipid classes. Male rats were fed diets with three different dietary fats (OV, PHSO, LN/CB/CN) for 3 months. 2,4,8,24 and 48 h after intubation of 20 μCi [1-^{14}C]linoleic acid and 50 μCi [3H]oleic acid in a small dose of dietary fat, the animals were bled via aorta cannulation and serum was prepared. Lipids of serum obtained 24 h after the intubation were extracted and lipid classes were separated on TLC. The radioactivity in the lipid classes (PL = phospholipids; FFA = free fatty acids; TAG = triacylglycerol; CE = cholesterolesters) was counted with a liquid scintillation counter. All values are means of four animals per group

TABLE II

FATTY ACID COMPOSITION OF THE SERUM PHOSPHOLIPID FRACTION

The composition is given in % (w/w). The serum phospholipid fraction was isolated by TLC. The fatty acid composition was determined by GLC of individual samples. The values given represent the mean of four animals per group.

Type of fatty acid[a]	Groups		
	OV	PHSO	LN/CB/CN
12:0	-	-	-
14:0	0.1	0.2	-
16:0	20.6	15.2	22.7
16:1n-7	0.8	1.5	0.6
18:0	19.4	16.4	24.8
18:1 [b]	19.9	24.2	9.0
18:2 [b]	12.0	19.4	18.5
18:3n-6	-	-	-
18:3n-3	0.3	-	2.5
20:1n-9	-	-	-
20:2n-9	0.7	0.3	-
20:3n-6	0.6	1.9	0.9
20:4n-6	17.7	13.1	6.7
20:5n-3	0.9	1.1	3.1
22:4n-6	0.5	0.8	-
22:5n-6	1.0	1.9	-
22:5n-3	-	0.1	2.4
22:6n-3	3.8	2.3	6.9
sum	98.3	99.4	97.5

a The shorthand notation used for the fatty acids indicates chainlength: number of double bonds

b Both *cis* and *trans* isomers

\- not detected

Fatty acid composition of serum phospholipid

The serum phospholipid fraction shows a relatively low incorporation of linoleic acid into the OV-group (Table II), while partially hydrogenated soybean oil (PHSO) induced the highest linoleic acid level. The levels of n-3 fatty acids were clearly increased by the LN/CB/CN-diet, which was coupled with a low arachidonic acid content. The PHSO-diet also lowered arachidonic acid level compared to the OV-diet. We did not investigate the levels of *trans* isomers but Moore et al. [17] reported no *trans* 18:2 isomers in serum phospholipids of rats fed *trans* fatty acids. The 18:1 fraction probably contains a substantial amount of *trans* 18:1 [17].

Incorporation of ^{3}H and ^{14}C into total liver and liver lipid classes

Both labelled fatty acids ([1-^{14}C]linoleic acid and [^{3}H]oleic acid) were readily incorporated into the liver (Fig. 4). The maximum incorporation was reached within 4 h (the values for 2 h were not determined). The intubated amount of [1-^{14}C]linoleic acid and [^{3}H] oleic acid was ca. 44 * 10^{6} dpm and 110 * 10^{6} dpm respectively. After 4 h the liver took up approximately 22% and 12% of [1-^{14}C]linoleic acid and [^{3}H]oleic acid respectively. Then the levels of ^{14}C and ^{3}H decreased rapidly in time, indicating that the export and/or oxidation of labelled fatty acids is higher than the uptake. The differences between the groups are small, except for a higher content of ^{3}H in the livers of the OV-fed animals, 1 and 2 days after the intubation.

The ratio of ^{3}H/^{14}C in the liver was considerably lower than that in serum, indicating a preferential transport of linoleic acid to the liver. This effect was strongest in the PHSO-group.

The incorporation patterns of labelled fatty acids in the liver lipid extract were similar to those of the total liver (data not shown). In all groups the amount of ^{3}H and ^{14}C in the lipid extract was lower at all times compared to that in total liver, indicating that part of the label was present in the non-lipid phase of the liver (water and solid

Fig.

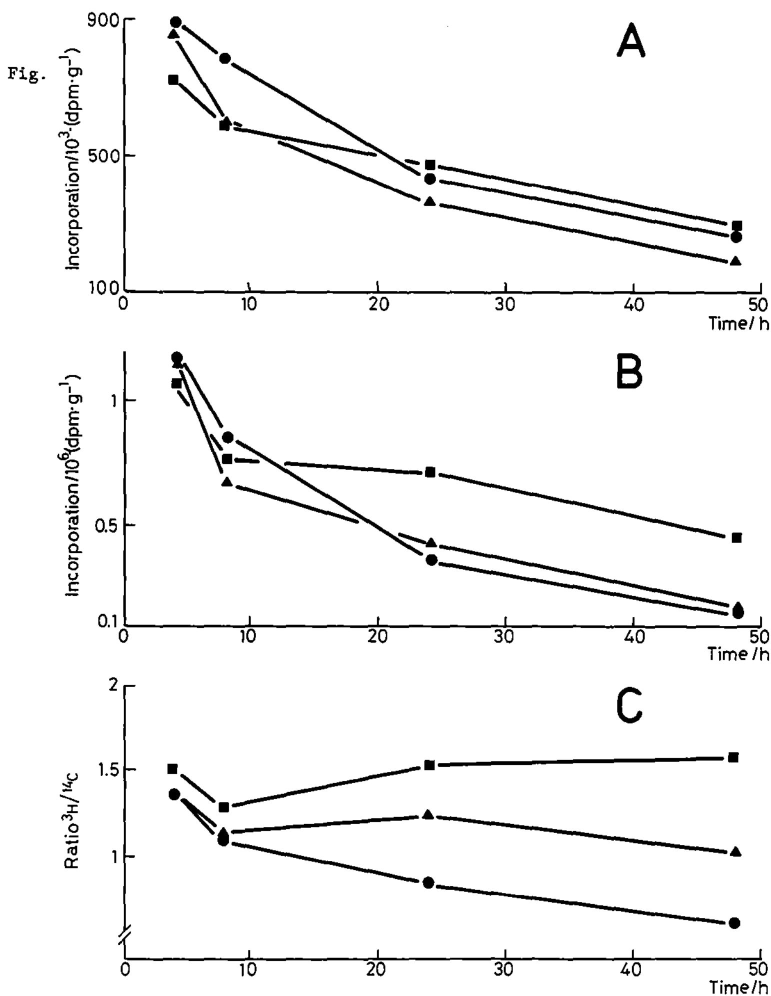

Fig. 4. Incorporation of ^{14}C (A) and ^{3}H (B) in total liver (dpm per g wet tissue) and the ratio of $^{3}H/^{14}C$ (C). Male rats were fed diets with three different dietary fats(■-OV,●-PHSO,▲-LN/CB/CN) for 3 months. 2,4,8,24 and 48 h after intubation of 20 μCi [1-^{14}C]linoleic acid and 50 μCi [^{3}H]oleic acid in a small dose of dietary fat, the animals were sacrificed; then their livers were quickly removed. The radioactivity in the livers was analyzed by incineration and counting with a liquid scintillation counter. All values are means of four animals per group

matter). The ^{14}C-ratio of the whole liver and the lipid extract was between 1 and 1.5 for all groups at t=2 h and increased slightly to between 1.4 (PHSO) and 2.3 (OV) at t=48 h.

[1-^{14}C]linoleic acid and its products were mainly incorporated into the phospholipidfraction of the liver (Fig. 5) and this incorporation increased slightly in time. The incorporation into cholesterolesters and free fatty acids was negligible. Compared to the other dietary groups, a lower ^{14}C incorporation of phospholipids into the LN/CB/CN-group and a higher incorporation into triacylglycerols was apparent. The extent of ^{3}H incorporation into the triacylglycerols was comparable to that into phospholipids (Fig. 6); here too, the LN/CB/CN-diet seemed to lower the incorporation of ^{3}H into the phospholipids. Cholesterolesters in the OV-group contained up to 6% of ^{3}H after 24 h; in the other groups this percentage was much less.

The preference of labelled linoleic acid and oleic acid (and their conversion products) for the lipid classes in the liver may be estimated from the ratio of $^{3}H/^{14}C$ in each lipid class (Fig. 7). This ratio clearly shows that linoleic acid has a strong preference for phospholipids. This preference was not influenced by the diets. Oleic acid is mainly incorporated in triacylglycerol. The LN/CB/CN-diet caused a relatively low preference for oleic acid incorporation in triacylglycerol compared to the other diets.

Distribution of ^{14}C over the fatty acids in the phospholipid fraction of the liver

Because in the liver the phospholipid fraction contained by far most of the [^{14}C]label, the distribution of this label over the fatty acids was investigated extensively in this lipid class. The fatty acids were separated into a few "bands" on $AgNO^{3}$-TLC, as shown in Table III. The fatty acids were identified by comparison with reference fatty acids.

In all groups labelled linoleic acid itself occurs most frequently in the first 24 h. However, significant incorporation of other polyunsaturated fatty acids also occurs. The first fatty acids to arise after

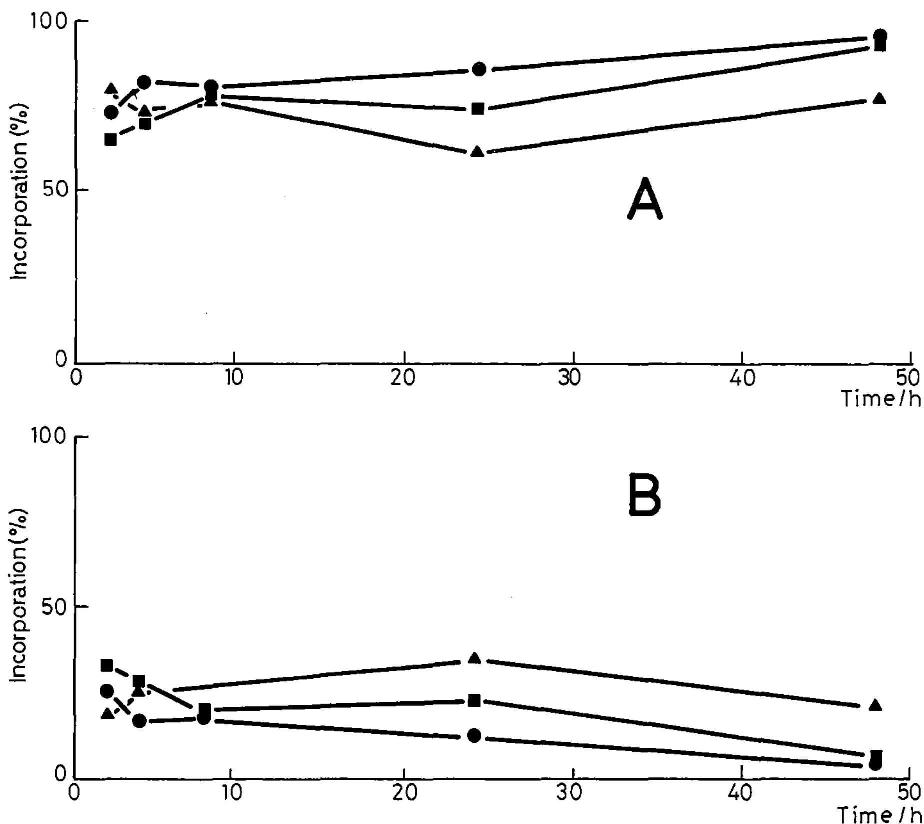

Fig. 5. Incorporation of ^{14}C in liver lipid classes, expressed as percentage of total radio-label in the lipid extract. Lipid classes were separated by TLC and analyzed for radioactivity. A: phospholipid fraction; B: triacylglycerol fraction. Each value represents the mean of four animals per group (■ -OV, ● -PHSO, ▲ -LN/CB/CN)

desaturation and elongation of linoleic acid are 18:3 and 20:3 n-6. Indeed, even after 2 h the triene fraction (mainly 18:3 n-6 and 20:3 n-6 as could be shown with reference fatty acids) contains a few percent of the [^{14}C]label and the relative amount of this label increases in time. Arachidonic acid (the predominant representative of the tetraene fraction), which is formed from 20:3 n-6, also shows a clear increase in time. Finally, other PUFAs (pentaenes and hexaenes) are formed. The labelled fatty acids in the first column are most likely of the n-6 family. 22:5 n-6 is the most likely candidate, having a similar retention time in this system as the n-3 fatty acids we used as references. The activity in the diene fraction (mainly linoleic acid) gradually decreased.

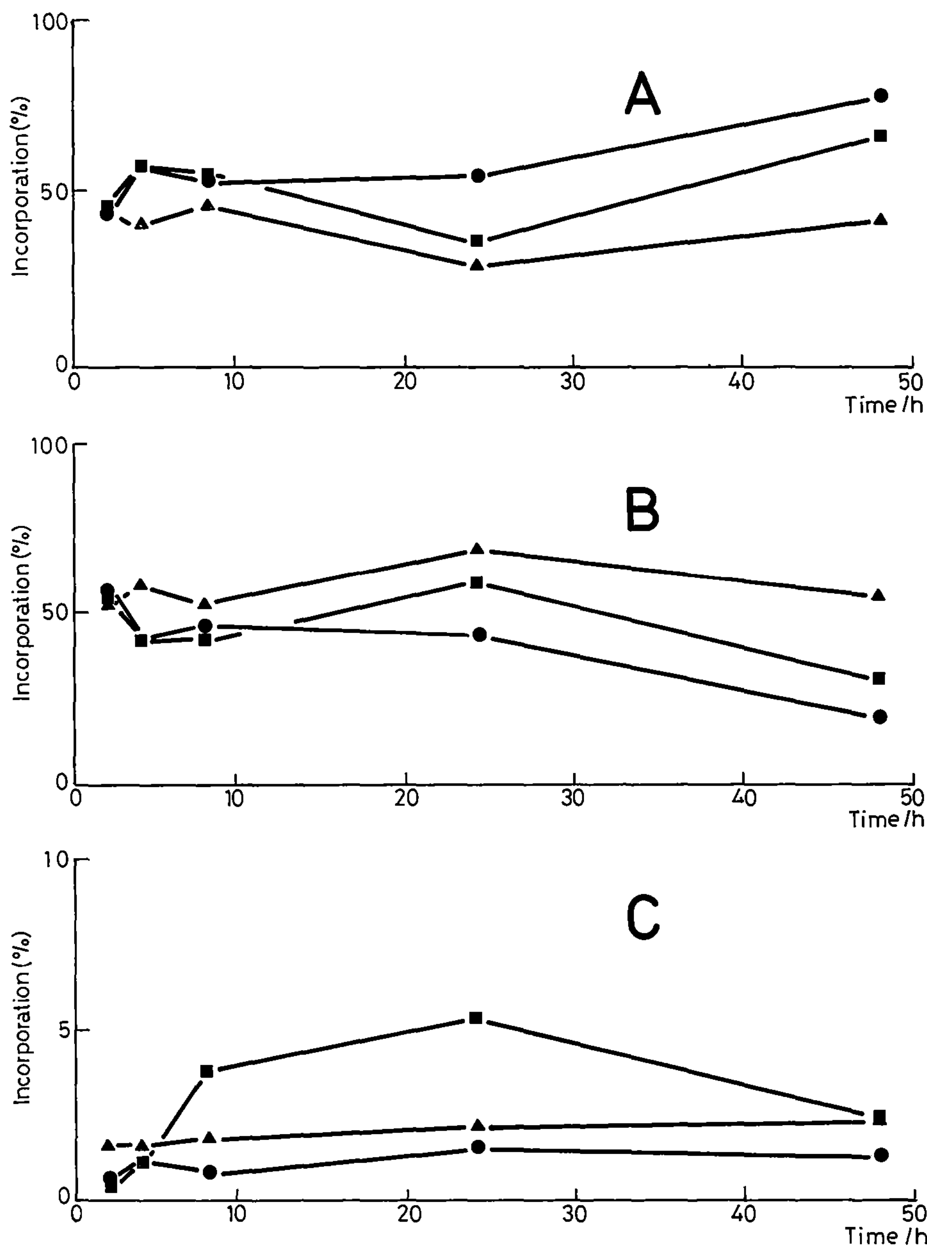

Fig. 6. Incorporation of ^{3}H in liver lipid classes, expressed as percentage of total radio-label in the lipid extract. Lipid classes were separated by TLC and analyzed for radioactivity. A: phospholipid fraction; B: triacylglycerol fraction; C: cholesterolester fraction. Each value represents the mean of four animals per group(■-OV,● -PHSO, ▲-LN/CB/CN)

TABLE III

DISTRIBUTION OF ^{14}C OVER THE FATTY ACIDS OF THE LIVER PHOSPHOLIPID FRACTION AS A FUNCTION OF TIME AND DIETARY FAT

Fatty acids of the liver phospholipid fraction were separated on $AgNO_3$-TLC into several groups and analysed for radioactivity. The values represent the mean ± SD of four animals per group and are expressed as the amount of [^{14}C]label in the fatty acid groups relative to the total amount of [^{14}C]label recovered in the fatty acids from liver phospholipids.

GROUP 1 (OV)	Fraction					
time/h	pentaene and hexaene	tetraene	triene	diene	monoene	saturated
2	-	6.8±0.9	4.8±1.3	82.5±6.3	0.8±0.1	1.5±0.5
4	0.4±0.1	4.9±1.2	4.4±2.4	89.1±4.2	0.3±0.1	1.1±0.3
8	0.8±0.3	9.6±2.4	7.5±0.6	80.2±3.3	0.4±0.1	1.4±0.3
24	1.8±0.4	37.8±4.5	10.1±0.8	47.3±3.9	1.1±1.3	1.7±0.4
48	7.6±2.4	50.0±5.3	9.7±1.4	30.0±2.7	1.3±1.0	1.7±0.5
GROUP 2 (PHSO)	Fraction					
time/h	pentaene and hexaene	tetraene	triene	diene	monoene	saturated
2	0.8±0.8	1.8±0.3	3.0±1.0	93.3±1.4	0.8±0.1	0.5±0.2
4	0.8±0.7	2.1±0.4	5.5±3.6	91.2±3.6	0.3±0.1	0.3±0.1
8	0.5±0.2	2.2±0.2	5.6±2.2	90.8±2.2	0.5±0.4	0.4±0.1
24	1.0±0.3	7.2±2.7	10.1±1.5	80.4±2.9	0.6±0.5	0.8±0.4
48	3.1±1.5	22.3±1.2	13.3±1.6	60.9±3.6	0.6±0.3	0.6±0.1
GROUP 3 (LN/CB/CN)	Fraction					
time/h	pentaene and hexaene	tetraene	triene	diene	monoene	saturated
2	0.4±0.1	0.4±0.1	2.4±1.4	96.1±1.4	0.6±0.1	0.3±0.1
4	0.4±0.1	0.5±0.1	5.0±1.2	93.5±1.2	0.4±0.1	0.2±0.1
8	0.3±0.2	0.7±0.7	2.7±2.4	95.4±3.6	0.3±0.2	0.3±0.3
24	1.2±0.3	8.0±1.5	9.6±2.3	79.3±2.3	0.9±0.9	1.0±0.4
48	3.1±0.9	14.5±3.1	12.1±1.4	66.8±3.7	1.2±0.5	1.3±0.1

- = missing value

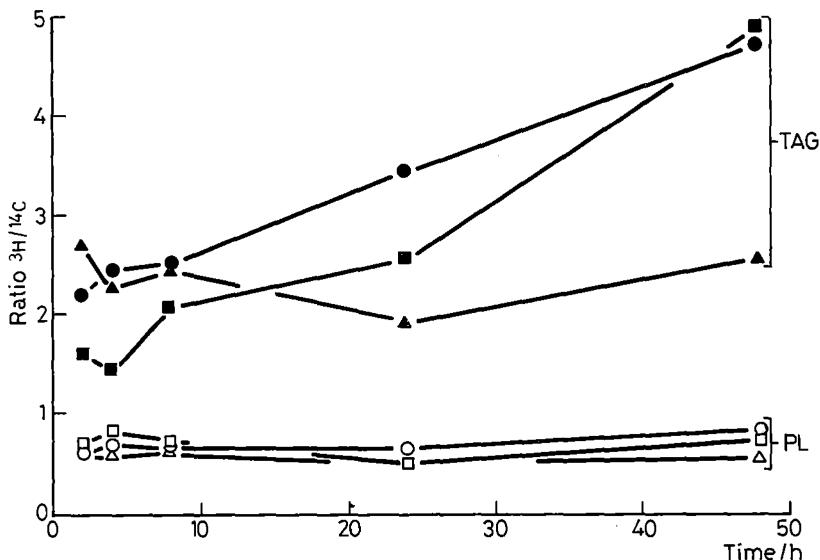

Fig. 7. Ratio of ^{3}H and ^{14}C in lipid classes of the liver. Lipid classes were separated by TLC and analyzed for radioactivity. The values represent the mean of four animals per group (■ □ -OV, ● ○ -PHSO, ▲ △ -LN/CB/CN; □ ○ △ phospholipid fraction, ■ ● ▲ -triacylglycerol fraction)

In a similar experiment as described here (to be reported), the fatty acids of the liver phospholipid fraction were separated on both $AgNO^{3}$-TLC and HPLC with a reverse phase column. Comparison of the results indicated that most of the activity (more than 80%) in the triene fraction could be ascribed to 20:3 n-6. The amount of labelled 20:2 n-6 in the diene fraction was very low (less than 1% of total labelled fatty acids), so most of the activity in this fraction in the present experiment was found in linoleic acid. The values found for arachidonic acid were very similar with both techniques.

