Metabolism of trans fatty acids with emphasis on the effects of trans,trans-octadecadienoate on lipid composition, essential fatty acid, and prostaglandins: an overview

J. E. Kinsella, G. Bruckner, J. Mai, and J. Shimp

ABSTRACT Information concerning the metabolism of trans isomers of dietary unsaturated fatty acids is presented. Dietary trans-octadecenoic and trans,trans-octadecadienoic acids are apparently absorbed, activated, oxidized, and acylated into ester lipids much like saturated fatty acids although differences have been observed with regard to their metabolism by different organs. Because of the important role of linoleic acid as the principal precursor of cyclic endoperoxides, prostaglandins and leukotrienes, the potential deleterious effects of trans isomers of this acid are discussed. High levels of dietary trans,trans-linoleate can impair δ6 desaturase activity and decrease prostaglandin production in rats on experimental diets. Am. J. Clin. Nutr. 34: 2307-2318, 1981.

KEY WORDS Essential fatty acids, trans fatty acids, acyldesaturase, prostaglandins, linoleic acid, trans linoleate, dietary trans fatty acids

Introduction

Consumption of nutrient fat has increased in the United States about 25% over the last 60 yr, and furthermore a definite shift in the sources of dietary fat has been noted (1). This large increase in nutrient fat consumption (approximately 55 kg per capita per annum) can be attributed mainly to the use of more vegetable fats, i.e., salad and cooking oils, shortenings, and margarine. Most of these products are made from soybean oil. There has been a concomitant increase in the consumption of polyunsaturated fatty acids (PUFAs), especially linoleic acid (1).

The relatively high content (8%) of linolenic acid occurring in soybean oil causes instability and contributes to oxidative rancidity. Therefore, to facilitate the utilization of soybean oil in the American diet, to improve chemical stability and manipulate physical properties in accordance with consumer and processing preferences, soybean oil is processed, i.e., hydrogenated to some degree. During processing, isomerization of the naturally occurring cis unsaturated fatty acids to the trans configuration and positional shifts of the double bonds may occur. Partial hydrogenation yields a wide range of both geometric (cis and trans) and positional isomers of oleic (18:1n9) and linoleic (18:2n6) acids in which double bonds may be shifted anywhere from C3 through C15 in the case of 18:2 (2). The distribution of positional isomers in various commercial products has recently been documented by Dutton (4).

The concentration of trans fatty acids varies with the extent and type of processing of the oil. Shortenings, margarine, and salad oils may contain from 14 to 60, 16 to 70, and 8 to 17% trans fatty acids, respectively. Most of the trans fatty acids are monoenoic (18:1) isomers while trans isomers of octadecadienoic (18:2) acids range from 0.4 to 5%, though some samples of shortenings and margarine may contain in excess of 10% trans isomers of 18:2 (4-7). It is estimated that up
to 10% of the fatty acids being ingested by the American consumer may be composed of \textit{trans} fatty acids (8); however, the general consensus is that the amount may be around 5\% \textit{trans} fatty acids.

Man and animals require certain unsaturated fatty acids (linoleic 18:2n6, and perhaps \(\alpha\)-linolenic 18:3n3) in their diets. The physiological roles of essential fatty acids (EFA), particularly linoleic, have been extensively reviewed (9–12). Animals, including humans, require a minimum supply (1 to 2\% dietary calories) of EFA (18:2n6) to prevent overt essential fatty acid deficiency (EFAD) symptoms, though recently 10\% was recommended as being effective for minimizing arteriosclerosis and ameliorating coronary heart disease (13). The requirements for EFA may vary with intake of \textit{trans} isomers since \textit{trans} isomers by inhibiting certain enzymes may increase the need for dietary EFA. \textit{Cis,trans} and \textit{trans,trans} linoleates 18:2 are devoid of EFA activity and as sole dietary sources of fat they retard growth to a greater degree than an EFA-deficient diet (14). Other dietary factors may also affect EFA requirements (15).

It is generally accepted that the consumption of \textit{trans} fatty acids, as dietary components, exerts no gross deleterious effects on growth and general well-being. However, while much data are available on the various effects of dietary \textit{trans} fatty acids, they are of questionable value because of inadequate dietary design, e.g., lack of appropriate controls; poor definition of dietary fat, variations in the fatty acids composition of control and experimental diets; short duration of most studies and the limited number of specific physiological parameters examined during these dietary studies. Consequently it is difficult to unequivocally conclude whether some reported observations (or negative results) are due to the \textit{trans} fatty acids or to other experimental inadequacies.

