TRANS MONOUNSATURATED FATTY ACIDS IN NUTRITION AND THEIR IMPACT ON SERUM LIPOPROTEIN LEVELS IN MAN

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I. INTRODUCTION

Trans fatty acids have been part of the human diet since times immemorial, because they occur in small amounts in the milk and body fat of ruminant animals, such as cows and sheep. However, large scale production and incorporation of fats high in trans fatty acids into foods started almost a hundred years ago, prompted by the requirements of the budding margarine industry. Margarine had first been manufactured in 1869 by the French scientist Mège Mouries from beef tallow and skimmed milk, the fluid left after churning of milk into butter. Margarine production was initially hampered by a lack of suitable butter-like fats and did not really take off until the introduction of hydrogenation. This is a widely used process to increase the melting range of oils so as to obtain solid fats and to increase the oxidation resistance of the oils. Hydrogenation now permitted industry to use a wide variety of marine and vegetable oils for the production of fats with the required texture and stability. Trans fatty acids from partially hydrogenated oils have been a component of the human diet for most of this century.
II. STRUCTURE AND CHEMISTRY

All unsaturated fatty acids have at least one carbon–carbon double bond. Because there is no rotation around the double bond, it is possible to create two molecules with a different configuration (Fig. 1). In the cis configuration the two carbon moieties are on the same side of the double bond and in the trans configuration they are on opposite sides. The cis configuration produces a bend in the molecule, whereas the trans configuration resembles more the straight chain of saturated fatty acids. In this way, two isomers with the same number of carbon, hydrogen and oxygen atoms can have a different three-dimensional structure. Such isomers that have their double bonds at the same position in the carbon chain, but have different configurations are called geometrical isomers. In addition to this spatial isomerism, double bonds may be located at any place along the molecule, so many positional isomers may also exist.

Geometrical and positional isomers have different physical and chemical characteristics. Oleic acid (cis-C18:1n-9) and elaidic acid (trans-C18:1n-9), for example, are geometrical isomers: both molecules have eighteen carbon atoms, thirty-four hydrogen atoms, two oxygen atoms and a single double bond present at the (n-9) position. The melting point of oleic acid is 13 °C, that of elaidic acid 44 °C and of the fully saturated stearic acid 70°C. This relatively high melting point makes trans isomers attractive for the production of semi-solid cooking fats and margarines.

III. HYDROGENATION OF OILS

A. Industrial Hydrogenation

Hydrogenation of vegetable and in some countries also of marine oils, is applied to modify their chemical, physical and sensory characteristics so as make them suitable for incorporation into foods. Depending on the hydrogenation conditions (hydrogen pressure, temperature, nature of the catalyst and stirring speed), three types of reaction may occur. Hydrogen may add to a cis carbon–carbon double bond, which then becomes saturated with hydrogen. For example, complete hydrogenation of linoleic acid (cis,cis-C18:2n-6) or α-linolenic acid (cis,cis,cis-C18:3n-3) produces stearic acid: no double bonds are left. Alternatively, the cis configuration may isomerize into a trans configuration, without net uptake of hydrogen. Finally, positional isomers can be formed through migration of the double bond along the length of the molecule. Strictly speaking, the latter two processes
**Trans monounsaturated fatty acids**

<table>
<thead>
<tr>
<th>Cis-monomounsaturates</th>
<th>Trans-monomounsaturates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cis-C18:1(n-7/Δ11)</td>
<td>Trans-C18:1(n-7/Δ11)</td>
</tr>
<tr>
<td>Cis-C18:1(n-8/Δ10)</td>
<td>Trans-C18:1(n-8/Δ10)</td>
</tr>
<tr>
<td>Cis-C18:1(n-9/Δ9) (Oleic acid)</td>
<td>Trans-C18:1(n-9/Δ9) (Elaidic acid)</td>
</tr>
<tr>
<td>Cis-C18:1(n-10/Δ8)</td>
<td>Trans-C18:1(n-10/Δ8)</td>
</tr>
<tr>
<td>Cis-C18:1(n-11/Δ7)</td>
<td>Trans-C18:1(n-11/Δ7)</td>
</tr>
</tbody>
</table>

**Stearic acid**

C18:0

**Isomers of linoleic acid**

Trans,trans-C18:2

Cis,trans-C18:2

Cis,cis-C18:2

**Linoleic acid**

Cis,cis-C18:2(n-6,9)

Fig. 2. Hydrogenation of linoleic acid leads to the formation geometrical and positional isomers of linoleic acid (trans,cis-C18:2, cis,trans-C18:2, trans,trans-C18:2 and cis,cis-C18:2), of elaidic acid and its positional isomers (trans-C18:1), of oleic acid and its positional isomers (cis-C18:1) and to stearic acid (C18:0).

should be called isomerization and not hydrogenation: although the oil is converted into a more solid product, no hydrogen is added to the fatty-acid molecule.