The differences between the groups were considerable. The OV-diet stimulated the incorporation of labelled arachidonic acid much more than the other diets, of which the LN/CB/CN-diet yielded the lowest amount of label in arachidonic acid. The amount of 20:3 and 18:3 n-6, however, did not seem to be influenced very much by the type of diet.

The absolute amounts of $[^{14}C]$labelled fatty acids in nmol per liver could be calculated from the absolute amounts of $[^{14}C]$label in the liver lipid extract, the relative amounts of ^{14}C in phospholipids and its

distribution over the fatty acids of the liver phospholipid fraction (Fig. 8). As depicted in this figure, the absolute amount of total [^{14}C]labelled fatty acids in the liver decreases in time (note variable scale on y-axis). At all times this parameter is significantly ($p<0.05$) higher in the PHSO-group than in the other groups. A similar pattern is found for linoleic acid. It appears that in the OV- and LN/CB/CN-group the maximum incorporation of arachidonic acid is obtained at t= 24 h; then it starts to diminish.

Comparison of the amounts of labelled linoleic acid and arachidonic acid between the groups shows remarkable differences. In this liver lipid, the OV-diet caused the lowest incorporation of labelled linoleic acid, but the highest incorporation of labelled arachidonic acid. The PHSO-diet, compared to the OV-diet, led to the highest incorporation of linoleic acid, but a significantly ($p<0.05$) lower arachidonic acid incorporation. The LN/CB/CN-group showed an even lower incorporation of arachidonic acid than the PHSO-group, but the linoleic acid level was also much lower.

Fatty acid composition of liver lipids

The fatty acid composition of liver lipids was determined in animals sacrificed at t=48 h. The linoleic acid level in liver phospholipid was highest in the PHSO-group and lowest in the OV-group (Table IV). The level of *trans* isomers of linoleic acid probably was around 2% in the PHSO-group [17]. The arachidonic acid level in the PHSO-group is only slightly lower than that in the OV-group. The LN/CB/CN-diet, however, more than halved the arachidonic acid level compared to the other diets. 22:5 n-6 is absent in this group. The reduction in n-6 PUFA is completely compensated for by a rise in n-3 PUFA.

In the triacylglycerol fraction of the liver, a similar effect of the dietary fats on the linoleic acid level (including approx. 4% *trans* isomers in the PHSO-group [17]) is seen (Table V).

As usual, other n-6 PUFA are nearly absent in this lipid class. After feeding of the LN/CB/CN-diet, a substantial incorporation of n-3 PUFA occurs, even of the highly unsaturated members of this fatty acid family. This results in a relatively high unsaturation index of 150 (cf. 82 and 91 in the OV- and PHSO-group respectively).

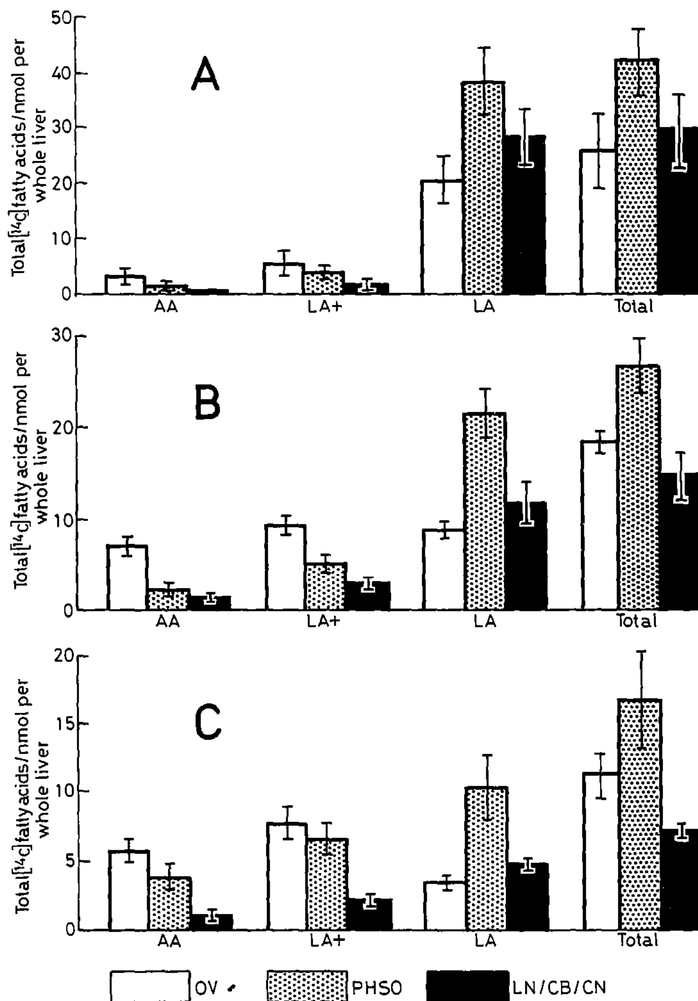

Fig. 8. Total amount of [^{14}C]labelled fatty acids in the phospholipid fraction of the liver, expressed as nmol fatty acid per whole liver. Fatty acids of the liver phospholipid fraction were separated on $AgNO_3$-TLC and analyzed for radioactivity. The absolute level of arachidonic acid (AA), linoleic acid (LA) and linoleic acid desaturation/elongation products (including arachidonic acid)(LA+) was calculated from the radioactivity in the liver lipid extract, the percentage of [^{14}C]label in the phospholipid fraction and the percentage of [^{14}C]label in the fatty acids. The values given are mean ± SD of four animals per group (OV, PHSO, LN/CB/CN). A: total amount of [^{14}C]labelled fatty acids 8 h after intubation; B: total amount of [^{14}C]labelled fatty acids 24 h after intubation; C: total amount of [^{14}C]labelled fatty acids 48 h after intubation (note differences in scale)

TABLE IV

FATTY ACID COMPOSITION OF THE LIVER PHOSPHOLIPID FRACTION

The composition is given in % (w/w). The liver phospholipid fraction was isolated by TLC. The fatty acid composition was determined by GLC of individual samples. The values given represent the mean of four animals per group.

Type of fatty acid[a]	Groups		
	OV	PHSO	LN/CB/CN
14:0	0.1	0.2	0.5
16:0	14.9	11.5	17.8
16:1n-7	0.8	1.8	0.6
18:0	21.0	17.6	24.6
18:1 [b]	14.0	19.9	7.2
18:2 [b]	6.4	13.1	11.2
18:3n-6	0.1	0.2	-
18:3n-3	0.1	0.2	0.9
20:1n-9	0.6	0.2	0.2
20:2n-6	1.1	1.3	0.5
20:3n-6	0.9	1.0	0.9
20:4n-6	26.1	23.3	11.1
20:5n-3	-	0.1	6.6
22:4n-6	1.2	0.7	0.3
22:5n-6	1.8	2.6	-
22:5n-3	0.3	0.2	2.9
22:6n-3	8.3	4.0	12.6
sum	97.5	97.9	97.9

a The shorthand notation used for the fatty acids indicates chainlength: number of double bonds

b Both *cis* and *trans* isomers

\- not detected

TABLE V

FATTY ACID COMPOSITION OF THE LIVER TRIACYLGLYCEROL FRACTION

The composition is given in % (w/w). The liver triacylglycerol fraction was isolated by TLC. The fatty acid composition was determined by GLC of individual samples. The values given represent the mean of four animals per group.

Type of fatty acid[a]	Groups		
	OV	PHSO	LN/CB/CN
12:0	0.2	0.3	0.7
14:0	0.7	0.9	2.0
16:0	18.6	19.1	23.5
16:1n-7	2.6	5.3	2.0
18:0	1.7	4.5	6.2
18:1 [b]	63.6	47.0	29.5
18:2 [b]	5.2	14.1	8.4
18:3n-6	0.2	0.6	0.1
18:3n-3	0.4	0.9	11.1
20:1n-9	1.0	1.1	1.2
20:2n-6	-	0.6	0.2
20:3n-6	-	-	0.1
20:4n-6	0.6	0.8	0.8
20:5n-3	-	-	2.7
22:4n-6	-	-	-
22:5n-6	-	-	-
22:5n-3	-	-	3.6
22:6n-3	-	-	5.2
sum	94.8	95.2	97.1

a The shorthand notation used for the fatty acids indicates chainlength: number of double bonds

b Both *cis* and *trans* isomers

\- not detected

As the phospholipid fraction and the triacylglycerol fraction are the two most important lipid classes in the liver, it is evident that the livers of the rats in the LN/CB/CN-group have taken up substantial amounts of n-3 PUFA. This take-up is predominantly at the expense of n-6 PUFAs other than linoleic acid, the level of which is not lowered by this diet relative to the OV-diet.

Incorporation of ^{3}H and ^{14}C into total heart and heart lipid classes

The incorporation of ^{3}H and ^{14}C in the heart is much less than that in the liver: the mean incorporation of ^{14}C per whole heart at 24 h was 35500, 51500 and 61970 dpm for the three groups, which is around 1% of that in the liver. The incorporation of ^{3}H was even less: ca. 32000 dpm for all groups (somewhat less than 1% of that in the liver). ^{14}C was mainly present in the phospholipid fraction (ca. 90% of the total label in the lipid extract; TAG contained ca. 7% and free fatty acids 3%) and the amount was hardly influenced by the diet. ^{3}H was more abundant in the triacylglycerol than ^{14}C (ca. 20% incorporation in TAG), but here, too, the phospholipid fraction contained by far most of the [^{3}H]label (ca. 75%).

Distribution of ^{14}C over the fatty acids in the phospholipid fraction of the heart

The relative incorporation of [^{14}C]arachidonic acid in heart phospholipids 24 h after the intubation (Table VI), was clearly lower than that in the liver (Table III). But here too, a significantly higher level of this fatty acid is found in the OV-group than in the other groups.

Fatty acid composition of heart phospholipid

The fatty acid composition of the heart phospholipids (determined in animals sacrificed at t=8 h) is very similar to that of the liver,

TABLE VI

DISTRIBUTION OF ^{14}C OVER THE FATTY ACIDS OF THE HEART PHOSPHOLIPID FRACTION 24 H. AFTER INTUBATION AS INFLUENCED BY DIETARY FAT

Fatty acids of the heart phospholipid fraction were separated on $AgNO_3$-TLC into several groups and analysed for radioactivity. The values represent the mean ± SD of four animals per group and are expressed as the amount of [^{14}C]label in the fatty acid groups relative to the total amount of [^{14}C]label recovered in labelled fatty acids in heart phospholipids.

	Fraction					
time/h	pentaene and hexaene	tetraene	triene	diene	monoene	saturated
OV	1.8±1.9	8.0±0.9	4.6±2.9	82.1±7.8	2.1±2.8	1.5±0.9
PHSO	0.5±0.1	1.9±0.5	2.5±0.7	91.9±5.7	0.5±0.1	0.3±0.1
LN/CB/CN	0.5±0.2	1.1±0.3	3.5±2.4	94.0±2.3	0.8±0.4	0.2±0.1

except for the very low incorporation of eicosapentaenoic acid (EPA; 20:5 n-3) in the liver, when 18:3 n-3 was present in the diet (Table VII). 22:5 n-3 and 22:6 n-3 occur even more frequently than in the liver. The dietary influence on the fatty acid composition of the phospholipid fraction of the heart is comparable to that of the liver.

TABLE VII

FATTY ACID COMPOSITION OF THE HEART PHOSPHOLIPID FRACTION

The composition is given in % (w/w). The heart phospholipid fraction was isolated by TLC. The fatty acid composition was determined by GLC of individual samples. The values given represent the mean of four animals per group.

Type of fatty acid[a]	Groups		
	OV	PHSO	LN/CB/CN
12:0	-	-	-
14:0	-	0.1	0.3
16:0	10.4	8.2	11.1
16:1n-7	0.9	1.4	0.7
18:0	22.6	15.4	29.6
18:1 [b]	13.6	20.3	7.1
18:2 [b]	9.3	18.0	14.6
18:3n-3	0.3	0.2	1.2
20:1n-9	0.3	0.2	0.2
20:3n-6	0.5	0.7	0.6
20:4n-6	25.9	24.7	11.4
20:5n-3	0.1	-	0.9
22:4n-6	1.1	0.9	0.2
22:5n-6	2.0	2.5	0.1
22:5n-3	0.7	0.2	5.0
22:6n-3	9.7	4.9	14.1
sum	97.4	97.7	97.1

a The shorthand notation used for the fatty acids indicates chainlength: number of double bonds

b Both *cis* and *trans* isomers

\- not detected

Discussion and Conclusions

The fate of radiolabelled linoleic acid in rats has been investigated in a few studies. In most of these studies [1-^{14}C]linoleic acid, either as methylester or as free fatty acid, was intubated intra-gastrically, dissolved in a small amount of carrier oil. The largest part of linoleic acid in our diet is esterified in triacylglycerol, however. The differences between the metabolism of linoleic acid administered in the form of either free fatty acid, methylester or triacylglycerol are insufficiently known.

The amount and type of carrier oil might affect the uptake of radiolabelled linoleic acid. However, Dittmer and Hanahan [18] reported that the type of carrier oil (olive oil or corn oil) did not influence the incorporation of [1-^{14}C]linoleic acid into liver lipids of rats. In our experiment all three carrier oils contained the same amount of linoleic acid, so no effect due to differences in isotope dilution of the [^{14}C]labelled linoleic acid could occur. In a previous experiment we did not find major differences in linoleic acid metabolism induced by the type of carrier oil (OV or LN/CB/CN; same composition as in the present experiment) when intubated in rats with the same dietary background (unpublished results). We concluded that the administration of radiolabelled linoleic acid in a small amount of carrier oil is very suitable for the comparative study of linoleic acid metabolism in rats with different dietary backgrounds.

In the present study, contrary to most studies of this type described in the literature, rats were fasted for 18 h prior to the intubation of [^{3}H] and/or [^{14}C]labelled fatty acid(s); an empty stomach facilitates the uptake of these radiolabels. However, fasting and refeeding have a significant effect on both carbohydrate and fat metabolism. Most of the changes in liver weight were probably due to depletion and refilling of glycogen stores, but the fat content may have been altered as well [19].

Fasting decreases the Δ6-desaturation activity, while refeeding (especially high-carbohydrate meals) reactivates this system [20,21]. So the desaturation capacity of the rats deprived of food after the intubation

(animals sacrificed at t=2 and 4 h) may have been decreased compared to the animals that were offered food after the intubation (animals sacrificed at t=8, 24 and 48 h).

Both labelled fatty acids ([1-^{14}C]linoleic acid and [^{3}H]oleic acid) were taken up rapidly from the gastro-intestinal tract into the serum. Within 2 h after the intubation the maximal incorporation of these labels was reached. We did not ascertain which fraction of the radiolabel had been taken up by that time, but as the amount of ingested lipid was only small, we expect the largest part of both ^{3}H and ^{14}C to be taken up within these 2 h. In a similar experiment, Illingworth and Glover [22] found a recovery of less than 1% of the original dose of [^{14}C]linoleic acid and [^{3}H]oleic acid in the intestinal washings 8 h after dosing.

At t=4 h only approx. 3-4% of the original dose of [^{3}H]- and [^{14}C]labels was still present in serum (assuming approx. 12 ml of circulating serum in the animals [23]). At that time (4 h), the liver contained more of the [^{14}C]label than of the [^{3}H]label (22 and 12%, respectively). Since the amount of ^{3}H and ^{14}C in the liver at t= 8 h was lower than that at t=4 h - Illingworth and Glover [22] found a maximum incorporation already after 1 h - either the export of ^{3}H and ^{14}C to other tissues via the blood was bigger than the uptake from the blood, or else a significant oxidation must have taken place after t=4 h. The total oxidation of orally administered [^{14}C]linoleic acid by the whole body in the first few hours after administration accounts for only a few percent of the total administered dose [24]. It may therefore be assumed that after the initial built-up of ^{3}H and ^{14}C, a net export from the liver to other organs occurred. The heart contained only a minor amount of ^{3}H and ^{14}C at t=24 h (approx. 1% of that of the liver). According to Becker [25] and Becker *et al.* [26] skeletal muscle and skin contained most of the [^{14}C]label (11 and 9% of the administered dose), 20 h after intubation; 4% of this label was present in liver and less than 1% in serum.

Oxidation of fatty acids will finally lead to the formation of H_2O, CO_2 or ketone-bodies, which are all soluble in water [27]. Some intermediary reaction products of the β-oxidation (eg. acetate) can be

used for anabolic processes such as de-novo fatty acids synthesis [28]. The amount of [^{14}C]labelled water-soluble products in the liver was small, because the ratio of ^{14}C-activity in the whole liver (lipid and water phase, and solid matter) to that of the lipid extract is only slightly above 1. This ratio was hardly different between the groups, indicating a comparable rate of linoleic acid catabolism in the liver. Fatty acid synthesis from [^{14}C]acetate was probably no major pathway in our experiments, as hardly any [^{14}C]labelled saturated or monounsaturated fatty acid could be found in liver lipids, not even after 48 h. Of course most of the newly synthesized saturated fatty acids would have been incorporated into the triacylglycerols and exported into the blood. However, triacylglycerol in the liver did not contain detectable amounts of [^{14}C]labelled saturated fatty acids 8 h after administration of the label (data not shown).

[^{14}C]labelled n-6 fatty acids were found to be preferentially incorporated into the phospholipids of liver and heart. This preference has also been found by others [29-32] and is in accordance with the relatively high content of n-6 fatty acids in phospholipids and the low content of these fatty acids in triacylglycerol. Illingworth and Glover [22] demonstrated that the phospholipid fraction of the liver was more extensively labelled than the triacylglycerol fraction, except for the first hour after administration of [^{14}C]linoleic acid. [^{3}H]labelled fatty acids derived from [^{3}H]oleic acid were equally divided over the triacylglycerol and phospholipid fraction in the liver, but in the heart 75% of this label was associated with phospholipids. This difference demonstrates that each organ has a specific fatty acids incorporation preference.

The catabolism of arachidonic acid is reported to be low compared to that of other fatty acids [29,30,31]. The total oxidation of labelled arachidonic acid in rats fed adequate amounts of linoleic acid is much lower than that of other fatty acids. Coots [30] found a recovery of approx. 25% of ^{14}C from labelled arachidonic acid in expired CO_2 after 24 h compared to approx. 50% for other fatty acids. In the above experiments [29,30,31] the catabolism of arachidonic acid was measured

by administration of the fatty acid itself. In our experiment, the degree of oxidation of [^{14}C]labelled arachidonic acid was probably much lower than that in the experiments in which the labelled arachidonic acid must first be biosynthesized from [^{14}C]linoleic acid. The arachidonic acid turnover due to synthesis of prostaglandins and related compounds is low [32]. The total eicosanoid production from [^{14}C]arachidonic acid derived from its precursor linoleic acid was probably very low compared to the amount of ^{14}C present in the liver.

Liver is one of the most active and important organs in the biosynthesis of arachidonic acid, as it is very likely to serve as supplier of arachidonic acid to other tissues [10,33]. The biosynthesis of arachidonic acid from linoleic acid in the liver must proceed at a rather high rate, because 24 h after the intubation, 2.3% of the ^{14}C is found in the arachidonic acid of the liver phospholipids (in the OV-group). When we extrapolate it to the whole body, this finding indicates that in rats fed a moderate amount, at least 5% of the dietary linoleic acid is converted to arachidonic acid each day.

Arachidonic acid is readily taken up from serum by most tissues [29,32,34]. However, our heart data reveal that the heart tissue takes up only a small amount of [^{14}C]labelled arachidonic acid in comparison with liver tissue. It may be assumed that tissues like those of muscle and skin contain a substantial pool of [^{14}C]arachidonic acid, whereas fat tissue will be relatively devoid of this fatty acid [34].

The amount of radiolabelled arachidonic acid in the liver is the net result of its biosynthesis in the liver, net export to other tissues and its degradation, which is slow. Because of this slow degradation it seems justified to regard the amount of [^{14}C]arachidonic acid in liver phospholipid as a measure for its biosynthesis by the liver in the first 24 h after administration of [^{14}C]linoleic acid. This assumption implies that the PHSO-diet and the LN/CB/CN-diet both caused a reduction in the rate of arachidonic acid biosynthesis by the liver compared to the OV-diet.

The specific activity of linoleic acid in liver phospholipid drops rapidly in time, but the specific activity of 18:3 and 20:3 remains rather constant. Moreover, the latter is higher than that of linoleic

acid after 8 h (Fig. 9), which is an indication of the direct product-precursor relationship as described by Zilversmit et al. [35]. The influence of the dietary fats on the specific activities of linoleic acid and its products (18:3 and 20:3) is only indicated by a slightly increased specific activity of 18:3 and 20:3 in the PHSO group. The specific activities of arachidonic acid on the other hand are one order of magnitude lower and the differences between the groups are apparent. The influence of the dietary fats on the total arachidonic acid synthesis is therefore not likely to be caused by an effect on the first steps of the linoleic acid conversion (Δ6-desaturation and elongation), but on events taking place after formation of 20:3.