The purpose of this paper is to review the effects of \textit{trans} fatty acids as they specifically affect the metabolism of linoleic acid and prostaglandins (PGs).

**Metabolism of \textit{trans} fatty acid**

The dietary \textit{trans} fatty acids are, for the most part, readily absorbed and incorporated into tissue lipids (7, 8, 16, 17). The absorption coefficient of \textit{trans} 18:1 is around 95\% and Emken (17) reported that trielaiden was absorbed as readily as triolein. The amounts of \textit{trans} fatty acids absorbed and incorporated into tissue lipids depends on their concentration in the diet (17). Human tissue samples contained \textit{trans} fatty acid concentrations (primarily t18:1) ranging from 2.4 to 12.2, 4.0 to 14.4, 4.9 to 9.3, and 2.3 to 8.8\% for adipose, liver, heart, and aortic tissue, respectively (18). Human serum lipid samples had 1.9\% \textit{trans} 18:1 and 0.8\% \textit{trans,trans} 18:2 while the corresponding erythrocytes contained 2.4\% \textit{trans} 18:1 and 0.7\% \textit{trans,trans} 18:2 (19).

Several workers (20–24) have reported that \textit{trans} fatty acids are oxidized at rates equivalent to the corresponding \textit{cis} isomers. The \textit{cis} isomer of \(^{14}\text{C}\)-labeled octadecenoic acid was catabolized to \(^{14}\text{CO}_2\) to a slightly greater extent than was the \textit{trans} isomers, and more of the \textit{trans} isomer was retained in the carcass of rats (22). This suggests that animals consuming a diet containing \textit{trans} octadecenoate for a prolonged period might accumulate the \textit{trans} acids in their depot fat. However, Sterns et al. (20) reported that \textit{trans} 18:2 was rapidly catabolized in rat liver, whereas \textit{cis} 18:2 was elongated and acylated into glycerolipids. Complete oxidation of \textit{trans} isomers may not occur in all instances and shortened isomers, i.e., \textit{trans} 16:1 and \textit{trans} 14:1 may accumulate (23). Mitochondria isolated from rat hearts catabolized CoA esters of 18:1 at different rates depending upon the geometry and position of the double bond. The \textit{cis} monoenes were the preferred substrates and of the \textit{trans} isomers, vaccenoyl-CoA was oxidized more rapidly than elaidoyl-CoA (24).

The \textit{trans} fatty acids are converted to CoA esters (25) and as such act as substrates for acyl transferases and some desaturases. In general the \textit{trans} 18:1 seems to be treated similarly to saturated fatty acid in the acylation of cholesterol and lysophosphatidylcholine (25–27).

Acylation enzyme systems can differentiate between \textit{cis,trans} isomers (26, 28). The hydrolysis of cholesterol esters containing \textit{trans} fatty acids was significantly lower than those with \textit{cis} double bonds (28) and \textit{trans} isomers also depressed the esterification of cholesterol in the liver. In vivo, \textit{trans} fatty acids are preferentially esterified into the Sn-1 position.
of phospholipids though trans-cis isomers of unsaturated acids may be acylated into Sn-2 position, particularly when saturated acids occupy position Sn-1 (29).

The deposition of trans fatty acids in tissues may be selective. Thus adipose tissue and liver generally contain higher levels than other tissues (30, 31). Minimum deposition of trans 18:1 occurs in the brain (32). The differences in tissue levels may reflect the preferential incorporation of trans fatty acids into particular lipid classes, e.g., in cholesterol esters in the case of adrenal tissue (33) or phospholipids in the case of liver tissue (34). However, this variation of trans fatty acids incorporation may merely reflect the turnover rates of lipid classes and thus may be appreciably influenced by the duration of feeding. Depletion of trans 18:1 from tissues, after cessation of feeding, occurs at a rate equivalent to that observed for saturated fatty acids, normally taking 4 to 8 wk (35).

The incorporation of trans fatty acids into membrane phospholipids may alter the packing of the phospholipid and possibly influence the physical properties of the membrane as well as the activities of the membrane associated enzymes (36), e.g., elongase, desaturase and PG synthetase.

The trans fatty acids are incorporated into serum lipoproteins and the possible significance of this in relation to atherosclerosis has been discussed (8). Jackson et al. (37) reported that dietary trans fatty acids (mostly C18:1) though incorporated into the lipoprotein fractions had no significant effect on their physical properties and little effect on the development of atherosclerotic lesions in swine.