Thus, partial hydrogenation of vegetable oils, which is applied widely in the edible fats industry, produces a complex mixture of fatty acids (Fig. 2). The hardening conditions as well as the fatty-acid composition of the feedstock oil determine the exact composition of the partially hydrogenated product (Table 1). *Trans* monounsaturated fatty acids with eighteen carbon atoms (trans-C18:1) are typically abundant in hydrogenated vegetable oils. However, positional *cis* isomers of oleic acid are formed as well.

**B. Biohydrogenation**

Hydrogenation of polyunsaturated fatty acids also occurs in the rumen of cows, sheep and other ruminants. In the absence of oxygen, bacteria use the double bonds of fatty acids as acceptors for the hydrogen produced during metabolism. This leads to saturation of dietary unsaturates as well as to the formation of *trans* fatty acids.

**TABLE I. Fatty-acid Composition of Soybean Oil hydrogenated to Various Degrees**

<table>
<thead>
<tr>
<th>Degree of hydrogenation</th>
<th>Nil</th>
<th>Low</th>
<th>Mid</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>g per 100 g of fatty acids</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>11</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>4</td>
<td>4</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>cis-C18:1*</td>
<td>22</td>
<td>29</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>trans-C18:1*</td>
<td>0</td>
<td>12</td>
<td>12</td>
<td>51</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>54</td>
<td>31</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>trans-C18:2*</td>
<td>0</td>
<td>8</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>8</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Cis-C18:1 refers to oleic acid and its positional isomers, trans-C18:1 to elaidic acid and its positional isomers, and trans-C18:2 to all possible geometrical and positional isomers of linoleic acid.

Samples were analyzed by Unilever Research Laboratory, Vlaardingen, The Netherlands.17
IV. ANALYSIS OF TRANS FATTY ACIDS

Infra-red absorption spectrophotometry is the classical technique for the quantification of trans fatty acids. It is based on the specific absorption band of the trans double bond at about 970 cm\(^{-1}\) (10.3 \(\mu\)m). The result is expressed in terms of grams of elaidic acid per 100 g of fatty acids. Biased values may thus be expected for fatty acids that have a longer chain length and/or more than one trans double bond per molecule, such as can be found in partially hydrogenated fish oils. The infra-red technique is not sensitive enough for reliable quantification of levels of 5% or less. Sensitivity can be improved by the use of Fourier-transform infra-red spectroscopy.

Gas–liquid chromatography appears a convenient technique for the identification and quantification of individual trans fatty acids. However, it is complicated by the frequent overlap of peaks, especially of the n-11 and n-12 (\(\Delta 7\) and \(\Delta 6\)) cis with the n-6 to n-8 (\(\Delta 12\) to \(\Delta 10\)) trans isomers of C18:1. Gas-chromatographic figures for trans fatty acids should therefore be approached with caution and should preferably be backed up by infra-red spectroscopy.

Trans fatty acids with twenty or twenty-two carbon atoms as present in hydrogenated fish oils cannot be reliably resolved by gas chromatography; here infra-red spectroscopy is the chosen method.

V. TRANS FATTY ACIDS IN FOODS

A. Plants and Vegetables

Unsaturated fatty acids in vegetable and animal tissues typically have the cis configuration, while the double bonds are usually located at the n-3, n-6, and/or n-9 positions. There are, however, some exceptions. Trans isomers of palmitoleic acid, oleic acid and polyunsaturated acids have been identified in leaves and seeds of several plant species, as summarized by Sommerfeld.\(^{46}\) The fat content of leaves, however, is low and although seed oils of plants rich in trans fatty acids do exist,\(^{18}\) they do not contribute significantly to dietary fat intake in man.