The above findings are in agreement with the lack of difference in the Δ6-desaturation capacity of the liver microsomes in rats fed olive oil or *trans* fatty acids at an adequate level of linoleic acid [36], but they do not comply with the findings of others [37]. The *trans* monoenoic acids were extensively investigated with respect to their effects on the conversion of linoleic acid *in vitro*; they were found to possess no greater inhibitory potency than the *cis* monoenoic acids (isomers of oleic acid) [38,39]. Therefore, the so-called Δ6-inhibitory effect of *trans* fatty acids *in vivo* cannot be explained by these *in vitro* experiments. The present *in vivo* experiment shows that trans fatty acids and α-linolenic acid have no effect on the synthesis of γ-linolenic acid and dihomo-γ-linolenic acid, the Δ6-desaturation products. It may be speculated that either the Δ5-desaturation of 20:3 or the incorporation of arachidonic acid into liver phospholipid is different.

Dietary fatty acids significantly influence the fatty acid composition of the liver phospholipids. *Trans* fatty acids, which are primarily incorporated at the 1-position of phospholipids [40], compete to a lesser extent with linoleic acid for incorporation than oleic acid (incorporated at both the 1- and 2-position). As a consequence the linoleic acid level of phospholipids is higher in the animals fed *trans* fatty acids than in those fed olive oil. α-Linolenic acid and

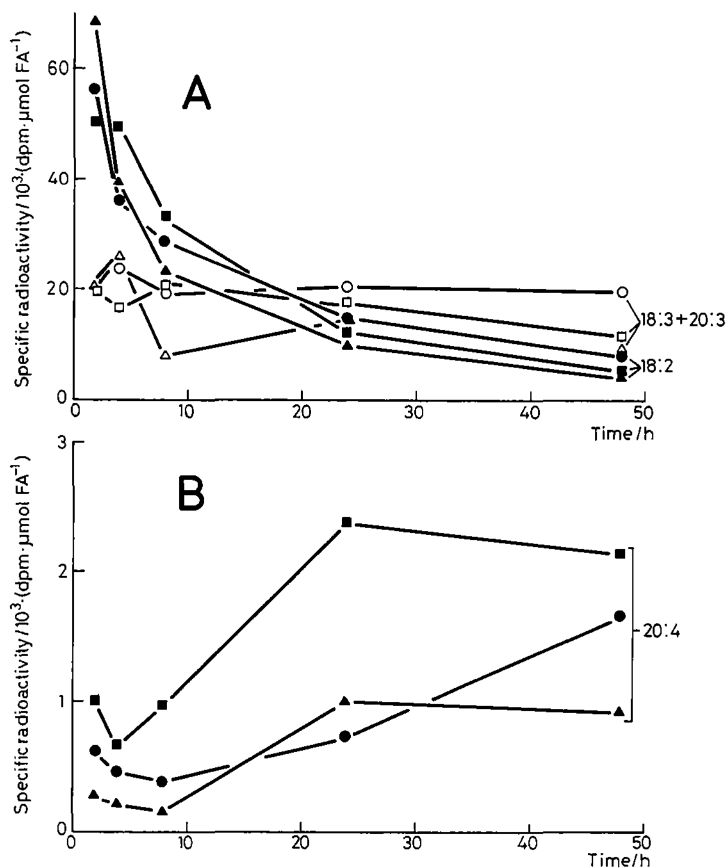

Fig. 9. Specific radioactivity of n-6 fatty acids (FA) in liver phospholipid as a function of time and diet. The specific activity of [^{14}C]labelled fatty acid was calculated by dividing the total radioactivity in linoleic acid, γ-linolenic acid plus dihomo-γ-linolenic acid (A) and arachidonic acid (B) in phospholipids of the whole liver (in dpm) at each timepoint by the amount of unlabelled phospholipid fatty acid in the liver (μmol) at t=24 h. The values represent the mean of four animals per group (■-OV, ●-PHSO, ▲-LN/CB/CN).

its product 20:5 n-3, on the other hand, compete very effectively with arachidonic acid for incorporation into phospholipids, thus lowering the arachidonic acid level [41]. The effect on the linoleic acid level is minor. The total amount of PUFA is very constant over the three groups

(approx. 47%). So, by considering the competition between the fatty acids for the available positions in the phospholipids without the necessity to take into account inhibitions of PUFA synthesising pathways, at least part of the changes in fatty acid composition can be explained.

In the literature the ratio of the amount of arachidonic acid and linoleic acid in liver phospholipids as determined by GLC is sometimes used as a measure for the activity of the Δ6-desaturation system [42-44]. This ratio is particularly used to assess the influence of dietary fats on the conversion of linoleic acid into arachidonic acid, the Δ6-desaturation being considered as the rate-limiting step [20]. Thus, according to these workers, *trans* fatty acids and α-linolenic acid inhibit the arachidonic acid biosynthesis via the Δ6-desaturation pathway (see Holman [45] for a review). In our experiments we indeed found a lower 20:4/18:2 ratio in the liver phospholipid fraction caused by diets rich in *trans* fatty acids (PHSO-diet) and α-linolenic (LN/CB/CN-diet) compared to the OV-diet (respectively 1.8, 1.0 and 4.1). However, as is evident from this experiment, these changes in ratio of 20:4/18:2 cannot be explained by an altered Δ6-desaturation. It is demonstrated that the effects of dietary fats on linoleic acid metabolism cannot be simply deduced from the fatty acid patterns of the organ lipids.

The results of this experiment show that studies like this one offer interesting possibilities to investigate the effects of dietary fats on linoleic acid metabolism. This may well be of significance for the assessment of the linoleic acid requirement as influenced by the diet.

References

1 Brenner, R.R. and Peluffo, R.O. (1966) J. Biol. Chem. 741, 5213-5219

2 Brenner, R.R. and Peluffo, R.O. (1967) Biochim. Biophys. Acta 137, 184-186

3 Brenner, R.R. and Peluffo, R.O. (1969) Biochim. Biophys. Acta 176, 471-479

4 Kurata, N. and Privett, O.S. (1980) Lipids 15, 512-518

5 De Schrijver, R. and Privett, O.S. (1982) J. Nutr. 112, 619-626

6 Blond, J.P., Ratieuville, P. and Bézard, J. (1985) Cah. Nutr. Diet. 20, 109-111

7 De Schrijver, R. (1985) Thesis, R.U. Gent, Gent, Belgium

8 Mohrhauer, H. and Holman, R.T. (1963) J. Nutr. 81, 67-74

9 Rahm, J.J. and Holman, R.T. (1964) J. Nutr. 84, 149-154

10 Naughton, J.M. (1981) Int. J. Biochem. 13, 21-32

11 Huang, Y.S., Nassar, B.A. and Horrobin, D.F. (1986) Biochim. Biophys. Acta 879, 22-27

12 Irritani, N. and Narita, R. (1984) Biochim. Biophys. Acta. 793, 441-447

13 Roshanai, F. and Sanders, T.A.B. (1985) Ann. Nutr. Metab. 29, 189-196

14 Zevenbergen, J.L. (1986) in Enzymes of Lipid Metabolism II, (Freysz, L. et al., eds.), pp. 647-649, Plenum Publishing Coorperation, New York

15 Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911-917

16 Christie, W.W. (1973) Lipid Analysis, p.p. 152-154, Pergamon Press, Oxford

17 Moore, C.E., Alfin-Slater, R.B. and Aftergood, L. (1980) Am. J. Clin. Nutr. 33, 2318-2323

18 Dittmer, J.C. and Hanahan, D.J. (1959) J. Biol. Chem. 234, 1976-1982

19 Strubelt, O., Dost-Kempf, E., Siegers, C.-P, Younes, M., Preuss, U. and Dreckmann, J.G. (1981) Toxicol. Appl. Pharmacol. 60, 66-77

20 Brenner, R.R. (1981) Prog. Lipid Res. 20, 41-47

21 De Gomez Dumm, I.N.T. De Alaniz, M.J.T. and Brenner, R.R. (1970) J. Lipid Res. 11, 96-101

22 Illingworth, D.R. and Glover, J. (1973) J. Neurochemistry 21, 1403-1414

23 Wang, L. (1959) Amer. J. Physiol. 196, 188

24 Coots, R.H. (1964) J. Lipid Res. 5, 473-476

25 Becker, W. (1984) J. Nutr. 114, 1690-1696

26 Becker, W. and Månson, J.E. (1985) J. Nutr. 115, 1248-1258

27 Bremer, J. and Osmundsen, H. (1984) in New Comprehensive Biochemistry: Fatty acid metabolism and its regulation, (Neuberger, A. and Van Deenen, L.L.M., eds.), pp. 113-147, Elsevier/North-Holland, Amsterdam

28 Wakil, S.J., Stoops, J.K. and Joshi, V.C. (1983) Ann. Rev. Biochem. 52, 537

29 Coniglio, J.G, Davis, J.T. and Aylward, S. (1964) J. Nutr. 84, 265-271

30 Coots, R.H. (1965) J. Lipid Res. 6, 494-497

31 Sinclair, A.J. (1975) Lipids 10, 175-184

32 Zijlstra, F.J and Vincent, J.E. (1985) Prostaglandins Leukotrienes and Medicine 20, 287-297

33 Brenner, R.R. (1971) Lipids 6, 567-575

34 Becker, W., Mohammed, A. and Slanina, P. (1985) Ann. Nutr. Metab. 29, 65-75

35 Zilversmit, D.B., Entenman, C. and Fishler, M.C. (1942) J. Gen. Physiol. 26, 325

36 Blomstrand, R., Diczfalusy, U, Sisfontes, L. and Svensson, L. (1985) Lipids 20, 284-293

37 Mahfouz, M.M., Smith, T.L. and Kummerow, F.A. (1984) Lipids 19, 214-222

38 Mahfouz, M.M., Johnson, S.B. and Holman, R.T. (1980) Lipids 15, 100-107

39 Mahfouz, M.M., Johnson, S.B. and Holman, R.T. (1981) Biochim. Biophys. Acta 663, 58-68

40 Wood, R. and Chumbler, F. (1978) Lipids 13, 75-84

41 Lands, W.E., Inoue, M., Sugiura, Y. and Okuyama, H. (1982) J. Biol. Chem. 257, 14968-14972.

42 Hill, E.G., Johnson, S.B., Lawson, L.D., Mahfouz, M.M. and Holman, R.T. (1982) Poc. Natl. Acad. Sci. USA 79, 953-957

43 Lawson, L.D., Hill, E.G. and Holman, R.T. (1983) J. Nutr. 113, 1827-1835

44 Mahfouz, M., Smith, T.L., Holmes, R.P. and Kummerow, F.A. (1983) Fed. Proc. 42, 1186

45 Holman, R.T. (1986) Prog. Lipid Res. 25, 24-39

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Chapter 7.

BIOLOGICAL EFFECTS OF *TRANS* FATTY ACIDS

J.L. Zevenbergen
Biological effects of *trans* fatty acids
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1. INTRODUCTION

Hydrogenation is widely applied in the edible fats industry. The aim of this process is to shift the melting range of edible oils to higher temperatures to obtain solid fats (hardstock) for production of margarines and shortenings. It also improves the flavour stability of oils, especially of those containing substantial amounts of linolenic acid, such as soybean oil.

During the hydrogenation process, saturated fatty acids and a variety of geometric (i.e. *trans*) and positional isomers of unsaturated fatty acids are formed in varying amounts, depending on the initial fatty acid composition of the oils and the process conditions. The most commonly used oils for hydrogenation are of vegetable origin, like soybean oil (1). It is estimated that in the U.S. 2.5 million tonnes of vegetable oil (44% of the total edible fat production) are hydrogenated per annum (2).

Vegetable oils contain unsaturated fatty acids predominantly with 18 carbon atoms (C18): oleic and linoleic acid with some linolenic acid. Hydrogenation of these oils can thus lead to the formation of monoenoic and dienoic *trans* fatty acids with the double bond(s) between carbon atoms 6 to 14 from the carboxyl end. The main isomeric fatty acids of partially hydrogenated vegetable oils are monoenoic *trans* fatty acids with the double bond at positions 9 to 12; furthermore much smaller amounts of *trans* dienoic acids, *cis*,*trans*- and *trans*,*cis*- and some *trans*,*trans*-linoleic acid isomers are present (3,4).

Partially hydrogenated marine oils contain C20 and C22 *trans* fatty acids. Very long chain fatty acids, irrespective of their geometrical configuration, show specific biological effects, which are caused by their chain length (5). These fatty acids will not be discussed in this review.

In this report the biological effects of *trans* fatty acids, as present in partially hydrogenated vegetable oils, will be reviewed. Data from the literature and our own work will be used to evaluate the safety of *trans* fatty acids for human consumption.

2. CONSUMPTION OF *TRANS* FATTY ACIDS

The content of *trans* fatty acids in commercially available edible products has been reported by several investigators (6-10). The *trans* fatty acids content in U.S. margarines ranges from almost 0 to 40%; as a guideline the average content of *trans* fatty acids in stick (hard) margarines amounts to 25% and in tub (soft) margarines to 17% (9,10). Shortenings contain ca. 16% on average. The average levels of *trans* fatty acids in European margarines differ from country to country. (6,7,11-14). Shortenings in Europe generally contain a higher level of *trans* fatty acids than in the U.S.

Trans fatty acids are not only present in partially hydrogenated vegetable oils, but also occur in ruminant fats and dairy products.
These fatty acids are mainly formed in a bio-hydrogenation process by microorganisms in the rumen. Ruminant milkfat may contain up to 8% (15) and meat up to 6% (10) of *trans* fatty acids. All the *trans* fatty acid isomers present in partially hydrogenated vegetable oils are also found in dairy products (16).

Because of the wide use of margarines, shortenings and ruminant- or milk-fat, *trans* fatty acids may be present in many edible products (9,13). Values for the total per capita daily consumption of *trans* fatty acids in the U.S. vary between 6.8 and 12.1 g, depending on the method of estimation (see 17 for recent review). However, on careful analysis of the available data, around 8 g seems to be the best estimate; this is about 6% of the total fat consumption (around 2.4% of energy). In Germany the estimated intake of *trans* fatty acids is 4.5-6.4 g per capita per day (18); in Sweden 5 g (19) and in the U.K. 12 g of *trans* fatty acids per capita per day (11).

3. NUTRITIONAL EVALUATION OF *TRANS* FATTY ACIDS

Since 1945 extensive long-term animal feeding studies of partially hydrogenated fats have been performed. Deuel et al. (20,21) and later

Alfin-Slater et al. (22-24) fed 46 generations of rats a diet containing margarine as the sole source of dietary fat. This fat contained 35% *trans* fatty acids. No adverse effects were found in reproductive performance, longevity and in the histopathological examination of many organs. Two additional 25-generation feeding studies with rats fed similar diets as in her previous experiments, were reported by Alfin-Slater et al. (25). Again no significant adverse effects could be attributed to the dietary margarines.

Vles and Gottenbos (26,27) reported long-term studies involving large groups of male and female, newly weaned, SPF Swiss mice and SPF Wistar rats fed diets containing soybean oil, three hydrogenated soybean oils containing different levels of *trans* fatty acids, coconut oil or butterfat. In each diet, 54% of the energy was provided by the experimental fat and 6% by soybean oil. The soybean oil ensured that the content of essential fatty acids was adequate. In both mice and rats, no significant differences were observed in mortality, lifespan, growth, organ weights or histopathology except for higher liver weights in mice fed the more extensively hydrogenated soybean oil containing 60% *trans* fatty acids. Histopathological examination of these livers revealed no abnormalities. No systematic differences in total tumor frequency were found between the dietary groups. Neither did obvious differences occur in the type and site of the neoplasms. In mice, leukemia and lung papiloma and, in rats, adenoma of the pituitary formed the most frequently occurring type of tumors. Other pathological (non-neoplastic) changes were randomly distributed among the groups.

In another experiment Vles et al. (28) fed female Viennese x Alaska rabbits diets containing the same fats (except butterfat) used in the rat and mice studies just described. The diets contained 22.5 energy% experimental fat plus 2.5 energy% soybean oil as a source of EFA. Diets were fed to the rabbits during their entire lifespan - up to 10 years. Differences in lifespan among the dietary groups were not significant. There were no systematic differences among the dietary groups in total tumor incidence. Atherosclerotic lesions were more severe in rabbits fed the diets with the lowest amounts of linoleic acid, i.e. coconut oil or hydrogenated soybean oil.

Nolen et al. (29) performed a 2-year chronic toxicity study of used frying fats (including a soybean oil and a partially hydrogenated soybean oil). Each fat was fed at the 28% of energy level in a semipurified diet to 50 male and 50 female, weanling Sprague-Dawley rats. Histopathological examination did not show significant differences attributable to diet.

The short term human studies with partially hydrogenated vegetable fats, to be discussed in the next section, did not reveal any adverse effects either. Therefore it can be concluded that neither partially hydrogenated fats nor the individual components present (isomeric fatty acids and the unsaponifiable part of the fats) show specific physiological or pathological effects. It has to be emphasized that in these long term experiments the dietary fats contained at least 5% *cis,cis*-linoleic acid to cover the requirement for EFA.

4. *TRANS* FATTY ACIDS, ATHEROSCLEROSIS AND CANCER

The effects of hydrogenated fats on serum cholesterol has been studied in both animals and man. Saturated fatty acids, especially lauric, myristic and palmitic acid increase serum cholesterol levels in man and animals, whereas linoleic acid has a serum cholesterol lowering effect. *Cis*-monounsaturated fatty acids, particularly oleic acid, appear to occupy an intermediate position in this respect.

Therefore when evaluating the effects of *trans* fatty acids on serum cholesterol levels one should study these effects relative to those of other comparable fatty acids, such as saturated or *cis*-monounsaturated fatty acids of the same chain length. Moreover, the experimental and control diet should be nutritionally adequate and contain equal amounts of linoleic acid and cholesterol.

Only a few human studies with well balanced diets containing hydrogenated fats have been performed. Mattson et al.(30) fed liquid formula diets containing a high level of either *trans* fatty acids or *cis*-monounsaturated fatty acids. After 21 days no significant difference in serum cholesterol levels was detected. Vergroesen and

Gottenbos (31) reported two studies in which they investigated the effect of *trans* fatty acids (elaidic acid) on serum cholesterol levels. They concluded that *trans* fatty acids (elaidic acid) induced a total serum cholesterol level lying between that induced by saturated and that by *cis*-monounsaturated fatty acids.

A rabbit experiment performed in our laboratory (32) was aimed at comparing the potential atherosclerotic effects of *trans* fatty acids with those of saturated fatty acids at the same linoleic acid level (10%). This experiment showed no significant difference in atherogenicity between *trans* fatty acids and saturated fatty acids. In a second experiment Hornstra and Vles (32) showed that linoleic acid also reduced the atherogenic effects of *trans* fatty acids as it does the atherogenicity of saturated fatty acids.

In two other animal studies, one with swine (33) and the other with Vervet monkeys (34), no significant differences in incidence and severity of atherosclerosis were found between animals fed partially hydrogenated fats and those fed control fats (oleic acid rich fats).

In conclusion, when *trans* fatty acids are compared with other fatty acids in well designed studies, it appears that they are certainly no more atherogenic than saturated fatty acids. The atherogenic effects of *trans* fatty acids are reduced by linoleic acid as are those of saturated fats.

The long-term experiments described in an earlier section never gave any indications of carcinogenic effects of *trans* fatty acids. Moreover, four additional studies on the tumor promoting effects of hydrogenated fats have been performed. These recent studies, two with mice (35,36) and two with rats (37,38), fed well balanced diets, showed no more tumors in the animals fed *trans* fatty acids than in those fed either *cis*-monounsaturated or saturated fatty acids.

5. BIOCHEMICAL ASPECTS OF *TRANS* FATTY ACIDS

In the first part of this review it has been discussed that the results of the long-term studies show that *trans* fatty acids have no

specific effects. For the evaluation of biochemical or physiological aspects of *trans* fatty acids, they can therefore be regarded as members of the fatty acid family. However, to regard *trans* fatty acids as one single group of fatty acids, as is often done in discussions on partially hydrogenated oils, is an oversimplification. A distinction has to be made, from a biochemical point of view, between different types of *trans* fatty acids. During hydrogenation of vegetable oils mainly C18 *trans* fatty acids are formed.
Two types can be differentiated: *trans* fatty acids with one double bond (isomers of oleic acid) and *trans* fatty acids with two double bonds (isomers of linoleic acid). The latter group consists of fatty acids with one *trans* double bond (*cis*,*trans* or *trans*, *cis*) or two *trans* double bonds (*trans*,*trans*, eg. 9*t*,12*t*-linoleic acid = linolelaidic acid).