Most studies with trans fatty acids have involved rats and while several reports indicated that trans acids have no adverse effects in rats when fed with adequate amounts of essential fatty acids (38–40), it is now recognized that trans fatty acids in the diet tend to increase the need for EFA. In EFA deficiency, trans fatty acids accentuate dermal symptoms and suppress growth (14) and recent studies in our laboratory showed that relatively low levels of dietary trans,trans 18:2 reduced liver desaturase activity (J. Shimp and J. E. Kinsella, unpublished data).

Dietary trans acids adversely affect various hemostatic and hematological properties of blood (41), enzyme activities (25, 42), and alter the properties of membrane phospholipids (36). Mitochondria from rats fed trans fatty acids are more susceptible to swelling and show lower rates of oxidative phosphorylation (42). Takatori et al. (42) concluded that the influence of trans fatty acids appeared to be out of proportion to their concentration in the diet because though relatively small concentrations accumulated in the tissues, metabolic effects were observed. This observation emphasizes the need to assess the effects of relatively small quantities of trans isomers in dietary fats on metabolic parameters in addition to clinical effects. This is particularly true where trans fatty acids may be consumed over a long period of time. However, Anderson et al. (43) reported that levels of trans fatty acids up to 18% of dietary fat had no gross ill effects on the well-being of rats when fed in the presence of adequate 18:2 (2.5%) for 26 weeks, but specific biochemical parameters were not studied.

Long-term multigeneration feeding of processed fats with up to 50% trans fatty acids in the presence of adequate linoleic acid to rats showed no evidence of any overall deleterious effects (35).

The trans isomers and desaturation of fatty acids

Small amounts of the CoA derivatives of trans monoenes can be elongated (44, 45). The trans isomers of 18:1 other than elaidic can be desaturated by Δ9 desaturase to yield trans,cis dienes (46). Dietary trans 18:1 inhibits the conversion of 18:1 to 20:3n9 and of 18:2n6 to 20:4n6 apparently by acting as a competitive inhibitor of the desaturase enzyme. This may partly explain the mechanism by which trans monoenes (and trans,trans dienes) exacerbate EFA deficiency symptoms.

Much of the available data on trans fatty acid metabolism refer to studies with elaidic acid or hydrogenated fat which contains mostly elaidic acid. Some recent studies have been conducted with diets containing trans isomers of linoleic acid. There are three common trans isomers of the naturally occurring linoleic acid (18:2n6). These are the trans,trans, the cis,trans and the trans,cis 9,12 octadecadienoic acids. The all cis fatty acid
(linoleic acid) is the only isomer that has essential fatty acid activity (12).

Interest in the metabolic effects of these isomers of 18:2n6 emanates from the knowledge that linoleic acid is the critical essential fatty acid which serves as the dietary precursor of arachidonic acid which is the principal precursor of the PGs, thromboxane, prostacyclin, and related endoperoxides (Fig. 1).

The trans,trans and the trans,cis isomers of

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**FIG. 1.** An outline of the pathway by which dietary linoleic acid (18:2n6) is desaturated/elongated to dihomoy-linolenic (20:3n6) and arachidonic acid (20:4n6). Dietary 18:2n6 and its products 20:3n6 and 20:4n6 are preponderantly acylated into the membrane PLs and thereby affect the fluidity of those membranes. Upon appropriate stimulation the esterified fatty acids, e.g., 20:4n6 (or 20:3n6) are released by phospholipase A2. The free 20:4n6 is then rapidly converted to the cyclic endoperoxides, 15-hydroperoxy-9a, 11a-peroxido-5 cis-13 trans prostadienic acid (PGG2) and 15-hydroperoxy-9a, 11a-peroxido-5 cis-12 trans (PGH2) by cyclooxygenase. In platelets these are converted to a potent proaggregatory compound (and vasoconstrictor) called thromboxane (TXA2) which in turn is rapidly converted to a stable inactive product, TXB2. Concurrently the endothelial cells lining blood vessels convert PGH2 to a potent antiaggregatory compound and vasodilator, prostacyclin (PGI2). This is rapidly converted to 6-keto PGF1, which maintains the integrity and functioning of the blood and cardiovascular system. The endoperoxides PGG2 and PGH2 are also converted to a series of PGs, e.g., PGE2 and PGG3, which exert potent regulatory effects on several physiological functions (renal, pulmonary, reproductive, digestive). A 17-carbon hydroxy fatty acid, hydroxy heptadecatrienoic acid (HHT), and malondialdehyde (MDA) are also produced during prostaglandin metabolism. Some free 20:4n6 may also be converted to hydroperoxy-eicosatetraenic acid (HPETE), by tissue lipoxygenase. These may be converted to hydroxy eicosatetraenic acid (HETE) by a peroxidase. Some of these compounds are involved in chemotaxis (leucocyte, macrophage functions) and allergic responses.