B. Hydrogenated Oils and Processed Foods

The trans fatty acid content of margarines varies from about 30% in brick-type hard margarines made from partially hydrogenated vegetable oil down to less than 1% in certain soft diet margarines. The latter are produced by mixing non-hydrogenated sunflower oil with a small amount of a fat high in stearic and palmitic acid. Hard, high-melting, margarines and shortenings made from partially hydrogenated vegetable oils typically contain quantities of trans fatty acids between 20 and 30% of total fatty acids, but levels up to 45% can be found.\(^{13,40,45}\) Margarines, however, are not the only source of trans fatty acids. Partially hydrogenated oils may also be used for the production of breads and rolls, parfried French fries, candies, cookies, crackers, mayonnaise, cakes and related products and for the preparation of deep-fat fried fast foods. It should be emphasized, however, that such products may vary widely in their composition.\(^{13}\)

The double bond distribution of positional trans and cis isomers also varies. Most positional trans isomers, however, have their double bond at the n-8 (\(\Delta 10\)), n-9 (\(\Delta 9\)) and n-10 (\(\Delta 8\)) position, and most positional cis isomers at the n-9 (\(\Delta 9\)), n-8 (\(\Delta 10\)), n-7 (\(\Delta 11\)) and n-6 (\(\Delta 12\)) position.\(^{42}\)

Traditionally, marine oils have been an important raw material for the manufacture of margarines. The use of partially hydrogenated fish oil is nowadays largely restricted to the United Kingdom, Norway and the Netherlands. During hydrogenation, eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), characteristic for fish oils, become partially saturated as well as isomerized to trans isomers. The level of trans fatty acids of such fats as measured by infra-red absorption is typically 25–45 g
per 100 g of fatty acids,\textsuperscript{20} most of which is contributed by monounsaturated fatty acids with a chain length of 20 or 22 carbon atoms.\textsuperscript{44}

\textbf{C. Dairy and Meat}

Bacteria in the rumen of animals like cows and sheep produce \textit{trans} fatty acids that can be found in their milk and meat. Levels of \textit{trans}-C18:1 in milk fat range from 4 to 10\% of total fatty acids,\textsuperscript{13,46} although large seasonal variation exists.\textsuperscript{38} The isomer composition does not vary as much as that of industrially hydrogenated fats. Most \textit{trans} double bonds are located at the (n-7) and to a lesser extent at the (n-9) position. Small amounts of other \textit{trans} isomers, as well as positional \textit{cis} isomers, are also found in dairy products.\textsuperscript{38,46}

\textbf{D. Trans Fatty Acids in Human Milk}

\textit{Trans} fatty acids in human milk are derived from the diet. Koletzko \textit{et al.}\textsuperscript{27} studied the fatty-acid composition of milk from 15 German mothers during the third or fourth month of lactation. The median value for total fat content was 53.5 g/l. \textit{Trans} fatty acids contributed 4.4\% of all fatty acids. Seven different \textit{trans} fatty acids were identified. The level of \textit{trans}-C18:1 was 3.1\%, but small amounts of less than 0.5\% of \textit{trans}-C14:1, \textit{trans}-C16:1, \textit{trans}-C20:1 and \textit{trans} isomers of linoleic acid were also reported. Chappell \textit{et al.}\textsuperscript{6} found levels of 2.8\% of \textit{trans}-C18:1 in breast milk samples from U.S.A. mothers. Several positional isomers of \textit{trans}-C18:1 were observed, although elaidic acid accounted for as much as 2.6\%. Also small amounts of \textit{trans} isomers of linoleic acid were detected, but no \textit{trans}-C14:1 and \textit{trans}-C16:1. Craig-Smith \textit{et al.}\textsuperscript{7} fed eight healthy mothers, 2 months post-partum, two diets either high or low in hydrogenated fats. \textit{Trans}-C18:1 provided on average about 3.5\% of total daily energy on the hydrogenated-fat diet, while the other diet was practically free of \textit{trans}-C18:1. An increase in the level of \textit{trans}-C18:1 in breast milk was already observed on the second day on the high-\textit{trans} diet and a decrease on the low-\textit{trans} diet. This short-term study suggests that the content of \textit{trans}-C18:1 in the milk is related to its level in the diet of the previous day. It was estimated that each increment of 1\% of energy from \textit{trans}-C18:1 in the diet increased the level in milk by 0.42\%.\textsuperscript{7}

Infant formula diets may also contain \textit{trans} fatty acids, although levels are generally lower than in human milk.\textsuperscript{26,39}