Emken (39,40) recently reviewed the biochemistry of *trans* fatty acids. In vivo investigations have indicated that hydrogenated fats and specific fatty acid isomers can influence the activity of the desaturases, elongases, acyltransferases, oxygenases and prostaglandin synthetases. This is often interpreted as an indication of undesirable effects of *trans* fatty acids on essential fatty acid metabolism (41,42). Indeed, in essential fatty acid deficiency *trans* fatty acids aggravate the characteristic symptoms of this disease (43). For proper lipid metabolism, however, the organism needs to have ample choice of fatty acids; the more the dietary composition restricts the variation of fatty acids available to the organism, the more limited the possibilities to carry out proper metabolic processes. For example, in the absence of essential fatty acids dietary stearic and oleic acid can be readily converted into a polyunsaturated fatty acid: Δ9,12,15-eicosatrienoic acid. Elaidic acid can only be converted marginally to a polyunsaturated fatty acid (44). Because large amounts of dietary fatty acids (any fatty acid) inhibit the fatty acid synthesis, *trans* fatty acids fed to EFA-deficient animals in considerable amounts, decrease the animals' ability to produce polyunsaturated fatty acids and as a result will aggravate the EFA-deficiency symptoms. However, if sufficient

amounts of linoleic acid are fed, *trans* monoenoic fatty acids do not adversely influence these lipid metabolizing enzymes (45).

The effects of 9-*trans*, 12-*trans*-18:2 appears to be more potent than those of other *trans* fatty acids, but this fatty acid is of no nutritional importance because it is present in the human diet in trace amounts only (46).

Trans fatty acids can be incorporated in biomembranes of all the investigated human and animal tissues. Extensive data is reported on the incorporation of both monoenoic *trans* fatty acids (47-51) and dienoic *trans* fatty acids (41,52) in lipid classes of many organs. The amount of *trans* fatty acids present in biomembranes largely depends on the amount in the diet, but even when very high amounts are fed, no excessive deposition occurs.

Emken states in his review (40) that large amounts of partially hydrogenated vegetable oils generally decrease the arachidonic acid content in tissue phospholipids somewhat. So *trans* fatty acids are capable of altering the fatty acid composition of biomembranes. But, even in much smaller amounts, some other fatty acids like α-linolenic acid and eicosapentaenoic acid have a much more profound effect on membrane arachidonic acid content than *trans* fatty acids (53,54).

More important than the fact that the fatty acid composition of biomembranes can be changed, is the question whether the function of those membranes is also altered. The mere fact that the fatty acid compostion of a biomembrane is altered does not neccesarily imply that its function is disturbed. The fatty acid composition of the mitochondrial membrane for example can be changed by feeding *trans* fatty acids (48,55). Despite these changes, the oxidative phosphorylation of mitochondria, which is strongly dependent on a high structural integrity of the inner mitochondrial membrane, is not influenced (55,56).

6. LINOLEIC ACID REQUIREMENT IN *TRANS* FATTY ACIDS-FED RATS

As is apparent from the previous part of this paper and from recent reviews dealing with the biological effects of *trans* fatty acids

(4,11,39,46) no undesirable effects occurred provided a sufficient amount of essential fatty acids (linoleic acid) was present in the diet. To define the minimum requirement for linoleic acid necessary to prevent specific or adverse effects (like aggravation of essential fatty acid deficiency) of *trans* fatty acids, we performed two rat feeding studies.

In the first study 8 groups of weanling, male SPF Wistar rats were fed semi-synthetic diets (with 40% of energy from fat) for 3 months. Six of those groups received a diet containing large amounts of *trans* fatty acids (around 50 % of total fat) as present in a partially hydrogenated soybean oil. By using variable amounts of sunflower seed oil, olive oil or coconut oil, the linoleic acid content of the diets varied from 0.4 to 7.1% of total energy. One group was fed a diet containing 40% of energy as olive oil (giving 2 energy% linoleic acid); and finally one group received a diet with a mixture of coconut oil and olive oil (giving 5 energy% linoleic acid content). The last two groups served as references.

Growth, food- and water- consumption were not systematically different and neither did (histo-) pathological examination revealed any abnormalities in any of the groups. After the feeding period the fatty acid composition of phospholipids of heart and liver mitochondria, blood platelets and segments of aorta were investigated. It was observed that *trans* fatty acids were incorporated in all investigated phospholipids and that the content of *trans* monoenoic acids was not influenced by the amount of linoleic acid in the diet. The level of *trans* isomers of linoleic acid was low, and tended to decrease with increasing amounts of linoleic acid in the diet. The linoleic acid content in these phospholipids hardly changed with the increase of its content in the diet.

The arachidonic acid level in the phospholipids generally increased with increasing amounts of dietary linoleic acid, and reached a constant level at a dietary linoleic acid level between 2 and 5 energy%, depending on the tissue and phospholipid class. Surprisingly, the arachidonic acid level in some phospholipids was lower in the reference-

group fed olive oil than in the reference-group fed the mixture of coconut and olive oil, despite the higher linoleic acid level in the first diet (5 and 2 energy% respectively).

Trans fatty acids increased the linoleic acid level of all investigated phospholipids. The arachidonic acid level in most phospholipids was lower in the group fed *trans* fatty acids and 2 energy% linoleic acid than in the reference-group fed the same amount of linoleic acid. With 5 energy% linoleic acid in the diet the effect of *trans* fatty acids on phospholipid arachidonic acid levels had disappeared or had become significantly smaller. This confirms other investigations in which it was found that *trans* fatty acids increase the linoleic acid level and decrease the arachidonic acid level in phospholipids (45,57). This effect decreases when the linoleic acid content of the diet is increased.

As stated before, much more important than the observation that *trans* fatty acids can influence the essential fatty acid composition of membrane phospholipids is whether *trans* fatty acids also influence the membrane functions. For this purpose we measured the respiration capacity and ATP-synthesis of liver and heart mitochondria. These functions are closely linked to the mitochondrial membrane and are therefore sensitive to changes in the composition of that membrane. Despite significant changes in the fatty acid composition of the mitochondrial membranes, the respiration and ATP-synthesis were not significantly influenced by the dietary *trans* fatty acids at any level of dietary linoleic acid investigated. This is in agreement with other investigations, in which it was found that dietary *trans* fatty acids in the presence of sufficient linoleic acid did not decrease the mitochondrial function (55,56).

The effect of *trans* fatty acids on eicosanoid synthesis was investigated by measuring the production of both lipoxygenase and cycloxygenase products by stimulated blood platelets and aorta-segments, as described by Hornsta et al. (54). The production of both hydroxy eicosatetraenoic acid and hydroxy heptadecatrienoic acid (indicative of lipoxygenase and cyclooxygenase activity, respectively) by blood platelets and prostacyclin (measured in a bio-assay) by segments of

aorta was linear with the arachidonic acid level in the phospholipid fraction. Because *trans* fatty acids lowered the arachidonic acid level, they also reduced the production of these eicosanoids. However, this decrease was not greater than could be predicted from the decrease in the arachidonic acid level, indicating that *trans* fatty acids do not have a direct effect on the prostaglandin or hydroxy fatty acid production. Neither did Blomstrand et al. (45) find any interference of high amounts of *trans* fatty acids with platelet cyclooxygenase and lipoxygenase activity provided sufficient amounts of linoleic acid were available.

In a following experiment we wanted to compare the effects of *trans* fatty acids with those of saturated or *cis*-monounsaturated fatty acids at the level of 2 energy% linoleic acid. In that experiment we investigated the same parameters as described above, in four groups of 40 male, weanling Wistar rats. The animals received semi-synthetic diets containing 40% of energy as fat. Three of the four dietary fat blends were composed mainly of a partially hydrogenated soybean oil (containing 50% of total fat as *trans* fatty acids), olive oil (special variety with only 5.5 % linoleic acid and nearly 80 % oleic acid) or cocoabutter (containing a high amount of saturated fatty acids), respectively. The fourth fat blend was a mixture of the other three, in order to obtain a diet with half the amount of *trans* fatty acids (25% of total fat) compared with the first blend. All blends were prepared in such a way as to give diets containing approximately 2 energy% linoleic acid.

We found that *trans* fatty acids decreased the arachidonic acid level in phospholipids when compared with saturated fatty acids; compared with *cis*-monounsaturated fatty acids the arachidonic acid level was equal or only slightly lower. Linoleic acid levels were generally increased by *trans* fatty acids. No systematic effects of *trans* fatty acids were found on the mitochondrial function or on the eicosanoid production by blood platelets and segments of aorta. From the results of these experiments we concluded that 2 energy% of linoleic acid is sufficient to prevent undesirable effects of dietary *trans* fatty acids.

7. CONCLUSIONS

From the recent reviews dealing with *trans* fatty acids and our own work in this field, we conclude that in view of the level of linoleic acid in the average diet (normally more than 2 energy%) *trans* fatty acids (average amount in the human diet less than 5 energy%) do not present a nutritional problem. The recent review of the ad-hoc panel of the FASEB (Federation of American Societies for Experimental Biology) (17) supports this conclusion by stating: "The available scientific information suggests little reason for concern with the safety of dietary *trans* fatty acids both at their present and expected levels of consumption and at the present and expected levels of consumption of linoleic acid".

8. REFERENCES

1. Applewhite, T.H. (1981) Nutritional effects of hydrogenated soya oil. J. Am. Oil Chem. Soc. 58, 260-269.

2. USDA (1980) US Department of Agriculture, Science and Education Administration: Food and Nutrient Intake of Individuals in 1 day in the United States, Spring 1977. Nationwide food consumption survey 1977-1978, Preliminary Report no. 2, September, Washington, DC, US Department of Agriculture.

3. Dutton, H.J. (1979) Hydrogenation of fats and its significance, in: Geometric and Positional Fatty Acid Isomers (eds. E.A. Emken and H.J. Dutton), pp. 1-16, American Oil Chemists' Society, Champaign, Illinois.

4. Gottenbos, J.J. (1983) Biological effects of *trans* fatty acids. in: Dietary fats and health. (eds. Perkins, E.G. and Visek, W.J.) pp. 375-390, American Oil Chemists' Society, Champaign, Illinois.

5. Sauer, F.D. and Kramer, J.K.G. (1983) In: High and low erucic acid rapeseed oils (eds. Kramer, J.K., Sauer, F.D. and Pigden, W.J.), pp. 253-292, Academic Press, New York.

6. Heckers, H. and Melcher F.W. (1978) *Trans*-isomeric fatty acids present in West German margarines, shortenings, frying and cooking fats. Am. J. Clin. Nutr. 32, 1041-1049.

7. Thomas, L.H., Jones, P.R., Winter, J.A. and Smith, H. (1981) Hydrogenated oils and fats: the presence of chemically modified fatty acids in human adipose tissue. Am. J. Clin. Nutr. 34, 877-886.

8. Davignon, J., Holub, B., Little, J.A., McDonald, B.E. and Spence, M. 1980) Report of the Ad Hoc Committee on the Composition of Special Margarines. Dept. of Health and Welfare, Minister of Supply and Services, Ottawa, Canada.

9. Enig, M.G., Pallansch, L.A., Sampugna, J. and Keeney, M. (1983) Fatty acid composition of the fat in selected food items with emphasis on *trans* fatty acids. J. Am. Oil Chem. Soc. 60, 1788-1795.

10. Slover, H.T., Thompson, R.H. Jr., Davis, C.S. and Merola, G.V. (1985) Lipids in margarines and margarine-like foods. J. Am. Oil Chem. Soc. 62, 775-786.

11. Gurr, M.I. (1983) *Trans* fatty acids. International Dairy Federation Document 166, 5-18.

12. Katan, M.B., van Bovenkamp, P. and Brussaard, J.H. (1983) Fatty Acid Composition, *Trans* Fatty Acid and Cholesterol Content of Margarines and other Edible Fats. In: Voedingsmiddelenanalyses van de Vakgroep Humane Voeding, Landbouwhogeschool, Wageningen, The Netherlands.

13. Kochar, S.P. and Matsui, T. (1984) Essential fatty acids and *trans* content of some oils, margarine and other food fats. Food Chemistry 13, 85-101.

14. Druckrey, F., Høy, C.-E. and Hølmer, G. (1985) Fatty acid composition of Danish margarines. Fette Seifen Anstrichm. 87, 350-355.

15. Parodi, P.W. (1976) Distribution of isomeric octadecenoic fatty acids in milk fat. J. Dairy Sci. 59, 1870-1873.

16. Sommerfeld, M. (1983) *Trans* unsaturated fatty acids in natural products and processed foods. Prog. Lipid Res. 22, 221-233.

17. FASEB Report of the ad-hoc Committee of the Federation of American Societies for Experimental Biology (1985) Health Aspects of Dietary *Trans* Fatty Acids (ed. F.R. Senti) Contract Number FDA 223-83-2020.

18. Heckers, H., Melcher, F.W. and Dittmar, K. (1979) Zum Täglichen Verzehr *trans*isomere Fettsäuren. Eine Kalkulation unter Zurgrundelegung der Zusammensetzung handelsüblicher Fette und verschiedener menschlicher Depotfette. Fette Seife Anstrichm. 6, 217-226.

19. Åkesson, B., Johansson, B.M., Eng, M., Svensson, M. and Ockerman, P.A. (1981) Content of *trans*-octadecenoic acid in vegetarian and normal diets in Sweden, analyzed by the Duplicate Portion technique. Am. J. Clin. Nutr. 34, 2517-1520.

20. Deuel, H.J., Hallman, L.F. and Movitt, E. (1945) Studies on the comparative nutritive value of fats. VI. Growth and reproduction over 10 generations on Sherman diet B where butterfat was replaced by a maragrine fat. J. Nutr. 29, 309-320.

21. Deuel, H.J., Greenberg, S.M., Savage. E.E. and Bavetta, L.A, (1950) Studies on the comparative nutritive value of fats. J. Nutr. 42, 239-255.

22. Alfin-Slater, R.B., Wells, A.F., Aftergood, L. and Deuel, H.J. (1957) Nutritive value and safety of hydrogenated vegetable fats as evaluated by long-term feeding experiments with rats. J. Nutr. 63, 241-261.

23. Alfin-Slater, R.B., Wells, A.F. and Aftergood, L. (1970) Longevity and multigeneration studies in rats fed hydrogenated fats of increasing polyunsaturated fat content. J. Am. Oil Chem. Soc. 47, 274.

24. Alfin-Slater, R.B., Aftergood, L. and Whitten, T. (1976) Nutritional value of *trans* fatty acids. J. Am. Oil Chem. Soc. 53, 658A.

25. Alfin-Slater, R.B., Wells, A.F. and Aftergood, L. (1973) Dietary fat composition and tocopherol requirement IV. Safety of polyunsaturated fats. J. Am. Oil Chem. Soc. 50, 479-484.

26. Vles, R.O. and Gottenbos, J.J. (1972) Long-term effects of feeding butterfat, coconut oil, and hydrogenated or non hydrogenated soyabean oils. I. Eighteen-month experiment in mice. Voeding 33, 428-433.

27. Vles, R.O. and Gottenbos, J.J. (1972) Long-term effects of feeding butterfat, coconut oil, and hydrogenated or non hydrogenated soyabean oils. II. Life-span experiment in rats. Voeding 33, 455-465.

28. Vles, R.O., Gottenbos, J.J. and van Pijpen, P.L. (1977) Aspects nutritionels des huiles de soja hydrogenees et de leur acides gras insatures isomeriques. Bibl. Nutr. Dieta 25, 186-196.

29. Nolen, G.A., Alexander, J.C. and Atman, N.R. (1967) Long-term rat feeding study with used frying fats. J. Nutr. 93, 337-348.

30. Mattson, F.H., Hollenbach, E.J. and Klingman, A.M. (1975) Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels in man. Am. J. Clin. Nutr. 28, 726-731.

31. Vergroesen, A.J. and Gottenbos, J.J. (1975) The role of fats in human nutrition: an introduction. in: The role of fats in human nutrition. (Ed. Vergroesen, A.J.), pp. 1-41, Academic press, New York.

32. Hornstra, G. and Vles, R. (1978) Effects of dietary fat on atherosclerosis and thrombosis. in: International Conference on Atherosclerosis (eds. Carlson, L.A. et al.), Raven Press, New York.

33. Royce, S.M., Holmes, R.P., Takagi, T. and Kummerow, F.A. (1984) The influence of dietary isomeric and saturated fatty acids on atherosclerosis and eicosanoid synthesis in swine. Am. J. Clin. Nutr. 39, 215-222.

34. Kritchevsky, D., Davidson, L.M, Weight, M., Kriek, N.P.J. and du Plessis, J.L. (1984) Effect of *trans*-unsaturated fats on experimental atherosclerosis in vervet monkeys. Atherosclerosis 51, 123-133.

35. Brown, R.R. (1981) Effects of dietary fat on incidence of spontaneous and induced cancer in mice. Cancer Res. 41, 3741-3742.

36. Erickson, K.L., Schlanger, D.S., Adams, D.A., Fregeau, D.R. and Stern, J.S. (1984) Influence of dietary fatty acid concentration and geometric configuration on murine mammary tumorigenesis and experimental metastasis. J. Nutr. 114, 1834-1842.

37. Selenskas, S.L., Ip, M.M. and Ip, C. (1984) Similarity between *trans* fat and saturated fat in the modification of rat mammary carcinogenesis. Cancer Res. 44, 1321-1326.

38. Watanabe, M., Koga, T. and Sugano, M. (1985) Influence of dietary *cis*- and *trans*- fat on 1,2-dimethylhydrazine-induced colon tumors and fecal steroid excretion in Fischer 344 rats. Am. J. Clin. Nutr. 42, 475-484.

39. Emken, E.A. (1983) Biochemistry of unsaturated fatty acid isomers. J. Am. Oil. Chem. Soc. 60, 995-1004.

40. Emken, E.A. (1984) Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. Annu. Rev. Nutr. 4, 339-376.

41. Kinsella, J.E., Bruckner, G. Mai. J. and Shimp, J. (1981) Metabolism of *trans* fatty acids with emphasis on the effects of *trans*,*trans*-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview. Am. J. Clin. Nutr. 34, 2307- 2318.

42. Holman, R.T., Mahfouz, M.M., Lawson, L.D. and Hill, E.G. (1983) Metabolic effects of isomeric octadecenoic acids. in: Dietary fats and health. (eds. Perkins, E.G. and Visek, W.J.), pp. 320- 340, American Oil Chemists' Society, Champaign, Illinois.

43. Holman, R.T. and Aaes-Jorgensen, E. (1956) Effects of *trans* fatty acid isomers upon essential fatty acid deficiency in rats. Proc. Soc. Exp. Biol. Med. 93, 175-179.

44. Mahfouz, M.M., Valicenti, A.M. and Holman, R.T. (1980) Desaturation of isomeric *trans*-octadecenoic acids by rat liver microsomes. Biochim. Biophys. Acta 618, 1-12.

45. Blomstrand, R., Diczfalusy, U., Sisfontes, L. and Svensson, L. (1985) Influence of dietary partially hydrogenated vegetable and marine oils on membrane composition and function of liver microsomes and platelets in the rat. Lipids 20, 283-295.

46. Beare-Rogers, J.L. (1983) *Trans-* and positional isomers of common fatty acids in: Advances in nutritional research. (ed. Draper, H.) Vol. 5., pp. 171-200, Plenum Publishing Corporation, New York.

47. Alfin-Slater, R.B. and Aftergood, L. (1979) Nutritional role of hydrogenated fats (in rats). In: Geometric and Positional Fatty Acid Isomers (eds. E.A. Emken and H.J. Dutton), pp. 53-74, American Oil Chemists' Society, Champaign, Illinois.

48. Høy, C.-E. and Hølmer, G. (1979) Incorporation of *cis-* and *trans-* octadecenoic acids into the membranes of rat liver mitochondria. Lipids 4, 717-733.

49. Wood, R. (1979) Incorporation of dietary *cis* and *trans* octadecenoate isomers in the lipid classes of various rat tissues. Lipids 14, 975- 982.

50. Emken, E.A. (1981) Influence of *trans*-9-, *trans*-12-, and *cis*-12- octadecenoic acid isomers on fatty acid composition of human plasma lipids. Prog. Lipid Res. 20, 135-141.

51. Ohlrogge, J.B., Gulley, R.M. and Emken, E.A. (1982) Occurence of octadecenoic fatty acid isomers from hydrogenated fats in human tissue lipid classes. Lipids 17, 552-557.

52. Anderson, R.L., Fullmer, C.S. and Hollenbach, E.J. (1975) Effects of the *trans* isomers of linoleic acid on the metabolism of linoleic acid in rats. J. Nutr. 105, 393-400.

53. Høy, C.-E, Hølmer, G., Kauer, N., Byrjalsen, I. and Kirstein, D. (1983) Acyl group distributions in tissue lipids of rats fed evening promrose oil (γ-linolenic plus linoleic acid) or soybean oil (α-linolenic plus linoleic acid). Lipids 18, 760-771.

54. Hornstra, G., Christ-Hazelhoff, E., Haddeman, E., ten Hoor F. and Nugteren, D.H. (1981) Fish oil feeding lowers thromboxane- and prostacyclin production by rat platelets and aorta and does not result in the formation of prostaglandin I3. Prostaglandins 21, 727-737.

55. Blomstrand, R. and Svensson, L. (1983) The effects of partially hydrogenated marine oils on the mitochondrial function and membrane phospholipid fatty acids in rat heart. Lipids 18, 151-170.

56. Royce, S.M. and Holmes, R.P. (1984) The saturation and isomerization of dietary fatty acids and the respiratory properties of rat heart mitochondria. Biochim. Biophys. Acta 792, 371-375.