Dietary trans fatty acids may interfere with or disrupt these normal pathways at several points denoted by asterisk. Thus, upon acylation into phospholipids they may alter the physical properties and fluidity of membranes which in turn, may affect the properties of enzymes associated with these membranes. Trans fatty acids, particularly dienoic species, are competitive inhibitors of some of the enzymes, i.e., Δ6 desaturase and cyclooxygenase, required for the metabolic conversion of dietary 18:2 to the various products of 20:4n6. In this way dietary trans fatty acids may affect essential fatty acid metabolism as discussed in the text.
linoleic acid are not readily converted to isomers of arachidonic acid (46, 47), whereas there is general agreement that the cis,trans isomer of linoleic acid can be desaturated and elongated to the cis 5, cis 8, cis 11, trans 14 isomer of arachidonic acid (47, 48).

As previously mentioned, hydrogenation of cis fatty acids yields not only the geometric but the positional isomers as well. To date, there is little information available on the relative distribution of these positional isomers in commercial products (4). Their specific influence in desaturation reactions has been discussed by Mohfouz et al. (49).

Various positional isomers of trans 18:1 can be desaturated by Δ9 desaturase, and thus may compete with stearic acid (18:0) which is the normal substrate for this desaturase. Some of these positional isomers of 18:1, i.e., Δ5 and Δ13, which readily allow the introduction of a cis Δ9 double bond are strong inhibitors of palmitic acid desaturation whereas the Δ8 and Δ9 isomers are weak inhibitors (50). Therefore, these unusual dienoic acids formed from the desaturation of trans 18:1 may perturb metabolism of essentially fatty acids.

Effects of trans fatty acids on metabolism of linoleic acid

Because PGs, thromboxane, and prostacyclin are synthesized mostly from arachidonic acid (Fig. 1), it is most important that the effects of dietary trans 18:2 isomers, which may affect the availability, elongation and desaturation of 18:2 to arachidonic acid, be carefully studied.

Liver microsomes have three desaturases which act on carbons 5, 6, and 9 of dietary fatty acids. The Δ6 desaturase which acts on dietary 18:2n6 is a rate-controlling enzyme in PUFA synthesis and Brenner (51) has reviewed its role and regulatory properties. Since the Δ6 and Δ5 desaturases are involved in the conversion of essential fatty acids to PG precursors [eicosatrienoic acid (20:3n6)] and arachidonic acid (20:4n6), and since trans isomers of mono and dienoic acids may inhibit these desaturases, careful consideration must be given to the effects of these unnatural fatty acids isomers on PG metabolism and their diverse functions.

1) Effects of trans fatty acids in vitro.

There is considerable information available regarding the effects of various trans fatty acid isomers on the Δ6 and Δ5 desaturase enzymes in vitro. The trans,trans linoleic acid significantly decreased the conversion of linoleic to γ-linolenic acid (18:3n6) by rat liver microsomal preparations (52). The trans,cis 18:2 isomer also inhibits the desaturation of linoleic acid (52).

Elaidic acid (t11:1) may inhibit Δ9 and Δ6 desaturases; however, the extent of the inhibition, at least for the Δ6 desaturation of linoleic acid to γ-linolenate, appears to be much less than the inhibition obtained with t18:2 (52). The inhibition of these desaturases not only by geometric isomers, but also by various positional isomers of trans 18:1 has been recently reported by Mahfouz et al. (50).

2) Effects of trans fatty acids in vivo. Privette et al. (47) reported that cis,trans 18:2 (which can be further elongated and desaturated) did not inhibit the conversion of cis,cis 18:2 to longer chain polyunsaturated fatty acids. Privett et al. (47) showed that dietary trans linoleate impaired the conversion of oleic (18:1) to eicosatrienoic (20:3n9); of linoleic (18:2) to arachidonic (20:4n6) acids and inhibited the incorporation of 20:3n9 into cholesterol esters. These authors concluded that trans fatty acids exhibited specific effects on the metabolism of fatty acids and thereby aggravated the symptoms of EFA deficiency. This probably reflected the inhibition of the desaturase enzymes (particularly Δ6) by the dietary trans linoleate (52, 53).