\section*{VI. CONSUMPTION OF \textit{TRANS} FATTY ACIDS}

\textbf{A. Intake in Various Countries}

The \textit{trans}- fatty acid content of foods is not given in most nutrient data banks, which makes it difficult to obtain a reliable estimate of the current intake. Based on published data, the estimated intake of \textit{trans}-C18:1 in Western countries varies between 2 and 12 g per day or 5–7\% of total fatty acids (Mensink and Katan, in preparation). The variation in intake between individuals is also very large.\textsuperscript{2,52}

\textbf{B. Contribution of Specific Food Items}

Margarine and spreads are a major source of \textit{trans} fatty acids in the diet. Hunter and Applewhite\textsuperscript{31} estimated the contribution of these two food items for the U.S.A. to total \textit{trans} intake at 31\%, of industrial fats and oils—used for baked goods, deep-fat frying, etc.—at 27\% and of meat and dairy products at 17\%. For the United Kingdom it was estimated that margarines and other fats and oils contributed about 42\% to total \textit{trans} intake, milk, butter and other dairy products 14\% and meat and meat products also 14\%.\textsuperscript{5}
VII. METABOLISM

Conversion into a trans isomer is an obligatory step in the \(\beta\)-oxidation of cis-unsaturated fatty acids. Human cells would therefore be expected also to metabolize exogenous trans fatty acids. This is indeed what has been found.

Aspects of the metabolism of positional cis-C18:1 and trans-C18:1 isomers have been reviewed in detail. In man Emken and co-workers have compared the uptake, distribution and turnover in human plasma lipids of positional and geometrical isomers of oleic acid. For this purpose deuterium-labelled isomers of oleic and elaidic acid were synthesized and converted into triacylglycerols. A single dose of these triacylglycerols was then fed as liquid-formula diets to two or three healthy volunteers and blood was sampled over the next 48 hr.

In general, the evidence suggested that there is no difference between the absorption of oleic acid and its positional or geometrical isomers. After absorption, however, incorporation of the isomers into plasma lipids was different. Oleic acid was preferentially incorporated into plasma triglycerides and cholesteryl esters as compared with its geometrical and positional isomers. Results for other plasma lipid components were less consistent. Oxidation rates and turnover rates may also be slightly different, although this does not lead to accumulation of trans fatty acids in human tissues over the years. If these differences in oxidation rate are real, certain unknown adaptive mechanisms must therefore exist to prevent isomeric fatty acids from accumulating. Elongation and desaturation occurred, but at very low rates. In a study with free-living subjects Melchert et al. have compared the fatty acid spectra of serum cholesteryl esters, triglycerides, free fatty acids, diglycerides and phosphatidylcholine from the plasma of male and female vegetarians and non-vegetarians. Levels of elaidic acid were slightly lower in vegetarians than in non-vegetarians. The proportion of elaidic acid, however, differed widely and inconsistently between the different lipid fractions.

The metabolism of the very long chain trans fatty acids from partially hydrogenated fish oil in man has been studied much less intensively. However, the fact that these fatty acids did not accumulate in the fat tissue of Dutchmen despite a life-long intake of some 5 g per day suggests that their catabolism does not present a major problem.

VIII. TISSUE LEVELS AND DIET

Trans fatty acids in human tissue lipids probably originate solely from dietary sources. This intake of trans fatty acids can therefore be estimated by analysis of the fatty-acid spectrum of subcutaneous adipose tissue, which is relatively easy to sample. The half-life of this fat depot is more than 2 years and its proportion of essential fatty acids—which are also completely supplied by the diet—is considered a valid indicator of the average intake over the preceding 1–3 years. The correspondence between levels of trans fatty acids in the diet and the levels present in human adipose tissue has not been adequately established. Studies in rats and mice, however, suggest that the relative proportion of trans-C18:1 in the diet exceeds that in adipose tissue. In humans, London et al. also noted a higher proportion of trans fatty acids in the diet than in subcutaneous adipose tissue. In this study with 125 post-menopausal women dietary fatty-acid intake was estimated by a semi-quantitative questionnaire over the past year. The level of trans-C18:1 in adipose tissue was positively related to the estimated intake of trans-C18:1 in the diet, expressed as g per day or as percentage of total fat intake. The Spearman correlation coefficient was about 0.50 (\(P < 0.001\), similar to values found for the relation between linoleic acid in the diet and in adipose tissue. These results suggest that the level of trans-C18:1 in the diet is reflected in adipose tissue.
A. Adipose Tissue Levels in Various Countries

Table 2 shows the trans-fatty acid level in adipose tissue of subjects from the U.S., the U.K., Germany, The Netherlands and Israel. Elaidic acid and its positional isomers (trans-C18:1) are the most abundant and levels range between 2 and 4%. The proportion of trans isomers of linoleic acid varies between 0.4 and 1.3% and the contribution of trans-C16:1 is less than 1%. Two studies also reported the presence of trans-C14:1 at levels of 0.05 and 0.12%, respectively.