57. Lawson, L.D., Hill, E.G, and Holman, R.T. (1985) Dietary fats containing concentrates of *cis* or *trans* octadecenoates and the patterns of polyunsaturated fatty acids of liver phosphatidylcholine and phosphatidylethanolamine. Lipids 20, 262-267.

Chapter 8.

GENERAL DISCUSSION AND CONCLUSIONS

8. GENERAL DISCUSSION AND CONCLUSIONS

As discussed in Chapters 1 and 7, at the start of the work described in this thesis, most reviews on *trans* fatty acids agreed that these isomeric fatty acids do not induce undesirable effects, provided sufficient linoleic acid is present in the diet. Our first objective was to determine the minimal amount of linoleic acid required to prevent specific or adverse effects of *trans* fatty acids.

Secondly, since it was obvious from many studies that *trans* fatty acids could influence linoleic acid metabolism (see Chapter 1), we wanted to gain more insight into effects of *trans* fatty acids on linoleic acid metabolism.

8.1. Linoleic acid requirement of rats fed *trans* fatty acids

To fulfil the first objective, we started with a feeding study with a dose-response set-up in male Wistar rats. For three months, eight groups of animals were fed semi-synthetic diets with 40% of energy (en%) from fat. Six of those groups received a diet containing a large amount of *trans* fatty acids (20 en%) as present in a special partially hydrogenated soybean oil. By using variable amounts of sunflower seed oil, olive oil or coconut oil, the linoleic acid content of these diets was varied from 0.4 to 7.1 en%. Two groups of animals served as references as they were fed diets without *trans* fatty acids: one group was given a mixture of olive oil and coconut oil (rich in oleic acid and medium chain saturated fatty acids) with 2 en% linoleic acid, the other an olive oil, rich in oleic acid, with 5 en% linoleic acid.

By comparing the *trans* fatty acids-fed groups with the two reference groups with identical linoleic acid levels (either 2 or 5 en% linoleic acid), we concluded that *trans* fatty acids did not affect growth or water- or food-consumption, nor did they induce essential fatty acid deficiency symptoms (see Chapter 2). The trans-epidermal water loss was

not increased by dietary *trans* fatty acids, thus indicating no effect on skin functionality. The clinical observation of the animals the examination at autopsy, the organ weights and the histo-pathological examinations did not give any indication of adverse effects of *trans* fatty acids. None of the clinical chemistry or haematological parameters investigated were affected. These observations are in line with many other studies with *trans* fatty acids in which sufficient linoleic acid was used (Alfin-Slater et al, 1957; Thomasson et al, 1966; Nolen et al, 1967; Vles and Gottenbos, 1972; Nolen, 1981; Bruckner et al, 1983). Moreover, they indicate that 2 en% linoleic acid is sufficient to prevent the aggravation of EFA-deficiency symptoms as was found after feeding *trans* fatty acids to EFA-deficient rats, such as poor growth, decrease in food intake, deteriorated skin, haematological changes and impairment of spermatogenesis (Aaes-Jørgensen and Hølmer, 1969; Privett et al, 1977; Hill et al, 1979; Bruckner et al, 1983). This part of the experiment closely resembled a short-term toxicity study: a method commonly used to evaluate the safety of food ingredients (Food Safety Council, 1978).

Trans fatty acids might have effects on the functionality of organs or organelles, without apparent effects on tissue structure or changes in haematological or clinical chemistry parameters. From the many function tests available, we chose a widely used and rather sensitive test to evaluate effects of diet on membrane functionality: mitochondrial respiration. Neither at the 2 en% nor at the 5 en% linoleic acid level, we found any effect of 20 en% *trans* fatty acids on the liver and heart mitochondrial oxygen uptake, ADP/O-ratio or the respiratory control ratio (see Chapter 3). This is in agreement with other studies with *trans* fatty acids in non-essential fatty acid deficient animals (Blomstrand and Svensson, 1983; Entressangles et al, 1984; Royce and Holmes, 1984) and indicates that 2 en% linoleic acid is enough to prevent effects of *trans* fatty acids on mitochondrial function. In this respect the incorporation of *trans* fatty acids in the mitochondrial membranes does not have consequences such as were found upon incorporation of very-long-chain monounsaturated fatty acids which were shown to depress the rate of ATP-synthesis (Houtsmuller et al, 1970; Clandinin, 1978; Renner et al, 1979).

Because it has frequently been suggested that *trans* fatty acids interfere with eicosanoid metabolism, we were particularly interested in the determination of the in vitro production of eicosanoids by platelets and aorta pieces (Chapter 4).
The hydroxy fatty acid-production (HHT and 12-HETE) by stimulated platelets did not differ between the *trans* fatty acids fed-groups and the reference groups. With 5 en% linoleic acid, no effect of *trans* fatty acids could be observed on the prostacyclin-production by pieces of aorta, as measured by a bio-assay. This has also been reported by Blomstrand et al. (1985). However, at the 2 en% linoleic acid level, we found a significantly lower production of prostacyclin by pieces of aorta in the group fed *trans* fatty acids compared to that in the reference group fed the mixture of olive oil and coconut oil. This indeed suggests an effect of *trans* fatty acids on eicosanoid synthesis. As will be discussed below, this may be due to an effect of these isomeric fatty acids on the aorta fatty acid composition.
Platelet aggregation is a process highly influenced by eicosanoids (see for reviews Needleman et al, 1976 and 1979) and, therefore, might be affected by dietary *trans* fatty acids. However, in our experiment the delay of the in vitro platelet aggregation, induced with three different doses of collagen, was not influenced by *trans* fatty acids.

It is well established that dietary lipids can affect tissue lipid composition, but in general only extreme measures (e.g. EFA-deficiency) may affect phospholipid class composition. Our studies indeed did not reveal a change in phospholipid classes, but the fatty acid composition was significantly affected by the feeding of *trans* fatty acids. Irrespective of the dietary linoleic acid level, these isomeric fatty acids are incorporated in all tissues and organelles we have investigated. The most striking effects of dietary *trans* fatty acids were on phospholipid levels of the essential fatty acids linoleic and arachidonic acid. The 18:2-fraction was increased in the phospholipids of all tissues examined, at all levels of dietary linoleic acid.

Capillary GLC clearly demonstrated that this increase in 18:2 was mainly due to an increase in 9*c*,12*c*-18:2 (linoleic acid), the precursor of all n-6 fatty acids. Incorporation of *trans* isomers of linoleic acid was very low. The level of arachidonic acid, the precursor of the eicosanoids measured in our study, was (modestly) decreased by *trans* fatty acids when the dietary level of linoleic acid was 2 en%. But at a dietary linoleic acid level of 5 en% this decrease was clearly diminished. As the in vitro biosynthesis of prostacyclin has been shown to be a linear function of the arachidonic acid level in aorta phospholipid (Hornstra et al, 1983 and see Chapter 4), this could provide an explanation for the lower production of prostacyclin by aorta pieces at the lower linoleic acid intake. A remarkable exception to the prevailing decrease in arachidonic acid was the rise of this fatty acid in PE of heart mitochondria, indicating that the effects of *trans* fatty acids on linoleic acid metabolism are very complex, as will be discussed in the next paragraph.

In our study the dietary linoleic acid level had generally more effect on the parameters investigated than *trans* fatty acids. The lowest linoleic acid level induced the highest trans-epidermal water loss (Chapter 2), suggesting that this level was indeed marginally EFA-sufficient only (Houtsmuller, 1975; Houtsmuller and van der Beek, 1981). An increase of the dietary linoleic acid level seemed to raise fat deposition in the liver; moreover it influenced the serum activity of two liver enzymes (sorbitol dehydrogenase and alkaline phosphatase). This may suggest an effect of linoleic acid intake on liver fat metabolism (Ringler and Dabich, 1979).
Although severe EFA-deficiency is known to impair mitochondrial respiration (Levin et al, 1957; Holman, 1968; Wollbeck et al, 1981), even the lowest dose of linoleic acid (0.4 en%) appeared to be sufficient for proper functioning of mitochondria in our experiment (Chapter 3). For both in vitro platelet aggregation and eicosanoid biosynthesis (HHT, 12-HETE and PGI_2) dose-response relationships with the dietary level of linoleic acid were obtained (Chapter 4). As could

be expected, the fatty acid composition of tissue lipids, particularly the content of polyunsaturated fatty acids, was clearly influenced by dietary linoleic acid (Chapters 3 and 4). The main effect of increasing the linoleic acid intake was a rapid increase in arachidonic acid level, which flattened off when the amount of linoleic acid in the diet was 2 - 3 en% (for an example see Fig. 1). This effect seemed to be similar in most tissues investigated. Dietary linoleic acid did generally not augment its own concentration in tissue phospholipids.

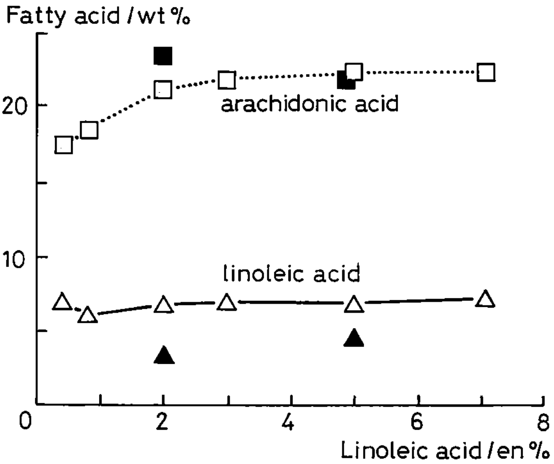

Fig. 1 Effect of increasing dietary levels of linoleic acid on tissue polyunsaturated fatty acid composition.
As an example the changes in total phospholipid of aorta are shown (data from first feeding experiment, see Chapter 4).
□, △ indicate the trans fatty acids-fed groups; ■, ▲ indicate the two reference groups. The values given are the mean of two pools of six animals per group.

One unexpected finding in this experiment was that in the reference group fed the mixture of hydrogenated coconut oil and olive oil with 2 en% linoleic acid, the phospholipid arachidonic acid level in platelets

and aorta was higher than that in the group fed olive oil with 5 en% linoleic acid. This suggested an arachidonic acid-increasing effect of the hydrogenated coconut oil compared to olive oil. As the saturated fatty acids in the former fat are mainly of medium chain length (C8-C12), we could not compare the effects of long-chain *trans* fatty acids (C16,C18) with long-chain saturated fatty acids. The metabolism of medium-chain fatty acids differs considerably from that of long-chain fatty acids (see for a review Bach and Babayan, 1982) which might well explain divergencies in effects on linoleic acid metabolism. The high arachidonic acid level caused by this diet resulted in a prostacyclin-production which was significantly higher than that in the olive oil-fed group, despite of a higher intake of linoleic acid in the latter group.

In conclusion, the first rat feeding study demonstrated that 2 en% linoleic acid is sufficient to prevent effects of a high level (20 en%) of *trans* fatty acids as present in partially hydrogenated soybean oil on parameters commonly used to evaluate safety of food ingredients. This supplemented the recent consensus on the general safety of *trans* fatty acids present in partially hydrogenated vegetable oils (FASEB, 1985; BNF, 1987). Moreover, we did not observe any effects of dietary *trans* fatty acids on membrane function or platelet aggregation, despite of significant alterations in membrane fatty acid composition. The capacity of *trans* fatty acids to influence linoleic acid metabolism is apparent from changes in the levels of various n-6 fatty acids in tissue phospholipids. Higher levels of linoleic acid diminished these effects. Differences in arachidonic acid level in tissue phospholipids affect in vitro biosynthesis of eicosanoids and are presumed to explain the lower in vitro biosynthesis of prostacyclin by pieces of aorta after feeding *trans* fatty acids at the 2 en% linoleic acid level.

In the first experiment we used one reference diet with 2 en% linoleic acid only. This diet contained a high level of medium-chain saturated fatty acids. In the second experiment we wanted to make a direct

comparison between long-chain *trans* fatty acids (C16-C18) and long-chain saturated and *cis*-monounsaturated fatty acids with respect to largely the same parameters as in the first experiment (described in Chapters 2-4).

For the *trans* fatty acid-containing diet, we chose the diet of the first experiment containing 20 en% *trans* fatty acids and 2 en% linoleic acid. One group of rats was fed a diet composed among others with a special olive oil, a very rich source of *cis*-monounsaturated fatty acids (mainly oleic acid) with only 5% linoleic acid, giving a diet with 2 en% linoleic acid. Cocoabutter, rich in C16 and C18 saturated fatty acids, was the main fat in the third diet. Another group was fed a diet with half the content of *trans* fatty acids (10 en%). This group was included to get some insight into the effect of the dose of *trans* fatty acids on the parameters investigated. To obtain such a diet with only 10 en% *trans* fatty acids, a mixture was prepared of the three dietary fats mentioned before.

Because of possible effects of the type of fat on fat metabolism in the liver, as suggested by the results of the first experiment, we extended the second experiment by performing enzyme histochemistry in the liver and more detailed clinical chemistry. Moreover, recent suggestions that *trans* fatty acids are more potent inducers of peroxisomal β-oxidation than their *cis* isomers (Thomassen et al, 1982), persuaded us to compare *trans* fatty acids with saturated or *cis*-monounsaturated fatty acids with respect to their effects on mitochondrial and peroxisomal β-oxidation in different tissues.

Trans fatty acids, at both levels of consumption (20 and 10 en%) did not significantly affect in vitro prostacyclin production, hydroxy fatty acid production or heart mitochondrial respiration (Chapters 3 and 4). This confirms our previous conclusion that *trans* fatty acids do not directly influence the enzymes involved in eicosanoid synthesis but exert an effect on eicosanoid formation by influencing tissue precursor levels, i.e. arachidonic acid. Fatty acid analyses of tissue lipids demonstrated similar changes induced by *trans* fatty acids as

observed in the first experiment. In all investigated lipids, the linoleic acid level was increased by feeding *trans* fatty acids. Trans fatty acids diets, compared to the cocoabutter diet, decreased the arachidonic acid level in phospholipids. However, the arachidonic acid level was not or only very slightly decreased by *trans* fatty acids when compared with *cis*-monounsaturated fatty acids. This would explain the absence of significant differences in the in vitro biosynthesis of eicosanoids between the *trans* fatty acids-fed group and the *cis*-monounsaturated fatty acids-fed group in this experiment.
In many respects the deviation of the cocoabutter group from the other three groups was greater than that of the *trans* fatty acids-fed groups from the two reference groups. The effect of cocoabutter on tissue fatty acid compositions was mostly similar to that of the hydrogenated coconut oil/olive oil-diet in the previous experiment. This suggests that the effect on fatty acid metabolism in the latter experiment is not specific for medium-chain fatty acids, but rather that saturated fatty acids in general have different properties in this respect than *cis*- or *trans*-monounsaturated fatty acids.

Trans fatty acids from the partially hydrogenated soybean oil did not influence the peroxisomal or mitochondrial β-oxidative capacity of heart, liver and muscle tissue (Chapter 5). Thomassen et al. (1982) reported a higher peroxisomal activity in rat liver after feeding partially hydrogenated soybean oil. In rat heart, however, a lower activity was observed (Norseth and Thomassen, 1983). Our results prompted us to conclude that high-fat diets only induce peroxisomal β-oxidation significantly if they contain monounsaturated fatty acids with 20 or 22 carbon atoms, irrespective of their configuration (*cis* or *trans*) (Chapter 5). As partially hydrogenated vegetable oils (excluding erucic acid-rich oils such as rapeseed or mustard oil) do not contain substantial amounts of very-long-chain fatty acids (C20, C22), they have no specific effect on peroxisomal β-oxidation (Chapter 5).

The incorporation of *trans* monoenoic fatty acids did not exceed 10% of total fatty acids in any lipid investigated in our experiments, despite the high level of these isomeric fatty acids in the diets used (about 16 en%). This is much lower than the maximum levels reported by others (Wood, 1979; Moore et al, 1980; Masuzawa et al, 1987). The sufficient supply of EFA to our animals may have contributed to the relatively low incorporation of *trans* fatty acids (Hill et al, 1979), but differences in rat strain and type of dietary fat could also have had an effect. The highest incorporation of t-18:1 was found in total liver lipids (9.9%) in the first radiolabel experiment (data not shown). The lowest level was found in aorta phospholipid (3.2%). Trans isomers of linoleic acid were also incorporated in lipids, be it to a much lower degree. Again the total liver lipid fraction incorporated most of these isomers (total *t*-18:2 3.5%). The level of linolelaidic acid (*tt*-18:2) was well below 1% both in heart mitochondrial PC and PE (the only lipids analyzed for this fatty acid). In most experiments with partially hydrogenated vegetable oils, *t*-18:2 levels were either not detected or not determined. A comparison of our groups fed 20 or 10 en% *trans* fatty acids revealed that the incorporation of *trans* isomers in tissue lipids is dose-dependent; this has also been reported by Hill et al. (1979) and Anderson et al. (1975). In our first experiment we analyzed the *t*-18:1 and *t*-18:2 levels in platelet lipid only; no conclusions can therefore be drawn on the effect of dietary linoleic acid on the incorporation of *trans* fatty acids for tissues other than platelets. The level of *trans* dienoic fatty acids seemed to decrease slightly with increasing linoleic acid consumption. This could be expected in view of the competition of some of the *trans* isomers of linoleic acid with linoleic acid for incorporation at the 2-position of phospholipids (Lands et al, 1966). The level of *trans* monoenes in platelets was not affected very much by dietary linoleic acid, as reported by others (Hill et al, 1982; Blomstrand and Svensson, 1983; Blomstrand et al, 1985).

In all lipids investigated, incorporation of *trans* fatty acids led to a decrease in stearic acid level; the palmitic acid level was much less affected. The high preference of both stearic acid and most

trans fatty acids for the 1-position of phospholipids is the most probable explanation (Blomstrand and Svensson, 1983; Blomstrand et al, 1985; Masazuwa et al, 1987).

Hence, irrespective of the linoleic acid level in the diet, *trans* fatty acids were incorporated in all tissues examined. No excessive accumulation of these isomeric fatty acids was observed. But, more importantly, membranes containing *trans* fatty acids did not have impaired functions.

In conclusion, the second experiment fully confirmed our previous findings that 2 en% linoleic acid is enough to prevent undesirable effects of high doses of *trans* fatty acids (20 and 10 en%) as present in partially hydrogenated soybean oil. With that level of linoleic acid in the diet, *trans* fatty acids, compared to *cis*-monounsaturated fatty acids, did not induce changes in parameters selected to assess the safety of food components, on membrane functioning (mitochondrial respiration), platelet aggregation, eicosanoid biosynthesis (HHT, 12-HETE and PGI_2) or peroxisomal and mitochondrial β-oxidation. Compared to saturated fatty acids, only a lower HETE-production by platelets was found.

On the basis of criteria like normal growth, absence of dermal symptoms and a ratio of 20:3n-9/20:4n-6 of below 0.4, Holman (1968) stated that the minimal requirement for linoleic acid in growing rats is 1 - 2 en%. Others have come to similar requirements (Pudelkewitz et al, 1968; Alling et al, 1972; Wollbeck et al, 1981). In view of our conclusion that *trans* fatty acids do not affect any criterium of good health when 2 en% linoleic acid is present, this would imply that *trans* fatty acids do not appreciably increase linoleic acid requirement in rats.

8.2. Effects of *trans* fatty acids on linoleic acid metabolism

The two feeding experiments with radiolabeled linoleic acid (described in Chapter 6) were designed to give us information on the effects of *trans* fatty acids on linoleic acid metabolism in vivo. If we assume

that the absolute amount of labeled arachidonic acid in liver phospholipids, measured 24 h after the intubation of labeled linoleic acid, reflects its biosynthesis by the liver (for explanation see Chapter 6b), these experiments point to significant differences in the effects of dietary fats on arachidonic acid synthesis. In the first experiment (Chapter 6a) we did not actually measure the absolute amounts of radiolabeled fatty acids in total liver lipid. We only determined the relative distribution of label over the fatty acids of liver lipid. But, as is evident from our second experiment, the effects of the dietary fats on the total incorporation of label in liver lipid were smaller than the effects on the distribution of label over the fatty acids. We therefore feel confident that both experiments can be used to evaluate effects of diet on arachidonic acid biosynthesis.