Dietary trans fatty acids apparently exert negligible adverse effects when ingested in the presence of sufficient linoleic acid (35, 43). However, dietary trans,trans at relatively low levels, even in the presence of cis,cis 18:2 (at 1.1% of dietary calories), caused a reduction in Δ6 desaturase activity in rat liver (J. Shimp and J. E. Kinsella, unpublished data).

Furthermore, the observation that the cis,trans isomer of 18:2 can be converted to 20:4 isomers, and that trans isomers can affect the synthesis of prostaglandin precursors (20:3n6 and 20:4n6) warrants careful study. There is evidence that the elongated, desaturated trans isomers of linoleic acid have specific effects on the PG synthetase involved in the formation of endoperoxides from 20:3n6 and 20:4n6 fatty acids. Nugteren (54) has
shown that 8 cis, 12 trans, 14 cis eicosatrienoic and 5 cis, 8 cis, 12 trans, 14 cis eicosatetraenoic acids are extremely inhibitory toward the conversion of cis 20:3n6 to PGE1. However, these specific isomers of trans fatty acids arising from desaturation and elongation of trans 18:2 are unlikely to occur in vivo as mentioned previously. The trans isomer most likely to occur could be the 5 cis, 8 cis, 11 cis, 14 trans 20:4n6 fatty acid. However, this fatty acid has not to date been fully tested for potential inhibition of PG synthesis.

If, as the evidence indicates, certain trans fatty acids are elongated and desaturated, then it is possible that these fatty acids may decrease the availability of the natural cis polyunsaturated fatty acids for PG synthesis by displacing them from the various phospholipid fractions. The possible inhibition of PG synthase by these trans fatty acids may further implicate them in the exacerbation of EFA deficiency symptoms.

**Effects of trans fatty acid on PG biosynthesis**

The finding that linoleic acid is the critical essential fatty acid that serves as the precursor of PGs, thromboxanes, prostacyclins, leukotrienes, hydroxy fatty acids, and related endoperoxides (10, 13) has stimulated a lot of research concerned with elucidating the regulation of linoleic acid metabolism. The available evidence indicates that the availability of precursor acids is one of the important factors regulating the biosynthesis of PGs (55–64). Thus, factors affecting the level of PG precursor fatty acids (i.e., 20:3n6 and 20:4n6) can influence the amount of PGs synthesized in tissues.

Lands et al. (65) reviewed the enzymes involved in PG metabolism. The PG synthetase requires unesterified fatty acid with at least three nonconjugated cis double bonds with 20:3n6 being optimum. Twenty carbon fatty acids with double bonds at 6, 9, 12 are best; additional double bonds either cis or trans toward the carboxyl end reduce the rates of synthesis. The 20:3n6 is converted to PGE1 much more rapidly than 20:4n6 is to PGE2. Conjugated 20:3n6 or 20:4n6 with trans double bonds at 9, 12, 15 are poor substrates, but significant amounts were converted to PG isomers (65).

Several fatty acids (18:3n6, 20:3n3, 20:5n3, 20:3n9, and to a lesser extent 18:2n6 and 18:1n3) may occupy the active site of cyclooxygenase and inhibit PG synthetase (65). PUFA of the n3 family, while not being readily available in mammalian tissue for PG synthesis, are potent competitive inhibitors of PG synthesis, i.e., 20:4n3, 20:5n3 and 22:6n3 had Ki’s of 6, 2.5, and 1.7 μM, respectively (66). Conjugated fatty acids and trans isomers of PG precursors inhibit the synthesis of PGs from normal substrates, e.g., conjugated trans 20:4n6 and 20:3n6, are most potent inhibitors of the enzyme having a high affinity for the active site of the enzyme (54). Both 20:3n6 (8 cis, 12 trans, 14 cis) and 20:4n6 (5 cis, 8 cis, 12 trans, 14 cis) inhibited PG synthesis by 74 and 54%, respectively (54), i.e., the presence of a trans or conjugated double bond in the usual PG precursors may cause a significant depression in synthesis.

These in vitro studies strongly indicate that various isomers of unsaturated fatty acids (which may occur in processed fats) and metabolic derivatives of these can impair prostaglandin homeostasis. This information emphasizes the need for in vivo studies. Little is known about the regulation of PG synthesis in vivo; however, accumulating data indicate a marked impact of dietary fatty acids (13, 67).