The range of values is similar for all countries, with no notable exceptions, which would suggest similar levels of dietary trans fatty acids. However, in view of the analytical problems involved in the identification of trans fatty acids (see above), these figures should be interpreted with caution. Studies from the U.K. reported also small amounts of trans-C20:1 and trans-C22:1, which are probably derived from hydrogenated marine oils.

The distribution of positional trans-C18:1 as well as cis-C18:1 isomers in human adipose tissue reflects that of partially hydrogenated soy bean oil, the major dietary source of trans fatty acids in the U.S.A. This also suggests that incorporation into human fat tissue is comparable for the various positional isomers.

B. Other Tissues

Levels of trans fatty acids have also been reported for liver, heart, aorta, brain, kidney and jejunum tissue lipids. For the same subjects levels were generally lower than those in adipose tissue, while trans-C18:1 was the most prominent trans-fatty acid. Heckers et al., however, noted higher trans-C16:1 than trans-C18:1 levels in jejunum and aortic tissue lipids.

IX. TRANS FATTY ACIDS AND SERUM LIPIDS AND LIPOPROTEINS

Cholesterol is transported in the blood in the form of lipoproteins. The risk of coronary heart disease is positively related to the total cholesterol and to the cholesterol level in the low-density lipoprotein (LDL) fraction, but negatively to that in the high-density lipoprotein (HDL) fraction. The major apoprotein in LDL is apoprotein B and in HDL apoprotein A-I. Another lipoprotein, lipoprotein(a) (Lp(a)), is an LDL-like particle. Its protein part is composed of apoprotein B and apoprotein(a). Although Lp(a) does not contribute significantly to total cholesterol levels, high levels are associated with increased risk for coronary heart disease.

As discussed above (Table 1), hydrogenation may increase dietary levels of trans mono- and trans polyunsaturated fatty acids, of stearic acid and of positional isomers of oleic acid and may decrease the level of ω-linolenic, of linoleic and of oleic acid. Many of the older studies on trans fatty acids compared the cholesterolemic potential of partially hydrogenated oil with that of the original oil. Thus, the level of trans fatty acids was not the sole variable between the diets, so that results could not be attributed to specific fatty acids.

A. Total Cholesterol

Two of the earlier studies that have examined the effect on serum total cholesterol levels of trans-C18:1 yielded conflicting results. Mattson et al. fed thirty subjects for three weeks a diet high in oleic acid. For the next four weeks 17 subjects continued on the high-oleic acid diet, while in the diet of the other men 14% of energy from cis-C18:1 was replaced by trans-C18:1. Cholesterol intake was 500 mg per day. Changes in serum total cholesterol and triglycerides were not different between the two groups. Vergroesen and co-workers tested the effects of trans-C18:1 on serum total cholesterol levels in two separate experiments. The first study suggested that 14% of energy from trans-C18:1 was only
### Table 2. Levels of Trans Fatty Acids in Human Depot Fat of Subjects from Various Countries