In both experiments, the diets with *trans* fatty acids (PHSO-diets) reduced arachidonic acid biosynthesis compared to the diets rich in *cis*-monounsaturated fatty acids (OV-diets). But also the diet rich in (medium-chain) saturated fatty acids (HCHO/OV-diet) and the α-linolenic acid-containing diet (LN/CB/CN-diet) reduced arachidonic acid biosynthesis compared to the OV-diets.
An inhibiting effect of α-linolenic acid on the in vitro Δ6-desaturation of linoleic acid has been well described (Brenner and Peluffo, 1966; see Chapter 1). Also in vivo this n-3 fatty acid has a strong influence on linoleic acid metabolism (Mohrhauer and Holman, 1963; De Schrijver and Privett, 1982; Holman et al, 1983). Therefore, the low conversion of radiolabeled linoleic acid to arachidonic acid after feeding a diet rich in α-linolenic acid (LN/CB/CN-diet; second label experiment) did not surprise us. However, the significant decrease in arachidonic acid biosynthesis caused by the replacement of about 45% of dietary oleic acid by (mainly medium-chain) saturated fatty acids (HCNO/OV-diet versus OV-diet in the first label experiment), was rather unexpected. Saturated fatty acids are generally considered to be neutral towards arachidonic acid biosynthesis. The inhibitory effect of the *trans* fatty acids-containing diet (PHSO-diet) was just as strong as that of the α-

linolenic acid rich-diet (LN/CB/CN). This could not be explained by the relative effects of these types of fatty acids on the in vitro Δ6-desaturation (see Chapter 1). The PHSO-diet, rich in *trans* fatty acids (20 en%) is also extremely low in oleic acid (7 en%). Surprisingly, a very strong correlation between the relative incorporation of labeled arachidonic acid in liver (phospho)lipid and the level of oleic acid in the diet was obtained (Fig. 2). This might indicate that instead of a strong depressing effect of *trans* fatty acids, oleic acid has a strong stimulatory effect on arachidonic acid biosynthesis. However, a number of authors concluded from fatty acid patterns in feeding experiments with guinea pigs and rats that oleic acid, when fed in large quantities, could depress linoleic acid metabolism (Dhopeshwarkar and Mead, 1961; Mohrhauer et al, 1967; Jeffcoat and James, 1977). Oleic acid was thought to compete with linoleic acid for the enzymes involved in the stepwise transformations of linoleic acid to arachidonic acid when a limited supply of linoleic acid was available.

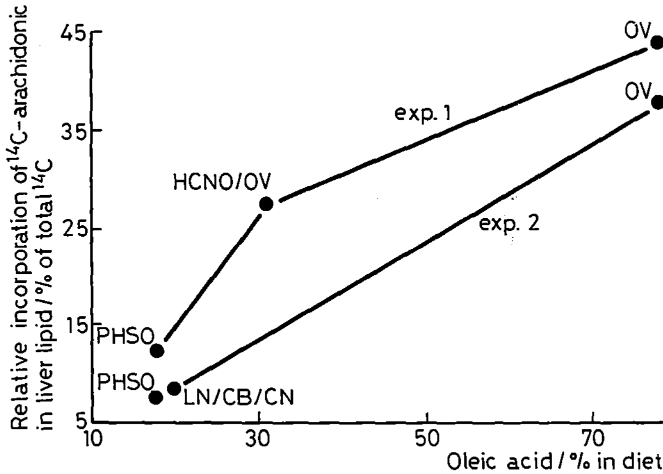

Fig. 2 Correlation of the relative incorporation of labeled arachidonic acid in liver (phospho)lipid after intubation of labeled linoleic acid with the level of oleic acid in the diet of rats

Our experiments in which a more direct method of measuring arachidonic acid biosynthesis was applied than looking at changes in fatty acid compositions, may indicate that actually the generally low level of oleic acid in *trans* fatty acids-containing diets causes a depression in arachidonic acid biosynthesis.

Whatever the cause, the large differences we observed in arachidonic acid biosynthesis after feeding the various dietary fats were not reflected by differences in arachidonic acid level in tissue lipids. The HCNO/OV-diet (first label experiment: Chapter 6a) gave a higher arachidonic acid level than the OV-diet, although the latter had a much more active arachidonic acid biosynthesis. The effect of α-linolenic acid on the synthesis of arachidonic acid in 24 h was similar to that of *trans* fatty acids (second experiment: Chapter 6b), but the arachidonic acid levels in the α-linolenic acid group were almost half of those in the *trans* group. And finally, the arachidonic acid synthesis was to a large extent decreased by the diet with *trans* fatty acids compared to the oleic acid-rich diet; nevertheless the effect on tissue arachidonic acid levels was only minor.

How can we explain these discrepancies between arachidonic acid biosynthesis and tissue levels? It is often stated that both *trans* fatty acids and α-linolenic acid inhibit the Δ6-desaturation, resulting in a reduced arachidonic acid synthesis which manifests itself by increased linoleic acid and reduced arachidonic acid levels in tissue lipids (see Holman (1986) for a review). In other words, this theory supposes that the Δ6-desaturase system actually determines the polyunsaturated fatty acid composition of tissue lipids by regulating the amounts of arachidonic acid synthesized. Our experiments, evidently do not support this supposition for three reasons. Firstly, we have demonstrated that large differences in arachidonic acid biosynthesis are not reflected in changes in arachidonic acid levels in tissue

lipids. Secondly, we calculated that rats fed 2 en% linoleic acid have the capacity to convert at least 5% of their daily intake of linoleic acid into arachidonic acid (Chapter 6b). In normal EFA-replete rats this capacity seems to be more than adequate to supply the required amounts of arachidonic acid and its metabolites (amount of arachidonic acid synthesized is about 6 mg/day; total eicosanoid production is estimated to be much lower than 1 mg/day (see Chapter 1)). Tissues will have ample supply of this fatty acid for incorporation into their phospholipids up to the required levels. Possibly the excess arachidonic acid produced is oxidized. Thirdly, also the finding that *trans* fatty acids reduced arachidonic acid incorporation in heart mitochondrial PC, but actually increased it in PE (Chapter 3; Blomstrand et al, 1985), makes it unlikely that the supply of arachidonic acid is the main factor determining phospholipid arachidonic acid levels.

An alternative hypothesis could be that competing effects of fatty acids on the level of incorporation in lipids actually determine polyunsaturated fatty acid composition of these lipids and that effects of dietary fats on the Δ6-desaturase system are of lesser importance. *Trans* fatty acids are predominantly incorporated at the 1-position of phospholipids (see Chapter 1) and, consequently, do not compete appreciably with linoleic acid for incorporation in phospholipids. Oleic acid, however, can occupy the 2-position of phospholipids and therefore is a competitor of linoleic acid for incorporation (Blomstrand and Svensson, 1983; see Lands (1979) for a review). An analogous competitive situation between linoleic acid and other (dietary) fatty acids may arise with respect to other lipid classes such as triglycerides and cholesterolesters. This implies that a high influx of oleic acid (after consumption of oleic acid-rich diets) would decrease linoleic acid deposition in complex lipids. Indeed, a reduction in linoleic acid level is often seen after feeding of oleic acid-rich diets (Mohrhauer et al, 1967; Chapters 3 and 4). In our second study we observed the lowest incorporation of labeled linoleic acid in liver phospholipid in the OV-group. Because after consumption of a high level

of oleic acid less linoleic acid is deposited in tissue lipids than after consumption of fats low in oleic acid (e.g. the *trans* fatty acids-rich fats), more linoleic acid will be available for other processes. One of these processes is the arachidonic acid biosynthesizing pathway and this would be reflected in the high amount of labeled arachidonic acid formed.

Another competitive pathway open to linoleic acid under these conditions could be the β-oxidative pathway. Indeed, olive oil, contrary to saturated and *trans* fats, stimulates the oxidation of labeled linoleic acid to CO_2 in the live rat (unpublished results, see Fig. 3).

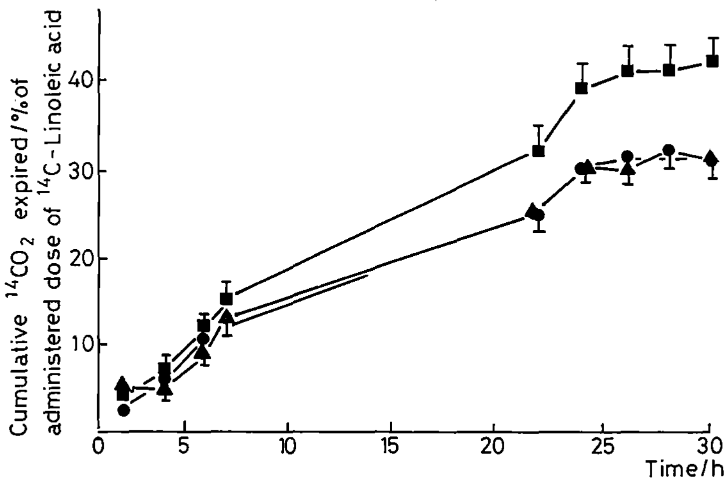

Fig. 3 Oxidation of labeled linoleic acid as influenced by the type of dietary fat in rats. After 5 weeks of feeding (■, OV-diet; ●, PHSO-diet; ▲, LN/CB/CN-diet; same diets as in radiolabel experiment 2) rats were given a single oral dose of 1-^{14}C-linoleic acid. The expiration of $^{14}CO_2$ was followed during 30 h. The values indicate means of 4 animals per group and the standard error of the mean.

With respect to α-linolenic acid, this fatty acid and its metabolite 20:5 n-3 can effectively compete with arachidonic acid for incorporation at the 2-position of phospholipids (Lands et al, 1982). This could explain the low levels of arachidonic acid in the group fed α-linolenic acid.

Unfortunately, the design of our label experiments does not permit a fair comparison of *trans* fatty acids and saturated fatty acids. The amount of oleic acid in the hydrogenated coconut oil/olive oil-diet in the first experiment was almost twice the amount of oleic acid in the PHSO-diet. As suggested earlier, this could explain the intermediate position of the HCNO/OV-diet in effect on arachidonic acid biosynthesis between olive oil and the *trans* fatty acids-rich diet.

A finding supporting the hypothesis that dietary fat influences fatty acid compositions of tissue lipids primarily via competitive effects between fatty acids at the incorporation level, is the differential effect of dietary fats on individual phospholipids (e.g. *trans* fatty acids cause a decrease in the arachidonic acid level in PC of heart mitochondria while that in PE was increased). It could very well be that preferences of the various types of phospholipids for specific fatty acids, the location of these phospholipids (pools, domains) and transport mechanisms of fatty acids and phospholipids play a much more important role in the determination of fatty acid compositions than is currently assumed. Anyhow, availability of fatty acids only is not sufficient to explain the complicated changes in fatty acid composition as observed with different dietary fats.

Conversely, fatty acid composition of tissue lipids cannot be used to deduce effects of dietary fats on one particular aspect of the linoleic acid metabolism. This would imply that the ratio of levels of arachidonic acid and linoleic acid in liver phospholipids does not give unequivocal information on effects of dietary fats on the activity of the Δ6-desaturation system (Hill et al, 1982; Lawson et al, 1983;

Mahfouz et al, 1983). Thus, in our view, the effects of dietary fatty acids on tissue fatty acid composition is not primarily brought about by changes in the Δ6-desaturation activity. This does not imply that the Δ6-desaturation does not play an important role in membrane composition. Various hormonal and nutritional factors influence the activity of this enzyme (see Brenner, 1981 for a review) and hence may also affect membrane composition. Also several disease conditions may involve altered Δ6-desaturation capacity (see Holman and Johnson, 1981 for a review). It should be noted, however, that although the 20:4/18:2-ratio in blood lipids may be changed in some disease states, this is not a reliable measure of arachidonic acid biosynthesis.

In conclusion, we have shown that the dietary fats investigated differ from each other with respect to effects on arachidonic acid biosynthesis. A fat rich in *trans* fatty acids compared to an oleic acid-rich oil inhibited the conversion of linoleic acid into arachidonic acid. In this respect the *trans* fatty acids-rich diet had almost the same effect as an α-linolenic acid-containing diet. As the dietary fats in our label experiments (Chapter 6) differed not only in content of *trans* fatty acids, saturated fatty acids or α-linolenic acid but also in oleic acid content, no definite conclusions as to which fatty acid(s) is (are) responsible for the observed effects can be drawn. Besides, we don't know whether a low arachidonic acid biosynthesis is an undesirable effect of *trans* fatty acids. As demonstrated, the differences in arachidonic acid biosynthesis were not reflected in major differences in arachidonic acid levels in phospholipids.
Trans fatty acids lower arachidonic acid levels slightly in most phospholipids compared to saturated fats or *cis*-monounsaturated fats by a mechanism not fully understood. However, there are phospholipids in which the arachidonic acid level is not decreased (heart mitochondrial PE and platelet lipid). And, more importantly, even with 2 en% linoleic acid in the diet, the *trans* fatty acids-diet did not significantly alter maximal eicosanoid synthesis in vitro (Chapter 4). So perhaps the differences in arachidonic acid synthesis we found are insignificant from a physiological point of view.

Trans fatty acids do not seem to exert effects on linoleic acid metabolism widely different from those of the common fatty acids. In many respects, the influence of n-3 fatty acids, such as α-linolenic acid and eicosapentaenoic acid, both common fatty acids, on tissue polyunsaturated fatty acid composition is much stronger. As our diets contained partially hydrogenated soybean oil including probably all the *trans* isomers of linoleic acid and oleic acid occurring in human diets in much higher quantities, a specific, adverse effect of (a) particular *trans* isomer(s) on linoleic acid metabolism of the rat is very unlikely.

8.3. Relevance of these rat studies for human nutrition

The comparison of linoleic acid metabolism in man and rat is difficult due to the very little information available on human linoleic acid metabolism. The enzyme systems to convert linoleic acid into arachidonic acid are probably the same in most mammals, including rat and man (De Gomez Dumm and Brenner, 1975; Sprecher and James, 1979). It is often suggested that the arachidonic acid synthesis in man is slower than that in rats, due to a lower Δ6-desaturation activity. In in vitro experiments, isolated human cells desaturated ^{14}C-linoleic acid much more slowly than rat cells (Cunnane et al, 1984). Also human liver microsomes displayed a Δ6-desaturation activity which was lower than that of rat liver microsomes (De Gomez Dumm and Brenner, 1975; Blond et al, 1981). Rats generally have higher levels of arachidonic acid and lower levels of γ-linolenic acid in phospholipids than healthy humans (Horrobin et al, 1984).

The capacity of man to convert linoleic acid into arachidonic acid has also been demonstrated in vivo in studies with radiolabeled linoleic acid (Nichaman et al, 1967). 24 h after the consumption of ^{14}C-linoleic acid, a small but definite amount of labeled arachidonate was found in plasma phospholipid. The time course of incorporation of radioactivity into blood lipids of human volunteers (Ormsby et al, 1963; Nichaman et al, 1967) and rats (Mead and Fillerup, 1959; Illingworth and Glover, 1973; Chapter 6b) was similar. Therefore, just as in rats, this has

possibly resulted in a far larger deposition of labeled arachidonic acid in the liver of the volunteers in Nichaman's experiment than found in plasma. The mechanism by which linoleic acid is handled in man, is in all probability the same as that in rats; quantitatively, however, significant differences between man and rat may exist.

To our knowledge no experiments comparable to ours in rats with the objective to elucidate effects of dietary fats on linoleic acid metabolism, have ever been described for humans.

Anyway, the linoleic acid intake required to prevent biochemical and clinical symptoms of essential fatty acid deficiency in man is roughly equal to that for rats, 1 - 2 en% (see Yamanaka et al, 1981 for a review). However, it has been suggested that the requirement of essential fatty acids is influenced by pregnancy, age, disease and dietary factors. The World Health Organization recommends a minimal consumption of 3 en% for adults and 1.5 en% more during pregnancy (FAO-report, 1977).

The incorporation of *trans* fatty acids in human tissues has been extensively investigated (Ohlrogge 1983; FASEB, 1985; Adlof and Emken, 1986). Just as in rats, these fatty acids are deposited in most tissues, the level depending on tissue, dietary history and duration of exposure. Generally, the levels in adipose tissue reflect the long term intake of *trans* fatty acids (Beynen et al, 1980). The highest levels of total *trans* fatty acids found by GLC in adipose tissue is 12% (Thomas et al, 1981). The depositions in other organs were generally less (Ohlrogge, 1983; FASEB, 1985). No individual has been identified whose tissues contained exceptionally high *trans* values (FASEB, 1985). *Trans* 18:2 isomers were identified in human tissues in very low amounts (up to 0.4% in adipose tissue), but no 18:3 isomers were detected (Adlof and Emken, 1986). The selectivity of tissues for incorporation and exclusion of positional isomers of 18:1 fatty acids is not widely different in man from rat (FASEB, 1985).

The evaluation of safety of macronutrients (such as fatty acids) differs in many aspects from that of food additives (see e.g. Verschuren, 1988). One of the main reasons is that macronutrients cannot be added to animal diets without altering the nutritional balance. This implies that the components to be tested (in casu *trans* fatty acids) have to be included as part of a well-balanced diet. One of the requirements of such a well-balanced diet is an adequate level of linoleic acid. Another reason is that the safety factor, used in classical additive toxicology to define the "acceptable daily intake" (in general 100) cannot be applied to macronutrient safety testing. For these reasons multidisciplinary experiments, involving analytical chemistry, nutrition, animal care, pathology, biochemistry and physiology, have to be designed, using criteria selected for their sensitivity to act as indicators of possible long-term consequences. Just as classical toxicology experiments play an important role in safety testing of additives, rat experiments as described in this thesis, that pay attention to the above considerations, are very valuable instruments to evaluate the safety of macronutrients for human consumption.
The consumption of *trans* fatty acids in western, industrialized societies is reported to be about 8 g/day; this is roughly 2.5 en% (see Chapter 7). An expert task force of the British Nutrition Foundation calculated that in extreme cases individuals might consume 27 g *trans* fatty acids per day (BNF, 1987). When these extreme intakes occur, a major proportion of isomeric fatty acids will often be derived from hydrogenated fish oils. As the consumption of hydrogenated fish oils is only relevant for a few European countries (Great Britain, Germany, Scandinavian countries and Holland), the maximum intakes in other societies will probably be much lower. The percentage of *trans* dienes of total dietary *trans* fatty acids is estimated at maximally 10% (Adlof and Emken, 1986). The average consumption of linoleic acid in the U.S. is estimated at about 5 - 7 en% (Fischer et al, 1985; Goor et al, 1985; Folsom et al, 1987). In European countries this value is somewhat lower; a low intake is reported for Scotland (3 en%) (Thomson et al, 1982). In our experiments rats were fed with 16 en% monoenoic

trans fatty acids and with maximally 4 en% dienoic *trans* isomers. This implies that our rats consumed on average about 8 times as much total *trans* fatty acids as western humans and at least 10 times as much *trans* dienes. We determined the minimal requirement for linoleic acid of rats fed 20 en% *trans* fatty acids to be 2 en%: the average linoleic acid consumption in western, industrialized societies is higher. Based on these considerations we conclude that *trans* fatty acids do not present a nutritional problem.

8.4. References

Aaes-Jørgensen, E. and Hølmer, G. (1969) Essential fatty acid-deficient rats: 1. Growth and testes development. Lipids 4, 501-506.

Adlof, R.O. and Emken, E.A. (1986) Distribution of hexadecenoic, octadecenoic and octadecadienoic acid isomers in human tissue lipids. Lipids 21, 543-547.

Alfin-Slater, R.B., Wells, A.F., Aftergood, L. and Deuel, H.J. Jr. (1957) Nutritive value and safety of hydrogenated vegetable fats as evaluated by long-term feeding experiments with rats. J. Nutr. 63, 241-261.

Alling, C., Bruce, Å., Karlsson, I., Sapia, O. and Svennerholm, L. (1972) Effect of maternal essential fatty acid supply on fatty acid composition of brain liver, muscle and serum in 21-day-old rats. J. Nutr. 102, 773-782.

Anderson, R.L., Fullmer, C.S. and Hollenbach, E.J. (1975) Effects of the *trans* isomers of linoleic acid on the metabolism of linoleic acid in rats. J. Nutr. 105, 393-400.

Bach, A.C. and Babayan, V.K. (1982) Medium-chain triglycerides: an update. Am. J. Clin. Nutr. 36, 950-962.

Beynen, A.C., Hermus, R.J.J. and Hautvast, J.G.A.J. (1980) A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man. Am. J. Clin. Nutr. 33, 877-886.

Blomstrand, R. and Svensson, L. (1983) The effects of partially hydrogenated marine oils on the mitochondrial function and membrane phospholipid fatty acids in rat heart. Lipids 18, 151-170.

Blomstrand, R., Diczfalusy, U., Sisfontes, L. and Svensson, L. (1985) Influence of dietary partially hydrogenated vegetable and marine oils on membrane composition and function of liver microsomes and platelets in the rat. Lipids 20, 283-295.

Blond, J.-P., Lemarchal, P. and Spielmann, D. (1981) Désaturation comparée des acides linoléique et dihomo-γ-linolénique par des homogénats de foie humain in vitro. C.R. Acad. Sc. Paris, 292, Série III, 911-914.

BNF (1987) *Trans* fatty acids. The report of the British Nutrition Foundation Task Force. The British Nutrition Foundation, London.

Brenner, R.R. (1981) Nutritional and hormonal factors influencing desaturation of essential fatty acids. Prog. Lipid Res. 20, 41-47.

Brenner, R.R. and Peluffo, R.O. (1966) Effect of saturated and unsaturated fatty acids on the desaturation in vitro of palmitic, stearic, oleic, linoleic, and linolenic acids. J. Biol. Chem. 22, 5213-5219.

Bruckner, G., Trimbo, S., Goswami, S. and Kinsella, J.E. (1983) Dietary trilinoelaidate: Effects on hematological parameters, serum eicosanoids and tissue fatty acid composition in rats. J. Nutr. 113, 704-713.

Clandinin, M.T. (1978) The role of dietary long chain fatty acids in mitochondrial structure and function. Effects on rat cardiac mitochondrial respiration. J. Nutr. 108, 273-281.