Dietary fatty acids by affecting availability of 20:3 and 20:4 influence PG biosynthesis (55). Hulan and Kramer (58) observed a positive correlation between dietary 18:2n6 and the synthesis of PGE in rat skin. Dupont et al. (68) showed a strong positive relationship between dietary 18:2n6 and the synthesis of PGE1, PGE2, PGF2α, and TXB2 by platelets. High dietary levels of 18:3n3 decreases the conversion of 18:2 to 20:3 and 20:4 and also impairs PG synthesis (59). EFA deficiency results in depressed PG synthesis (60, 62) due to unavailability of precursor FA or inhibition of cyclooxygenase by 20:3n9 (63) “PG synthetase” activity is apparently increased in EFAD (64).

The amount of dietary linoleic acid has been shown to exert a profound influence on the biosynthesis of serum PG’s (55, 61, 68–70). Disruption of PG biosynthesis has been implicated in renal failure as observed in rodents on an EFA-deficient diet (64). There is a growing body of data relating the involve-
ment of dietary fatty acids to PG biosynthesis and to subsequent related vascular and thrombotic phenomena, i.e., regulation of platelet aggregation (70). The delicate interrelationship between fatty acid levels and hemodynamic functions, as regulated by PG endoperoxides was extensively discussed in a review by Marcus (71).

Because of the potential influence of dietary trans fatty acids on the biochemical pathways regulating PG synthesis the effects of dietary trans fatty acids need to be carefully evaluated in vivo.

**Specific effects of dietary trans, trans-octadecadienoate on PG synthesis and tissue lipid composition**

Because the availability of precursor fatty acids is a major factor controlling the production of PGs, dietary components affecting the availability of these fatty acids should also influence the biosynthesis of PGs in animal tissues. Since isomers of trans linoleate aggravate the symptoms of EFA deficiency and inhibit conversion of cis linoleate to arachidonate it is important to determine if dietary trans linoleate affects the biosynthesis of PGs in vivo. In a study using very high concentrations of dietary trans, trans linoleate (alone) and in combination with cis, cis 18:2 we observed that this fatty acid affected the level of PG precursors in various tissues and the capacity of blood platelets to synthesize PGs (61).

Three groups of Sprague Dawley weanling male rats were fed balanced isocaloric diets containing all cis, cis 18:2 (CL); equal amounts of cis, cis 18:2 and trans, trans 18:2 (CL-TL) and all trans, trans 18:2 (TL) as sole sources of dietary fat supplying 11 cal percent (61). This extreme dietary level was employed to accentuate the impact of trans, trans 18:2. An EFA deficient diet [hydrogenated coconut oil (HCO)] was fed to a fourth group.

The growth pattern observed for rats (Fig. 2) on the various dietary treatments were consistent with earlier observations. Growth rates were depressed in rats receiving all trans, trans 18:2 to a significantly greater extent than rats maintained on the HCO diet. The early onset and severity of dermal symptoms and the decreased body weight gains of rats in the TL group indicated that the trans 18:2 aggravated EFA deficiency symptoms compared to the HCO (EFAD) group.

The weights, total lipid and fatty acid composition of the various organs from rats maintained on the different dietary fatty acids for 12 wk are shown in Table 1. The HCO, CL-TL, and TL groups generally showed a reduction in the total n6 family of fatty acids (i.e., 18:2, 20:4, 22:4, 22:5) and a concomitant increase in the n7 and n9 family fatty acids (i.e., 16:1n7, 18:1n9, and 20:3n9) in comparison to the CL group. Although the total amounts of n6 fatty acids in the TL group appeared to be greater than that for the HCO group, the symptoms of EFA deficiency were more severe for the TL animals. The data suggested that the trans fatty acids interfered with the metabolism of essential fatty acids, thereby impairing their conversion to PGs. The observed differences in the severity of EFAD symptoms was probably caused by the inhibition of the Δ6 desaturase enzyme by the trans fatty acids, as indicated by the higher 18:2/20:4 and 18:1n9/20:3n9 ratios in the organs from animals on the TL versus HCO diets, respectively. The inhibition of the Δ6 desaturase could also explain the somewhat lower 20:3/20:4 ratios in the TL compared to the HCO organs.