<table>
<thead>
<tr>
<th>First author</th>
<th>Country</th>
<th>Year</th>
<th>Method analysis*</th>
<th>Sampling side</th>
<th>No. of subjects†</th>
<th>Trans-C16:1</th>
<th>Trans-C18:2</th>
<th>Trans-C18:2 trans</th>
<th>Total trans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnston22</td>
<td>U.S.A.</td>
<td>1957</td>
<td>IR</td>
<td></td>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td>(2.4–12.2)</td>
</tr>
<tr>
<td>Johnston23</td>
<td>U.S.A.</td>
<td>1958</td>
<td>IR</td>
<td></td>
<td>6F</td>
<td></td>
<td></td>
<td></td>
<td>(4.6 ± 2.4)</td>
</tr>
<tr>
<td>Kaufmann25</td>
<td>Germany</td>
<td>1964</td>
<td>IR</td>
<td>Mesenterium</td>
<td>8M, 7F</td>
<td></td>
<td></td>
<td></td>
<td>(1.5–6.8)</td>
</tr>
<tr>
<td>Heckers16</td>
<td>Germany</td>
<td>1979</td>
<td>GC</td>
<td></td>
<td>16M</td>
<td>1.9 ± 0.8</td>
<td></td>
<td>(1.2–4.2)</td>
<td>(2.0–9.3)</td>
</tr>
<tr>
<td>Ohlrogge17</td>
<td>U.S.A.</td>
<td>1981</td>
<td>GC</td>
<td>Abdomen Omentum</td>
<td>5M, 3F</td>
<td>3.4</td>
<td></td>
<td>(2.0–5.8)</td>
<td></td>
</tr>
<tr>
<td>Thomas48,51</td>
<td>Wales/Engl</td>
<td>1981</td>
<td>GC, IR</td>
<td>Abdomen</td>
<td>231M</td>
<td>0.7</td>
<td>2.5</td>
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<td>5.4</td>
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<td>Enig1</td>
<td>Israël</td>
<td>1984</td>
<td>GC</td>
<td>Abdomen</td>
<td>1M, 7F</td>
<td>0.4 ± 0.1</td>
<td>3.5 ± 1.3</td>
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<td>(0.2–0.4)</td>
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<td>(1.7–5.8)</td>
<td>(0.2–0.8)</td>
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<td>(1.9–6.6)</td>
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<tr>
<td>Katan24</td>
<td>Netherlands</td>
<td>1986</td>
<td>GC</td>
<td>Buttock</td>
<td>66F</td>
<td>0.9 ± 0.2</td>
<td>3.1 ± 0.7</td>
<td>0.9</td>
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<tr>
<td>(0.5–1.6)</td>
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<td>(1.4–5.0)</td>
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<tr>
<td>Adlof1</td>
<td>U.S.A.</td>
<td>1986</td>
<td>GC</td>
<td></td>
<td>6M</td>
<td>0.3</td>
<td>4.0</td>
<td>0.4</td>
<td></td>
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<tr>
<td>Thomas49</td>
<td>Wales</td>
<td>1987</td>
<td>GC</td>
<td>Abdomen</td>
<td>120M</td>
<td>0.4</td>
<td>2.5</td>
<td>0.9</td>
<td></td>
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<td>Scotland</td>
<td>1987</td>
<td>GC</td>
<td>Abdomen</td>
<td>54M</td>
<td>0.7</td>
<td>2.8</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>Wahl19</td>
<td>Scotland</td>
<td>1990</td>
<td>GC</td>
<td>Abdomen</td>
<td>58M, 88F</td>
<td>0.7</td>
<td>2.6</td>
<td></td>
<td>4.3</td>
</tr>
<tr>
<td>Hudgins19</td>
<td>U.S.A.</td>
<td>1991</td>
<td>GC</td>
<td>Gluteal</td>
<td>76M</td>
<td>0.2 ± 0.1</td>
<td>2.7</td>
<td>1.1</td>
<td>4.1 ± 1.0</td>
</tr>
<tr>
<td>(0.0–0.4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.4–4.8)</td>
<td></td>
<td></td>
<td>(1.0–6.2)</td>
</tr>
<tr>
<td>London39</td>
<td>U.S.A.</td>
<td>1991</td>
<td>GC</td>
<td>Buttock</td>
<td>115F</td>
<td>0.1 ± 0.0</td>
<td>2.9 ± 0.8</td>
<td>1.3</td>
<td>4.4 ± 1.1</td>
</tr>
<tr>
<td>(0.0–0.2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.3–6.1)</td>
<td></td>
<td></td>
<td>(2.3–8.2)</td>
</tr>
<tr>
<td>Leichsenring29</td>
<td>Germany</td>
<td>1992</td>
<td>GC</td>
<td>Abdomen</td>
<td>26M, 21F</td>
<td>0.4 ± 0.1</td>
<td>2.4 ± 0.9</td>
<td>0.3 ± 0.1</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td>(0.1–0.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(1.0–6.2)</td>
<td></td>
<td>(0.2–0.5)</td>
<td>(1.3–7.0)</td>
</tr>
</tbody>
</table>

Values are means ± SD or (ranges) and are expressed in gram per 100 g of fatty acid methyl ester.