Cunnane, S.C., Keeling, P.W.N., Thompson, R.P.H. and Crawford, M.A. (1984) Linoleic acid and arachidonic acid metabolism in human peripheral blood lymphocytes: comparison with the rat. Br. J. Nutr. 51, 209-217.

De Gomez Dumm, I.N.T. and Brenner, R.R. (1975) Oxidative desaturation of α-linolenic, linoleic, and stearic acids by human liver microsomes. Lipids 10, 315-317.

De Schrijver, R. and Privett, O.S. (1982) Effects of dietary long-chain fatty acids on the biosynthesis of unsaturated fatty acids in the rat. J. Nutr. 112, 619-626.

Dhopeshwarkar, G.A. and Mead, J.F. (1961) Role of oleic acid in the metabolism of essential fatty acids. J. Am. Oil Chem. Soc. 38, 297-301.

Divakaran, P. and Venkataraman, A. (1977) Effect of dietary fats on oxidative phosphorylation and fatty acid profile of rat liver mitochondria. J. Nutr. 107, 1621-1977.

Emken, E.A. (1984) Nutrition and biochemistry of *trans* and positional fatty acid isomers in hydrogenated oils. Ann. Rev. Nutr. 4, 339-376.

Entressangles, B., Combe, N. and Wolff, R. (1984) Influence of dietary elaidic acid on the lipid composition and physical and functional properties of rat mitochondria. J. Am. Oil Chem. Soc. 61, 679.

FAO-report (1977) Dietary fats and oils. Report of the Food and Agriculture Organization of the United Nations and the World Health Organization, FAO, 1978.

FASEB (1985) Health aspects of dietary *trans* fatty acids. Report of the ad-hoc Committee of the Federation of American Societies for Experimental Biology, (Senti, F.R., ed.) FDA223-83-2020.

Fischer, D.R., Morgan, K.J. and Zabik, M.E. (1985) Cholesterol, saturated fatty acids, polyunsaturated fatty acids, sodium and potassium intakes of the United States population. J. Coll. Am. Coll. Nutr. 4, 207-224.

Folsom, A.R., Jacobs, D.R., Luepker, R.V., Kushi, L.H., Gillum, R.F., Elmer, P.J. and Blackburn, H. (1987) Nutrient intake in a metropolitan area, 1973-74 vs 1980-82: the Minnesota Heart Survey. Am. J. Clin. Nutr. 45, 1533-1540.

Food Safety Council (1978) Proposed system for food safety assessment. Food Cosmetic Toxicology 16, Suppl. 2, 83-92.

Goor, R., Hosking, J.D., Dennis, B.H., Graves, K.L., Waldman, G.T. and Haynes, S.G. (1985) Nutrient intakes among selected North American populations in the Lipid Research Clinics Prevalence Study: composition of fat intake. Am. J. Clin. Nutr. 41, 299-311.

Hill, E.G., Johnson, S.B. and Holman, R.T. (1979) Intensification of essential fatty acid deficiency in the rat by dietary *trans* fatty acids. J. Nutr. 109: 1759-1765.

Hill, E.G., Johnson, S.B., Lawson, L.D., Mahfouz, M.M. and Holman, R.T. (1982) Perturbation of the metabolism of essential fatty acids by dietary partially hydrogenated vegetable oil. Proc. Natl. Acad. Sci. USA 79, 953-957.

Holman, R.T. (1968) Essential fatty acid deficiency. Progr. Chem Fats Lipids 9, 275-348.

Holman, R.T. (1986) Nutritional and biochemical evidences of acyl interaction with respect to essential polyunsaturated fatty acids. Prog. Lipid Res. 25, 24-39.

Holman, R.T. and Johnson, S. (1981) Changes in essential fatty acid profile on serum phospholipids in human disease. Prog. Lipid Res. 20, 67-73.

Holman, R.T., Mahfouz, M.M., Lawson, L.D. and Hill, E.G. (1983) Metabolic effects of isomeric octadecenoic acids. In: Dietary fats and health, (Perkins, E.G. and Visek, W.J., eds.), pp. 320-339, American Oil Chemists' Society, Champaign, Il.

Hornstra, G., Haddeman, E., Kloeze, J. and Verschuren, P.M. (1983) Dietary-fat-induced changes in the formation of prostanoids of the 2 and 3 series in relation to arterial thrombosis (rat) and atherosclerosis (rabbit). In: Advances in Prostaglandins, Thromboxane, and Leukotriene Research, Vol. 12 (Samuelsson, B., Paoletti, R. and Ramwell, P., eds.) pp. 193-202, Raven Press, New York.

Horrobin, D.F., Huang, Y.-S., Cunnane, S.C. and Manku, M.S. (1984) Essential fatty acids in plasma, red blood cells and liver phospholipids in common laboratory animals as compared to humans. Lipids 19, 806-811.

Houtsmuller, U.M.T. (1975) Specific biological effects of polyunsaturated fatty acids. In: The role of fats in human nutrition (Vergroesen, A.J., ed.) pp.331-352, Academic Press, London.

Houtsmuller, U.M.T., Struyk, C.B. and Van der Beek, A. (1970) Decrease in rate of ATP synthesis of isolated rat heart mitochondria induced by dietary erucic acid. Biochim. Biophys. Acta 218, 564-566.

Houtsmuller, U.M.T. and van der Beek, A. (1981) Effects of topical application of fatty acids. Prog. Lipid Res. 20, 219-224.

Illingworth, D.R. and Glover, J. (1973) Simultaneous incorporation of orally administered [9,10-$^3H_2$]oleic and [1-^{14}C]linoleic acids into lipids of the adult rat brain. J. Neurochem. 21, 1403-1414.

Jeffcoat, R. and James, A.T. (1977) Interrelationships between the dietary regulation of fatty acid synthesis and the fatty acyl-CoA desaturases. Lipids 12, 469-474.

Lands, W.E.M. (1979) Selective recognition of geometric and positional isomers of fatty acids in vitro and in vivo. In: Geometric and positional fatty acid isomers (Emken, E.A. and Dutton, H.J., eds.), pp.181-212, American Oil Chemists' Society, Champaign, Il.

Lands, W.E.M, Blank, M.L., Nutter, L.J. and Privett, O.S. (1966) A comparison of acyltransferase activities in vitro with the distribution of fatty acids in lecithins and triglycerides in vivo. Lipids 1, 224-229.

Lands, W.E.M., Inoue, M., Sugiura, Y. and Okuyama, H. (1982) Selective incorporation of polyunsaturated fatty acids into phosphatidylcholine by rat liver microsomes. J. Biol. Chem. 257, 14968-14972.

Lawson, L.D., Hill, E.G. and Holman, R.T. (1983) Suppression of arachidonic acid in lipids of rat tissues by dietary mixed isomers of *cis* and *trans* octadecenoates. J. Nutr. 113, 1827-1835.

Levin, E., Johnson, R.M. and Albert, S. (1957) Mitochondrial changes associated with essential fatty acid deficiency in rats. J. Biol. Chem. 228, 15-21.

Mahfouz, M., Smith, T.L., Holmes, R.P. and Kummerow, F.A. (1983) Effects of dietary fats on desaturase activities and the biosynthesis of fatty acids in rat liver microsomes. Fed. Proc. 42, 1186.

Masuzawa, Y., Prasad, M.R. and Lands, W.E.M. (1987) Distribution of dietary *trans*-octadecenoate among acyl-CoA and other lipid fractions of rat liver and heart. Biochim. Biophys. Acta 919, 297-306.

Mead, J.F. and Fillerup, D.L. (1959) The transport of fatty acids in the blood. J. Biol. Chem. 234, 1983.

Mohrhauer, H. and Holman, R.T. (1963) Effect of linolenic acid upon the metabolism of linoleic acid. J. Nutr. 81, 67-74.

Mohrhauer, H., Rahm, J.J., Seufert, J. and Holman, R.T. (1967) Metabolism of linoleic acid in relation to dietary monoenoic fatty acids in the rat. J. Nutr. 91, 521-527.

Moore, C.E., Alfin-Slater, R.B. and Aftergood, L. (1980) Incorporation and disappearance of *trans* fatty acids in rat tissues. Am. J. Clin. Nutr. 33, 2318-2323.

Needleman, P., Minkes, M. and Raz, A. (1976) Thromboxanes: selective biosynthesis and distinct biological properties. Science 193, 163-165.

Needleman, P., Raz, A., Minkes, M.S., Ferrendelli, J.A. and Sprecher, H., (1979) Prostaglandin and thromboxane biosynthesis and unique biological properties. Proc. Nath. Acid Sci. USA 76, 944-948.

Nichaman, M.Z., Olson, R.E. and Sweeley, C.C. (1967) Metabolism of linoleic-1-^{14}C in normolipemic and hyperlipemic humans fed linoleate diets. Am. J. Clin. Nutr. 20, 1070-1083.

Nolen, G.A. (1981) Biological evaluation of hydrogenated rapeseed oil. J. Am. Oil Chem. Soc. 58, 31-37.

Nolen, G.A., Alexander, J.G. and Artman, N.R. (1967) Long-term rat feeding study with used frying fats. J. Nutr. 93, 337-348.

Norseth, J. and Thomassen, M.S. (1983) Stimulation of microperoxisomal β-oxidation in rat heart by high-fat diets. Biochim. Biophys. Acta 751, 312-320.

Ohlrogge, J.B. (1983) Distribution in human tissues of fatty acid isomers from hydrogenated oils. In: Dietary fats and health (Perkins, E.G. and Visek, W.J., eds.) pp. 359-374, American Oil Chemists's Society, Campaign, Il.

Ormsby, J.W., Schnatz, J.D. and Williams, R.H. (1963) The incorporation of linoleic-1-C^{14} acid in human plasma and adipose tissue. Metabolism 12, 812-820.

Privett, O.S., Phillips, F., Shimasaki, H., Nozawa, M.D. and Nickell, E.C. (1977) Studies on the effects of *trans* fatty acids in the diet on lipid metabolism in essential fatty acid deficient rats. Am. J. Clin. Nutr. 30, 1009-1017.

Pudelkewitz, C., Seufert., J. and Holman, R.T. (1968) Requirement of the female rat for linoleic acid and linolenic acids. J. Nutr. 94, 138-146.

Renner, R., Innis, S.M. and Clandinin, M.T. (1979) Effects of high and low erucic acid rapeseed oils on energy metabolism and mitochondrial function of the chick. J. Nutr. 109, 378-387.

Ringler, D.H. and Dabich, L. (1979) Hematology and clinical biochemistry. In: The laboratory rat, Vol. I. (Baker, H.J., Lindsey, J.R. and Weisbroth, S.H., eds.) pp. 112-121, Academic Press Inc., Orlando.

Royce, S.M. and Holmes, R.P. (1984) The saturation and isomerization of dietary *trans* fatty acids and the respiratory properties of rat heart mitochondria. Biochim. Biophys. Acta 792, 371-375.

Sprecher, H. and James, A.T. (1979) Biosynthesis of long chain fatty acids in mammalian systems. In: Geometric and positional fatty acid isomers (Emken, E.A. and Dutton, H.J., eds.), pp.303-338, American Oil Chemists' Society, Champaign, Il.

Thomas, L.H., Jones, P.R., Winter, J.A. and Smith, H. (1981) Hydrogenated oils and fats: the presence of chemically-modified fatty acids in human adipose tissue. Am. J. Clin. Nutr. 34, 877-886.

Thomassen, M.S., Christiansen, E.N. and Norum, K.R. (1982) Characterization of the stimulatory effect of high-fat diets on peroxisomal β-oxidation in rat liver. Biochem. J. 206, 195-202.

Thomasson, H.J., Gottenbos, J.J., Kloeze, J. and Vles, R.O. (1966) Nutritional evaluation of hydrogenated fats. Proc. Nutr. Soc. 25, 1-4.

Thomson, M., Logan, R.L., Sharman, M., Lockerbie, L., Riemersma, R.A. and Oliver, M.F. (1982) Dietary survey in 40-year old Edinburgh men. Human Nutr: Applied Nutr. 36A, 272-280.

Verschuren, P.M. (1988) Safety evaluation of macronutrients. Human Toxicol. 7, 63.

Vles, R.O. and Gottenbos, J.J. (1972) Long-term effects of feeding butterfat, coconut oil and hydrogenated or non- hydrogenated soyabean oils 2. Life span experiment in rats. Voeding 33, 455-465.

Wollbeck, D., Kleist, F.V. and Elmadfa, I. (1981) Biochemische Parameter zur Ermittlung des Mindestbedarfs an Linolsäure. Fette Seifen Anstrichm. 83, 317-323.

Wood, R. (1979) Incorporation of dietary *cis* and *trans* octadecenoate isomers in the lipid classes of various rat tissues. Lipids 14, 975-982.

Yamanaka, K.W., Clemans, G.W. and Hutchinson, M.L. (1981) Essential fatty acid deficiency in humans. Prog. Lipid Res. 19, 187-215.

SUMMARY

This thesis describes a series of experiments aimed at determining the possible effects of *trans* fatty acids on linoleic acid metabolism in the rat. We had two objectives with these studies: to define the minimum requirement for linoleic acid necessary to prevent adverse effects of *trans* fatty acids, and to gain more insight into the influence of *trans* fatty acids on linoleic acid metabolism in the rat.

To fulfil the first objective, we started with a feeding study with a dose-response set-up in male Wistar rats. For three months, eight groups of animals were fed semi-synthetic diets with 40% of energy (en%) from fat. Six of those groups received a diet containing a large amount of *trans* fatty acids (20 en%) as present in a special partially hydrogenated soybean oil. By using variable amounts of sunflower seed oil, olive oil or coconut oil, the linoleic acid content of these diets was varied from 0.4 to 7.1 en%. Two groups of animals served as references as they were fed diets without *trans* fatty acids: one group was given a mixture of olive oil and coconut oil (rich in oleic acid and medium chain saturated fatty acids) with 2 en% linoleic acid, the other an olive oil, rich in oleic acid, with 5 en% linoleic acid.

By comparing the *trans* fatty acids-fed groups with the two reference groups with identical linoleic acid levels (either 2 or 5 en% linoleic acid), the effects of *trans* fatty acids at two levels of linoleic acid on various parameters could be established.

The high level of *trans* fatty acids (20 en%) did not affect growth, water- or food consumption, nor did it induce essential fatty acid deficiency symptoms (Chapter 2). The trans-epidermal water loss was not increased by dietary *trans* fatty acids, indicating no effect on skin functionality. At the end of the feeding period the animals were sacrificed and autopsy was performed. Organs were weighed and histologically examined, and several haematological and clinical chemistry parameters were determined (Chapter 2). This part of the experiment closely resembled a sub-chronic toxicity study: a method commonly used to evaluate the safety of food ingredients. The clinical

observation of the animals, the examination at autopsy, the organ weights and the histopathological examinations did not give any indication of adverse effects of *trans* fatty acids. None of the clinical chemistry or haematological parameters investigated were affected. These results indicate that *trans* fatty acids from partially hydrogenated vegetable oils do not cause adverse effects with regard to general health, pathology, clinical chemistry and haematology when rats are fed 2 en% linoleic acid.

Another part of the study consisted of the investigation of the lipid composition of heart and liver mitochondria, segments of aorta and blood platelets (Chapters 3 and 4). The distribution of phospholipid classes in mitochondria was not affected by *trans* fatty acids. At all levels of dietary linoleic acid, *trans* fatty acids were incorporated in all the tissues and organelles we have investigated. These isomeric fatty acids were mostly monoenes; incorporation of *trans* isomers of linoleic acid was very low. The most striking effects of dietary *trans* fatty acids were an increase in the linoleic acid level and a decreased incorporation of arachidonic acid in most phospholipids. The linoleic acid level was increased in the phospholipids of all tissues examined, irrespective of the dietary linoleic acid level. A (modest) decrease in arachidonic acid in most phospholipids was induced by *trans* fatty acids when the dietary level of linoleic acid was 2 en%. With the higher dietary linoleic acid level (5 en%) this effect was clearly diminished. In contrast to all other phospholipids investigated, in heart mitochondrial phosphatidyl-ethanolamine the content of arachidonic acid went up by *trans* fatty acids-feeding.

Much more important than the observation that *trans* fatty acids can influence the essential fatty acid composition of membranes is the question whether *trans* fatty acids affect the function of those membranes. To investigate possible effects of *trans* fatty acids on membrane function of organs or organelles, the respiration of heart and liver mitochondria, a function which is closely linked to the mitochondrial membrane and sensitive to changes in that membrane, was measured (Chapter 3). Despite significant effects of *trans* fatty acids

on membrane composition, we found no effects on heart and liver mitochondrial respiration: the QO2, the ADP/O and the respiratory control ratios (RCR) were not affected.
Finally, we determined the effects of *trans* fatty acids on the eicosanoid production by aorta and platelets and the platelet aggregation (Chapter 4). The delay of the in vitro platelet aggregation, induced with three different doses of collagen, was not influenced by *trans* fatty acids. Also the hydroxy fatty acid-production (hydroxy-eicosatetraenoic acid and hydroxy-heptadecatrienoic acid) by stimulated platelets did not differ in the *trans* fatty acids fed-groups compared to the reference groups. With 5 en% linoleic acid, no effect of *trans* fatty acids could be observed on the prostacyclin-production by pieces of aorta, as measured by a bio-assay. However, at the 2 en% linoleic acid level, we found a significantly lower production of prostacyclin in the group fed *trans* fatty acids compared to that in the corresponding reference group (the group fed the mixture of olive oil and coconut oil). This could be explained by the linear relationship between the arachidonic acid level in aorta phospholipid and the prostacyclin production. This relationship was independent of the *trans* fatty acids content of the diet. As the arachidonic acid level in aorta phospholipid was lower in the *trans* group at the 2 en% linoleic acid level than in the corresponding reference group, the prostacyclin production was also lower.

In the second experiment we compared the effects of *trans* fatty acids with both saturated and *cis*-monounsaturated fatty acids of the same chain length (mainly C18) at a dietary level of 2 en% linoleic acid on largely the same parameters as in the first experiment in order to ascertain that 2 en% linoleic acid indeed is sufficient to prevent specific or adverse effects of long chain *trans* fatty acids (mainly C18) from partially hydrogenated vegetable oil. We prepared four diets, equal in fat content (40 en%) and linoleic acid level (2 en%). As a *trans* fatty acid-containing diet, we chose the same diet as in the first experiment; i.e. with 20 en% *trans* fatty acids (as present in the

partially hydrogenated soybean oil) and 2 en% linoleic acid. One group of rats was fed a diet characterized by a very high content of *cis*-monounsaturated fatty acids (from olive oil). Another group was fed a diet rich in (C16,C18) saturated fatty acids (mainly from cocoabutter). A fourth group receiving a diet with half the content of dietary *trans* fatty acids as the first diet was included to gain some insight into the effect of the dose of *trans* fatty acids on the parameters investigated. To obtain such a diet with only 10 en% *trans* fatty acids, a mixture of the three abovementioned dietary fats was prepared.

We extended the second experiment by comparing the effects of *trans* fatty acids with those of saturated or *cis*-monounsaturated fatty acids on mitochondrial and peroxisomal β-oxidation in different tissues. With 2 en% linoleic acid in the diet, again no effects of *trans* fatty acids on general health, pathology, clinical chemistry and haematology were observed (Chapter 2). Membrane functioning (mitochondrial respiration) (Chapter 3), platelet aggregation and peroxisomal and mitochondrial β-oxidation (Chapter 5) were not different between the groups.

In this experiment we found no significant differences in the production of prostacyclin by aorta segments between the groups (Chapter 4). Neither did *trans* fatty acids significantly affect the hydroxy-heptadecatrienoic or hydroxy-eicosatetraenoic acid-syntheses by platelets. This indicates that *trans* fatty acids do not directly influence enzymes involved in eicosanoid biosynthesis, but may exert an effect by reducing arachidonic acid levels.

The second experiment fully confirmed our previous findings that for the rat 2 en% linoleic acid is sufficient to prevent undesirable effects of high doses of *trans* fatty acids (20 and 10 en%) as present in partially hydrogenated soybean oil.