Because they represent structural components and provide the precursor fatty acids for PG synthesis, the fatty acids of the phospholipids (PL) from the various organs were also analyzed (Table 2). The influence of dietary trans fatty acids on the fatty acid composition of PL from various organs was very similar to that observed for the corresponding total lipids. There was a reduction of the total n6 family fatty acids and an increase in the n7 and n9 fatty acids in PL from EFAD rats (HCO in comparison to linoleic supplemented controls (CL)). The inhibition of the Δ6 desaturase by trans fatty acids was indicated by the increased 18:2/20:4 and 18:1n9/20:3n9 ratios in rats on the TL and CL-TL compared to CL and HCO dietary groups, respectively.

The observation that the various organs reflect different 18:2/20:4 ratios in linoleic supplemented animals (CL) indicates the importance of assessing the relative fatty acid distribution associated with the various PL classes in future trials. A better understanding of the relative abundance of the unsaturated
fatty acids associated with different PL classes could then be ascertained.

The data show that the triene to tetraene ratios (20:3/20:4) did not accurately reflect the EFA status of the animals in this study. These ratios are lower in all the organs from the rats consuming the all trans 18:2 (indicating a better EFA status for the TL group) compared to those from HCO rats, however the performance data (i.e., growth, dermal symptoms) indicated that the TL group had much more severe symptoms of EFAD. This observation may be partly explained by the lower 20:4 levels found in the organs from the all trans 18:2 diets compared to those from HCO diets, implying that EFAD symptoms are closely related to availability of 20:4n6. The lower levels of 20:4n6 and 20:3n9, as suggested, may be due to the inhibition of the desaturase enzyme. The fatty acid precursors of the PG were very much influenced by dietary trans octadecadienoic acid (Tables 1 and 2). The lower concentrations of 20:3n9 in rats receiving TL compared to those on HCO indicated that the trans,trans 18:2 inhibited desaturation and elongation of 18:1n9. This effect also invalidates the triene: tetraene ratio as a reliable index of EFAD status under these dietary conditions.

The alteration in platelet fatty acid composition and corresponding PG concentrations as influenced by dietary trans fatty acids are summarized in Table 3. The levels of 20:4n6 were significantly lower in platelets from rats receiving trans,trans 18:2. It should be mentioned that there is little desaturase and elongase activity associated with platelets (71) and therefore the fatty acid composition of...
TABLE 1
Fatty acid composition of total lipids from organs of rats after 12 wk on different dietary fatty acids

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Kidney</th>
<th>Lung</th>
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<tbody>
<tr>
<td><strong>Organ wt (g)</strong></td>
<td>CL</td>
<td>CL-TL</td>
<td>TL</td>
</tr>
<tr>
<td>mg/100g (body wt)</td>
<td>1.06</td>
<td>0.92</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>Fatty Acid wt (%)</strong></td>
<td></td>
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<tr>
<td>14:0</td>
<td>0.4</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>16:0</td>
<td>15.6</td>
<td>14.9</td>
<td>12.4</td>
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<td>18:0</td>
<td>22.6</td>
<td>18.1</td>
<td>15.2</td>
</tr>
<tr>
<td>16:1 n7</td>
<td>1.6</td>
<td>1.8</td>
<td>5.7</td>
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<tr>
<td>18:1 n9</td>
<td>13.5</td>
<td>15.4</td>
<td>36.4</td>
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<tr>
<td>20:3</td>
<td>5.1</td>
<td>18.2</td>
<td>1.4</td>
</tr>
<tr>
<td>18:1/20:3</td>
<td>t</td>
<td>t</td>
<td>t</td>
</tr>
<tr>
<td>18:2/20:4</td>
<td>0.98</td>
<td>1.07</td>
<td>1.50</td>
</tr>
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<td>18:1/20:3</td>
<td>7.13</td>
<td>1.64</td>
<td>36.87</td>
</tr>
<tr>
<td>20:3/20:4</td>
<td>0.98</td>
<td>2.84</td>
<td>0.61</td>
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* CL, CL-TL, TL, and HCO denote the rats receiving all cis, cis 18:2; 50% each of cis, cis 18:2 and trans, trans 18:2; all trans, trans 18:2 and hydrogenated coconut oil, respectively.