*IR: infrared, GC, gas-chromatography.
†F: female, M: male.
hypercholesterolemic in the presence of dietary cholesterol. The authors, however, reported that serum total cholesterol levels in a control group fluctuated more than normal, which may have biased the changes observed. In their second experiment it was shown that, when the intake of cholesterol was 220 mg, 14% of energy from trans-C18:1 increased serum total cholesterol levels relative to cis-C18:1. Cholesterol-free diets were not tested in that study. There is no obvious explanation for the discrepant findings of Mattson, Vergroesen and their co-workers: liquid formula diets were employed in both studies, subjects had normal total cholesterol levels and dietary periods were long enough for serum lipids to stabilize.

Anderson et al. reported that simultaneous replacement of 3.8% of energy from trans-C18:2 by linoleic acid and of 11.0% from trans-C18:1 by oleic acid decreased serum total cholesterol levels by 0.54 mmol/l (21 mg/dl). Although effects could not be attributed solely to trans-C18:1, these results did suggest that trans-C18:1 and/or trans-C18:2 raised serum cholesterol.

B. HDL and LDL Cholesterol

Mensink and Katan carried out a controlled trial with 25 men and 34 women. Subjects received in random order a diet high in cis-C18:1, one high in trans-C18:1 and a diet high in the cholesterol-raising saturated fatty acids lauric, myristic and palmitic acid. Each diet was fed for three weeks. The level of trans-C18:1 on the trans-diet was 11% of total energy, which is well above the average level of trans-C18:1 intake. HDL-cholesterol was 0.17 mmol/l (7 mg/dl) lower on the trans-diet than on the other two diets, while LDL cholesterol was 3.04 mmol/l (118 mg/dl) on the trans-diet, 2.67 mmol/l (103 mg/dl) on the cis-C18:1 diet and 3.14 mmol/l (121 mg/dl) on the high-saturated fat diet. Trans-C18:1 thus lowered HDL and raised LDL cholesterol. This study has been criticized, because of its high level of trans-C18:1. In a second experiment, Zock and Katan compared the effects of 8% of energy from trans-C18:1 on serum lipoproteins with those of linoleic acid and of stearic acid, a saturated fat with eighteen carbon atoms. Replacement of linoleic acid by trans-C18:1 or stearic acid caused lower HDL cholesterol and higher LDL cholesterol levels. This study confirmed the effects on HDL and LDL cholesterol at lower intakes of trans-C18:1.

Nestel and co-workers reported two studies of the effect of trans-C18:1 on plasma lipoprotein levels. In their first study with 26 mildly hypercholesterolemic males, two different fat blends were compared with a control fat. The fatty-acid composition of the two experimental fat blends was nearly identical and contained 7 g per 100 g more trans-C18:1, but also 17 g less palmitic acid and 16 g more linoleic acid as compared with the control fat. LDL levels were significantly lower on the test blends, while HDL cholesterol were slightly, but not significantly, lower. In view of the multiple changes in fatty-acid intake, these findings do not necessarily contradict our own results. In a second study 27 mildly hypercholesterolemic men each received diets enriched with 4.3% of oleic acid, elaidic acid or palmitic acid for 3 weeks. As compared with the oleic acid diet both the plasma total and LDL cholesterol increased by 0.36 mmol/l (14 mg/dl) on the elaidic acid diet and by 0.28 and 0.26 mmol/l (11 and 10 mg/dl) mg/dl, respectively, on the palmitic acid diet. HDL cholesterol levels were 0.10 mmol/l (4 mg/dl) higher on the palmitic acid diet than on the other two test diets. The authors concluded that elaidic and palmitic acid are equally hypercholesterolemic. Nestel et al. did not report a HDL-cholesterol lowering effect of trans-C18:1. Recently, Judd and co-workers at the Beltsville Agricultural Research Center of the U.S. Department of Agriculture completed a large, well-controlled trial on the effect of partially hydrogenated soybean oil on lipoprotein in volunteers. According to preliminary results (Food Chemical News, August 31, 1992, p. 3) this study supported our earlier findings that trans-C18:1 lower HDL cholesterol and raise LDL cholesterol.
C. Lipoprotein(a)

Plasma levels of Lp(a) were hitherto thought to be impervious to effects of diet. Two recent independent papers, however, reported that trans-C18:1 increases serum Lp(a) levels relative to cis-C18:1, to linoleic acid, or to saturated fatty acids. This effect was dose dependent.