For the investigation of the effects of *trans* fatty acids on linoleic acid metabolism, two feeding studies with male Wistar rats were performed (Chapter 6). In both experiments, three groups of rats were fed semi-synthetic diets with 40 en% fat and 2 en% linoleic acid for six weeks. In the first experiment the dietary fats comprised a mixture of

hydrogenated coconut oil and olive oil, a pure olive oil or the same mixture with the special partially hydrogenated soybean oil as in the two feeding studies described earlier. Thus, these diets were rich in (medium chain) saturated fatty acids, *cis*-monounsaturated fatty acids (oleic acid) and *trans* fatty acids, respectively. In the second experiment the same oleic acid-rich and *trans* fatty acids-rich diets were used. Now the third diet consisted of a saturated fat (mixture of coconut oil and cocoabutter) with linseed oil. This diet contained a substantial amount of α-linolenic acid. At the end of the feeding period, the animals were given a single oral dose of ^{14}C-radiolabeled linoleic acid. After 24 h (first experiment) or at several times after the intubation of the linoleic acid (2, 4, 8, 24 and 48 h; second experiment) the animals were sacrificed and the radioactivity of the fatty acids in liver and heart was measured. Moreover, the fatty acid composition of these tissues was determined by gas-liquid chromatography. In both experiments we observed that the type of fat significantly influenced the ratio of labeled arachidonic acid to linoleic acid in both liver and heart. As the amount of radiolabeled arachidonic acid in the liver is the net result of its biosynthesis in the liver, net export to other tissues and its degradation (which is slow), this amount reflects the rate of biosynthesis of arachidonic acid in the liver. Compared to the oleic acid-rich diets, all other diets clearly reduced the arachidonic acid synthesis. The α-linolenic acid-rich diet had the most potent effect; an almost 3-fold lower incorporation of radiolabeled arachidonic acid was found after 24 h. The *trans* fatty acids-rich diet reduced the arachidonic acid synthesis slightly less than the α-linolenic acid-rich diet, while the diet rich in saturated fatty acids had the least effect compared to the oleic acid-rich diet. However, these differences in rate of arachidonic acid synthesis in the liver were not reflected by similar changes in fatty acid composition. The diet rich in saturated fatty acids resulted in the highest arachidonic acid levels: slightly higher than those in the animals fed olive oil. *Trans* fatty acids slightly reduced the arachidonic acid level in liver lipid compared to dietary oleic acid,

while dietary α-linolenic acid reduced arachidonic acid levels by half. This could indicate that the rate of arachidonic acid synthesis from linoleic acid (via Δ6- and Δ5-desaturation and elongation) is not the main determinant of tissue polyunsaturated fatty acid composition under the present conditions. Competition between fatty acids for the incorporation in lipids probably plays a much more important role in the regulation of the fatty acid composition than is generally assumed. In any case, we have demonstrated that fatty acid composition of tissue lipids cannot be used to deduce effects of dietary fats on one particular aspect of linoleic acid metabolism, namely arachidonic acid synthesis. These experiments also demonstrated that *trans* fatty acids do not have effects on linoleic acid metabolism other than those of common fatty acids. Although *trans* fatty acids reduced arachidonic acid biosynthesis, the changes in fatty acid compositions induced by the high level of these isomeric fatty acids (20 en%) were much less profound than those induced by α-linolenic acid.

In conclusion, our experiments did not give any indication of specific effects of *trans* fatty acids as present in partially hydrogenated soybean oil on linoleic acid metabolism. Moreover, we determined that 2 en% linoleic acid is sufficient to prevent undesirable effects of high levels of dietary *trans* fatty acids (upto 20 en%). In view of the average level of linoleic in the diets of western, industrialized societies (normally more than 2 en%), we conclude that *trans* fatty acids (average intake less than 5 en%) do not present a nutritional problem.

SAMENVATTING

Dit proefschrift beschrijft een aantal experimenten om mogelijke effecten van *trans* vetzuren op het linolzuurmetabolisme in de rat te bepalen. Wij hadden twee doelstellingen voor ogen met deze studies: 1. het bepalen van de minimale behoefte aan linolzuur om ongewenste effecten van *trans* vetzuren te voorkomen en 2. het verkrijgen van meer inzicht in de invloed van *trans* vetzuren op het linolzuurmetabolisme in de rat.

Om de eerste doelstelling te bereiken hebben we een voedingsexperiment uitgevoerd met mannelijke Wistar-ratten op basis van een dosis-werking-ontwerp. Gedurende drie maanden werden acht groepen dieren gevoed met verschillende soorten semi-synthetisch voedsel; 40% van de energie (en%) in dit voedsel werd verkregen uit vet. Zes van die groepen kregen een voeding met een hoog gehalte aan *trans* vetzuren (20 en%), zoals aanwezig in een speciale, gedeeltelijk geharde soja-olie. Door verschillende hoeveelheden zonnebloemolie, olijfolie en kokosnootolie te gebruiken, lieten we de hoeveelheid linolzuur in de voedingen variëren van 0.4 tot 7.1 en%. Twee groepen dieren dienden als referentie: hun voeding bevatte geen *trans* vetzuren. Een van die groepen werd een mengsel van olijfolie en kokosnootolie (rijk aan oliezuur en verzadigde vetzuren met middellange ketens) met 2 en% linolzuur gegeven, de ander een olijfolie, rijk aan oliezuur, met 5 en% linolzuur. Door de groepen die *trans* vetzuren kregen (2 of 5 en% linolzuur) te vergelijken met de twee referentiegroepen (ook met 2 of 5 en% linolzuur) konden effecten van *trans* vetzuren bij twee linolzuurniveaus worden bepaald op diverse parameters.

Het hoge gehalte aan *trans* vetzuren (20 en%) had geen effect op de groei en de water- en voedselopname, en bracht geen symptomen aan het licht die zouden kunnen duiden op een gebrek aan essentiële vetzuren (Hoofdstuk 2). Het trans-epidermale waterverlies werd niet verhoogd door *trans* vetzuren; dit geeft aan dat deze vetzuren geen effect hebben op de functionaliteit van de huid. Aan het einde van de voedingsperiode werden de dieren gedood en werd autopsie uitgevoerd. Diverse organen werden gewogen en histologisch onderzocht. Tevens werden diverse

hematologische en klinisch-chemische parameters bepaald (Hoofdstuk 2). Dit gedeelte van het experiment vertoonde vele overeenkomsten met een sub-chronische toxiciteitstest: een methode die veel wordt toegepast om de veiligheid van voedingsingrediënten te evalueren. De klinische observatie van de dieren, het onderzoek tijdens autopsie, de gewichten van de diverse organen en de histologische onderzoekingen wezen op geen enkele manier op schadelijke effecten van *trans* vetzuren. Van de onderzochte klinisch-chemische en hematologische parameters was geen enkele beïnvloed. Deze resultaten geven aan dat *trans* vetzuren in gedeeltelijk geharde plantaardige oliën geen schadelijke effecten veroorzaken op de algemene gezondheid van ratten indien 2 en% linolzuur in de voeding aanwezig is.

Een ander gedeelte van dit experiment bestond uit een onderzoek naar de lipidesamenstelling van hart- en levermitochondriën, stukjes aorta en bloedplaatjes (Hoofdstukken 3 en 4). De verdeling van de fosfolipideklassen in de mitochondriën was niet veranderd door de *trans* vetzuren. *Trans* vetzuren werden opgenomen door alle onderzochte weefsels en organellen, ongeacht het linolzuurgehalte in de voeding. Deze isomere vetzuren waren voornamelijk monoenen; opname van *trans* isomeren van linolzuur kwam bijna niet voor. De meest opvallende effecten van *trans* vetzuren waren een verhoging van het linolzuurgehalte en een verlaging van het arachidonzuurgehalte in de meeste fosfolipiden. Het linolzuurgehalte was verhoogd in de fosfolipiden van alle onderzochte weefsels, ongeacht het linolzuurgehalte in de voeding. *Trans* vetzuren induceerden een matige verlaging van het arachidonzuurgehalte in de meeste fosfolipiden indien het linolzuurgehalte in de voeding 2 en% bedroeg. Bij een hoger gehalte (5 en%) werd dit effect van *trans* vetzuren duidelijk minder. In tegenstelling tot in alle andere onderzochte fosfolipiden ging het arachidonzuurgehalte in het fosfatidyl-ethanolamine van de hartmitochondriën omhoog wanneer de voeding *trans* vetzuren bevatte.

Veel belangrijker dan verschuivingen in de vetzuursamenstelling van de membranen zijn mogelijke effecten op de functie van deze membranen. Om effecten van *trans* vetzuren op de membraanfunctie van organen of

organellen te onderzoeken, hebben we de ademhaling van hart- en levermitochondriën gemeten. Deze ademhaling is nauw verbonden aan de functie van de mitochondriële membraan en derhalve gevoelig voor veranderingen in de samenstelling van dit membraan (Hoofdstuk 3). Ondanks grote effecten van *trans* vetzuren op de membraansamenstelling was de ademhaling van hart- en levermitochondriën (QO_2, ADP/O en RCR) niet beïnvloed.
Tenslotte hebben we effecten gemeten van *trans* vetzuren op de synthese van eicosanoïden door aorta en bloedplaatjes, alsmede de aggregatie van bloedplaatjes (Hoofdstuk 4). De vertraging van de in vitro plaatjesaggregatie, zoals veroorzaakt door drie verschillende doses collageen, werd niet beïnvloed door *trans* vetzuren. Ook de productie van hydroxy-vetzuren (hydroxy-eicosatetraeenzuur en hydroxy-heptadecatriëenzuur) door gestimuleerde plaatjes van de groepen gevoed met *trans* vetzuren verschilde niet van die van de referentiegroepen. Indien 5 en% linolzuur aanwezig was in de voeding, werd geen enkel effect van *trans* vetzuren op de prostacyclinesynthese door stukjes aorta waargenomen (gemeten met behulp van een bioassay). Met 2 en% linolzuur in de voeding vonden we echter wel een synthese van prostacycline in de *trans* vetzuren-groep die significant lager was dan die in de referentiegroep met dezelfde linolzuurconsumptie (de groep die een mengsel van olijfolie en kokosnootolie werd gevoed). Dit zou verklaard kunnen worden door het recht evenredige verband tussen het arachidonzuurgehalte van aorta-fosfolipide en de prostacyclineproductie. Dit verband werd niet beïnvloed door *trans* vetzuren in de voeding. Aangezien het arachidonzuurgehalte in aorta-fosfolipide in de groep gevoed met *trans* vetzuren bij een niveau van 2 en% linolzuur lager was dan dat in de overeenkomstige referentie-groep, was de prostacyclineproductie eveneens lager.

In het tweede experiment vergeleken we de effecten van *trans* vetzuren met die van zowel verzadigde als *cis*-mono-onverzadigde vetzuren van dezelfde ketenlengte (voornamelijk C18) bij 2 en% linolzuur in de voeding op grotendeels dezelfde parameters als in het eerste experiment.

Het doel van deze proef was vast te stellen of 2 en% linolzuur voldoende is om specifieke of ongewenste effecten van *trans* vetzuren (voornamelijk C18) in gedeeltelijk geharde plantaardige oliën te voorkomen. Daartoe werden vier voedingen bereid, alle gelijk in vet- (40 en%) en linolzuurgehalte (2 en%). Als voeding rijk aan *trans* vetzuren kozen we dezelfde voeding met 20 en% *trans* vetzuren (als aanwezig in de gedeeltelijk geharde soja-olie) en 2 en% linolzuur als in het eerste experiment. Eén groep ratten kreeg een voeding gekenmerkt door een zeer hoog gehalte aan *cis*-mono-onverzadigde vetzuren (olijfolie). Een andere groep werd gevoed met een voeding rijk aan C16,C18-verzadigde vetzuren (voornamelijk van cacaoboter). Een vierde groep ontving een voeding met de helft van de hoeveelheid *trans* vetzuren als voor de eerste groep om effecten van de hoeveelheid geconsumeerde *trans* vetzuren te bestuderen. Om een dergelijk voedingsvet met 10 en% *trans* vetzuren te verkrijgen werd een mengsel gemaakt van de drie bovenstaande voedingsvetten.
Het tweede experiment werd uitgebreid door de effecten van *trans* vetzuren en *cis*-mono-onverzadigde en verzadigde vetzuren op de mitochondriële en peroxisomale β-oxidatie in diverse weefsels te vergelijken.
Met 2 en% linolzuur in de voeding werden ook in dit experiment geen effecten van *trans* vetzuren gevonden op de algemene gezondheid en de klinisch-chemische en hematologische parameters (Hoofdstuk 2). De membraanfunctie (mitochondriële ademhaling) (Hoofdstuk 3), plaatjes-aggregatie en peroxisomale en mitochondriële β-oxidatie (Hoofdstuk 5) van de groepen waren onderling niet verschillend. Ook vonden we geen verschillen tussen de groepen wat betreft de synthese van prostacycline door stukjes aorta (Hoofdstuk 4). Evenmin was de synthese van hydroxy-heptadecatriëenzuur of hydroxy-eicosatetraeenzuur door plaatjes significant beïnvloed. Dit geeft aan dat *trans* vetzuren de enzymen die betrokken zijn bij de eicosanoïdesynthese niet beïnvloeden op een directe wijze. *Trans* vetzuren kunnen een effect hebben op de eicosanoïdesynthese via een verlaging van het arachidonzuurgehalte in fosfolipiden. Dit tweede experiment bevestigde volledig onze eerdere ervaringen dat 2 en%

linolzuur voldoende is om ongewenste effecten te voorkomen van grote hoeveelheden *trans* vetzuren (20 en 10 en%), zoals aanwezig in gedeeltelijk geharde soja-olie.

Voor het onderzoek naar de effecten van *trans* vetzuren op het linolzuurmetabolisme hebben we twee experimenten met mannelijke Wistar-ratten uitgevoerd (Hoofdstuk 6). In beide experimenten werden 3 groepen ratten gedurende 6 weken gevoed met semi-synthetische voeding, verschillend van samenstelling, maar met 40 en% vet en 2 en% linolzuur. In het eerste experiment bestonden de voedingsvetten uit een mengsel van geharde kokosnootolie en olijfolie, een pure olijfolie of hetzelfde mengsel als in de twee vorige experimenten, met de speciale, gedeeltelijk geharde soja-olie. Dientengevolge waren deze voedingen rijk aan respectievelijk verzadigde vetzuren (met middellange ketens), *cis*-mono-onverzadigde vetzuren (oliezuur) en *trans* vetzuren. In het tweede experiment gebruikten we dezelfde oliezuurrijke en *trans* vetzurenrijke voedingen. De derde voeding bestond uit een verzadigd vet (een mengsel van cacaoboter en kokosnootolie) met lijnzaadolie. Deze voeding bevatte een aanzienlijke hoeveelheid α-linoleenzuur. Aan het eind van de voedingsperiode werd de dieren een enkele dosis ^{14}C-radio-actief linolzuur gegeven via maagsonde. Na 24 uur (eerste experiment) of op verschillende tijden (na 2, 4, 8, 24 en 48 uur, tweede experiment) werden de dieren gedood en werd de radioactiviteit van de vetzuren in lever en hart gemeten. Tevens werd de vetzuursamenstelling van deze weefsels bepaald met behulp van gas-vloeistofchromatografie.
In beide experimenten zagen we dat het soort vet de ratio van 'gelabeld' arachidonzuur en linolzuur in lever en hart aanzienlijk had beïnvloed. Aangezien de hoeveelheid gelabeld arachidonzuur in de lever het netto resultaat is van de biosynthese in de lever, de netto export naar andere weefsels en de afbraak daarvan (verloopt traag) is deze hoeveelheid een maat voor de snelheid van biosynthese van arachidonzuur in de lever. Vergeleken met de voedingen rijk aan oliezuur verlaagden alle andere voedingen de arachidonzuursynthese aanzienlijk. De voeding rijk aan α-linoleenzuur gaf het grootste effect; de hoeveelheid radioactief

arachidonzuur die was ingebouwd in de lever 24 uur na toediening van het linolzuur, was 3 maal zo laag. De voedingen rijk aan *trans* vetzuren verlaagden de arachidonzuursynthese iets minder dan de voeding rijk aan α-linoleenzuur. De voeding rijk aan verzadigde vetzuren had het minste effect. Deze verschillen in snelheid van arachidonzuursynthese kwamen niet tot uiting via dergelijke verschillen in vetzuursamenstelling. De voeding rijk aan verzadigde vetzuren resulteerde in de hoogste arachidonzuurgehaltes: iets hoger dan die van de dieren die olijfolie hadden gekregen. *Trans* vetzuren verlaagden het arachidonzuurgehalte in leverlipiden slechts iets ten opzichte van olijfolie. De consumptie van α-linoleenzuur halveerde de arachidonzuurgehaltes. Dit zou een aanwijzing kunnen zijn dat onder de door ons gebruikte omstandigheden, de snelheid van arachidonzuursynthese vanuit linolzuur (via Δ6- en Δ5-desaturaties en elongatie) niet het belangrijkste mechanisme is dat de samenstelling aan meervoudig onverzadigde vetzuren in weefsellipiden bepaalt. Competitie tussen vetzuren voor opname in lipiden speelt waarschijnlijk een veel belangrijkere rol dan gewoonlijk wordt aangenomen. Hoe dan ook, wij hebben aangetoond dat de vetzuursamenstelling van lipiden niet kan worden gebruikt om de effecten van voedingsvetten op een bepaald aspect van het linolzuurmetabolisme -de arachidonzuur-synthese- te bepalen. Deze experimenten geven ook aan dat *trans* vetzuren niet wezenlijk andere effecten hebben op het linolzuur-metabolisme dan de meeste overige veelgebruikte vetzuren. Hoewel *trans* vetzuren de snelheid van de arachidonzuursynthese verlagen, veroorzaakt een hoge inname van die vetzuren (20 en%) minder grote verschuivingen in vetzuursamenstellingen dan α-linoleenzuur.

Onze experimenten geven geen aanwijzingen dat *trans* vetzuren in gedeeltelijk geharde soja-olie specifieke effecten op het linolzuur-metabolisme hebben. Tevens vonden wij dat 2 en% linolzuur voldoende is om ongewenste effecten van een hoge inname van *trans* vetzuren (20 en%) te voorkomen. Aangezien de gemiddelde consumptie aan linolzuur in de westerse, geïndustrialiseerde landen gewoonlijk meer is dan 2 en%, concluderen wij dat *trans* vetzuren (gemiddelde consumptie minder dan 5 en%) geen probleem vormen in voedingskundig opzicht.

CURRICULUM VITAE

Hans Zevenbergen werd geboren op 2 oktober 1956 te Utrecht. Na het diploma Atheneum B te hebben gehaald aan het Westfries Lyceum te Hoorn, begon hij in 1974 aan de studie Scheikunde aan de Rijksuniversiteit te Utrecht. In 1980 werd het doctoraalexamen afgelegd met als hoofdvak Biochemie. Hierna was hij gedurende een jaar in dienst bij de Rijksuniversiteit Utrecht als wetenschappelijk medewerker aan de Vakgroep Fysiologie. Op 1 december 1981 trad hij in dienst bij het Unilever Research Laboratorium te Vlaardingen in de sectie Fysiologie, alwaar hij meewerkte aan een onderzoek naar effecten van *trans* vetzuren. In het kader hiervan werden alle in dit proefschrift beschreven experimenten uitgevoerd. Na 2½ jaar kreeg hij de leiding van dit project. Eind 1986 werd het beëindigd. Sindsdien is hij projectleider van het onderzoek naar de rol van vetten in onze gezondheid.

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GLOSSARY OF TERMS AND LIST OF ABBREVIATIONS

Nomenclature of fatty acids

Common name	Systematic name of acid	Abbreviation
Palmitic acid	hexadecanoic	16:0
Stearic acid	octadecanoic	18:0
Oleic acid	9*cis*-octadecenoic	9c-18:1
Elaidic acid	9*trans*-octadecenoic	9t-18:1
Linoleic acid	9*cis*,12*cis*-octadecadienoic	9c,12c-18:2
Linolelaidic acid	9*trans*,12*trans*-octadecadienoic	9t,12t-18:2
α-Linolenic acid	all *cis* 9,12,15-octadecatrienoic	9c,12c,15c-18:3
γ-Linolenic acid	all *cis* 6,9,12-octadecatrienoic	6c,9c,12c;18:3
Gadoleic acid	11*cis*-eicosenoic	11c-20:1
Dihomo-γ-linolenic acid	all *cis* 8,11,14-eicosatrienoic	8c,11c,14c-20:3
Arachidonic acid Arachidonic acid	all *cis* 5,8,11,14-eicosatetraenoic	5c,8c,11c,14c-20:4
Eicosapenta-enoic acid (EPA)	all *cis* 5,8,11,14,17-eicosapentaenoic	5c,8c,11c,14c,17c-20:5
Erucic acid	13*cis*-docosenoic	13c-22:1
Cetoleic acid	11*cis*-docosenoic	11c-22:1
Brassidic acid	13*trans*-docosenoic	13t-22:1
Docosahexa-enoic acid (DHA)	all *cis* 4,7,10,13,16,19-docosahexaenoic	4c,7c,10c,13c,16c,19c-22:6

Abbreviations

ADP/O	Adenosine diphosphate/Oxygen-ratio
ATP	Adenosine triphosphate
CoA	Co-enzyme A
DHA	Docosahexaenoic acid (22:6)
EC	Enzyme classification
EFA	Essential fatty acid
EFAD	Essential fatty acid deficiency
en%	% of total energy
EPA	Eicosapentaenoic acid (20:5)
GLC	Gas liquid chromatography
HCNO/OV	Hydrogenated coconut oil/olive oil mixture
12-HETE	12-hydroxy-5,8,10,14-eicosatetraenoic acid
HHT	12-hydroxy-5,8,10-heptadecatrienoic acid
HPLC	High performance liquid chromatography
Km	Michaelis constant
LN/CB/CN	Linseed oil/cocoa butter/coconut oil mixture
OV	Olive oil
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Prostaglandin
PGI_2	Prostacyclin
PHPO	Partially hydrogenated peanut oil
PHRO	Partially hydrogenated rapeseed oil
PHSO	Partially hydrogenated soybean oil
PHVO	Partially hydrogenated vegetable oil
PL	Phospholipid
RIA	Radioimmuno assay
TLC	Thin layer chromatography
TXA_2	Thromboxane A_2