TABLE 2
Fatty acid composition of PLs from organs of rats on different dietary fatty acids

<table>
<thead>
<tr>
<th></th>
<th>Heart</th>
<th>Kidney</th>
<th>Lung</th>
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<tbody>
<tr>
<td><strong>Fatty acid wt %</strong></td>
<td>CL</td>
<td>CL-TL</td>
<td>TL</td>
</tr>
<tr>
<td>16:0</td>
<td>10.9</td>
<td>13.5</td>
<td>7.0</td>
</tr>
<tr>
<td>18:0</td>
<td>23.4</td>
<td>28.5</td>
<td>23.6</td>
</tr>
<tr>
<td>16:1 n7</td>
<td>7.0</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>18:1 n9</td>
<td>9.4</td>
<td>12.5</td>
<td>39.3</td>
</tr>
<tr>
<td>20:3</td>
<td>5.9</td>
<td>22.6</td>
<td>5.4</td>
</tr>
<tr>
<td>18:2/20:4</td>
<td>2.8</td>
<td>6.1</td>
<td>1.1</td>
</tr>
<tr>
<td>18:1/20:3</td>
<td>7.13</td>
<td>1.64</td>
<td>36.87</td>
</tr>
<tr>
<td>20:3/20:4</td>
<td>0.98</td>
<td>2.84</td>
<td>0.61</td>
</tr>
</tbody>
</table>

* See Table 1

TABLE 3
The effect of dietary fatty acids on serum PG levels and the concentration of their precursor acids in rat platelets (12 wk)*

<table>
<thead>
<tr>
<th>Dietary fatty acids</th>
<th>Platelet 20:3ω6</th>
<th>Serum PGE1</th>
<th>Platelet 20:4ω6</th>
<th>Serum PGE2, PGF2α</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>ng/ml</td>
<td>%</td>
<td>ng/ml</td>
<td>ng/ml</td>
</tr>
<tr>
<td>CL</td>
<td>0.33</td>
<td>5.69 ± 0.59</td>
<td>17.60</td>
<td>24.89 ± 4.35</td>
</tr>
<tr>
<td>CL-TL</td>
<td>0.10</td>
<td>3.51 ± 0.58</td>
<td>14.11</td>
<td>11.64 ± 2.63</td>
</tr>
<tr>
<td>TL</td>
<td>Trace</td>
<td>0.22 ± 0.02</td>
<td>1.43</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>HCO</td>
<td>Trace</td>
<td>1.10 ± 0.24</td>
<td>1.91</td>
<td>2.19 ± 0.85</td>
</tr>
</tbody>
</table>

* Hwang and Kinsella (61).
platelets reflect the pool from which they are derived, i.e., serum fatty acids.

The level of 20:4n6 was only slightly decreased in the platelet lipids of the CL-TL group compared to the CL group (20%) whereas the corresponding PGE2 level was drastically reduced (~64%). As the level of dietary trans,trans fatty acid was increased and/or the level of EFA in the diet decreased the influence of trans fatty acids became more striking, i.e., the PG levels for the TL compared to HCO groups were more depressed, especially when compared to the EFA-supplemented animals.

These data indicated that abnormally high levels of dietary trans fatty acids exerted a greater impact on PG production than is apparent from their effects on levels of the respective PG precursor fatty acids. The effect of 50% trans 18:2 in significantly decreasing all these prostaglandins, even when apparently adequate amounts of precursors were available, is noteworthy. This may indicate that the trans,trans 18:2 fatty acid or metabolic derivatives thereof inhibited some of the enzymes involved in prostaglandin synthesis. This may in part explain why the classical clinical symptoms of EFA deficiency are more severe in the rats receiving high dietary levels of trans,trans 18:2. Furthermore, it may be speculated that the EFA deficiency symptoms are also aggravated by the decreased desaturation and elongation of 18:1n9 to 20:3n9 which might result in decreased membrane integrity.

More recently we observed that increasing proportions of dietary trans,trans 18:2 when fed to rats receiving cis,cis 18:2n6 (1.1% of calories) and hydrogenated tallow to provide a total of 12.9% of total calories, progressively reduced hepatic Δ6 desaturase activity. However, this effect was observed only when the trans,trans 18:2 exceeded 20% of total calories from fat or 2.5% of total dietary calories (J. Shimp and J. E. Kinsella, unpublished data).

In light of the aforementioned evidence, it is extremely important to carefully reexamine the role of trans fatty acids on desaturase inhibition in vivo, because even small changes in fatty acid concentrations may result in large changes in the levels of PGs synthesized. Since PGs have been shown to play a critical role in modulating many physiological events, the importance of reexamining the influence of dietary trans fatty acids (as well as other dietary substances) on EFA metabolism and subsequent PG biosynthesis is extremely important.

References

19. Perkins EG, McCarthy T, O'Brien T, Kummerow F. The application of packed column gas chromato-
olenate and the positional distribution of linoleate isomers in liver lecithin. Biochim Biophys Acta 1965;106:56-61.