D. Triglycerides

Anderson et al. reported a triglyceride-elevating effect of trans fatty acids, but Mattson et al. found similar plasma triglycerides levels on the trans-C18:1-diet and the diet high in oleic acid. Mensink and Katan, however, found that trans-C18:1 raised serum triglycerides relative to oleic acid, but not relative to the cholesterol-raising saturated fatty acids. Zock and Katan also noted slightly higher serum triglyceride levels on the trans-C18:1-diet than on the linoleate-diet, although the difference did not reach statistical significance. Levels on the stearate-diet were comparable to those on the trans-C18:1 diet. Finally, Nestel et al. found that increasing the intake of trans-C18:1 by 4.3% of total energy at the expense of oleic acid increased plasma triglycerides by 0.08 mmol/l (7 mg/dl). Levels on the diet high in palmitic acid, however, were the lowest, but none of these differences did reach statistical significance. The results of these studies together suggest that trans-C18:1 isomers have the potential to raise serum triglycerides.

E. Plasma Apoproteins

In the study of Mensink and Katan apoprotein B levels were somewhat higher on the trans-C18:1 diet than on the saturated-fat diet, but considerably lower on the oleic acid diet. The changes observed did not entirely parallel those of LDL cholesterol, which was highest on the diet high in the cholesterol-raising saturated fatty acids. Zock and Katan showed that the trans diet also increased apoprotein B levels relative to the linoleate diet and were intermediate on the stearic diet. These findings together suggest that trans-C18:1 increases the ratio of apoprotein B to cholesterol in LDL relative to the most common saturated fatty acids in the diet. This could be interpreted as showing a shift toward smaller, denser LDL on diets high in trans-C18:1. However, this is a conjecture that needs to be investigated directly.

In both studies the diet high in trans-C18:1 caused the lowest apolipoprotein A-I levels and changes paralleled those of HDL cholesterol.

F. Positional Isomers

Theoretically, the different positional isomers of elaidic acid may have different effects on serum lipids and lipoproteins. In addition, the effects ascribed above to trans-C18:1 may also be, at least partly, due to the positional cis isomers invariably present in hydrogenated oils. These differences—if any—are probably not large: the spectrum of positional cis- and trans-C18:1 isomers in the fats used for our two studies was quite different, while the effect on serum HDL and LDL cholesterol levels, expressed per percent of energy, were nearly similar.

X. TISSUE LEVELS OF TRANS FATTY ACIDS AND CORONARY HEART DISEASE

A possible relationship between the intake of trans fatty acid and risk for coronary heart disease has been examined by analyzing post-mortem tissue samples. Heckers et al. could not demonstrate a relationship between the degree of atherosclerosis and the level of trans fatty acids in lipids from myocardium, jejunum or aorta tissue. In two studies Thomas et al. did find higher levels of trans-C16:1 from hydrogenated marine oils, but not of trans-C18:1, or total trans fatty acids, in adipose tissue of males who died of coronary
heart disease as compared with controls. Cases and controls, however, were not matched and effects of possible confounding variables were not accounted for. At present, there appears to be no strong evidence that high tissue levels of \textit{trans} fatty acids \textit{per se} are associated with increased risk for coronary heart disease.

\section*{XI. SUMMARY AND CONCLUSIONS}

\textit{Trans}-C18:1 in the diet originate predominantly from partially hydrogenated oils, with beef, mutton and dairy products being an additional source. These fatty acids are absorbed and incorporated into lipids. Their estimated consumption is about 5--7\% of total fatty acids, although reliable data are lacking. In addition, large variations between individuals exist. There is no evidence that \textit{trans} fatty acids accumulate in human tissues. Elaidic acid and its positional isomers do, however, raise LDL cholesterol and apoprotein B and Lp(a) and probably depress HDL cholesterol and apoprotein A-I, compared with the \textit{cis} isomer, oleic acid. In view of these adverse effects, patients at high risk for atherosclerosis, in addition to reducing their intake of saturated fatty acids and of cholesterol might also do well to avoid excessive intakes of \textit{trans} fatty acids. Still, \textit{trans} fatty acids form only a minor component of the diets of most patients and therefore even marked relative reductions in intake will probably have less of an impact on LDL cholesterol than a sizeable reduction in saturated fatty acids and cholesterol will produce.

Acknowledgements—Supported by a fellowship of the Royal Netherlands Academy of Arts and Sciences to Ronald P. Mensink.

(Received 20 January 1993)

\section*{REFERENCES}