Trans FA Content in Danish Margarines and Shortenings

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ABSTRACT: Margarines and shortenings have been major contributors to the intake by humans of the probably atherogenic *trans* FA (TFA). In 1999, all 73 brands of margarines and shortenings on the Danish market were analyzed by GLC on a 50-m highly polar capillary column, and the results were compared with similar investigations in 1992 and 1995. A gradual decline of TFA in Danish margarines was observed. From 1992 to 1995, a reduction of TFA from 10.4 to 3.6% took place in margarines with 20–40% linoleic acid. In 1999, TFA was practically absent in all the margarines, but it remained unchanged in shortenings, averaging about 6–7%. Long-chain TFA from hydrogenated fish oil, although present in 13 brands in 1995, were not found at all in the 1999 samples. *Trans*-linoleic acids or CLA were not found. The reduction in TFA content in margarines has not resulted in a systematic change over the years in the content of saturated FA, monounsaturated FA, or PUFA. Calculated from sales figures, the intake of TFA decreased from 2.2 g per capita per year in 1992, to 1.5 g in 1995, and to 0.4 g in 1999.

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Margarine products and shortenings contain *trans* FA (TFA) from the industrial hydrogenation of *cis* unsaturated vegetable and marine oils. Hydrogenation allows the FA to pack together more closely than their corresponding *cis* isomers, resulting in improved functional properties, including harder texture and longer shelf life (1). The most abundant *trans* isomers formed by hydrogenation are C18:1 with unsaturation in the 9, 10, and 11 positions (2). However, small amounts of *trans* C18:2 and C18:3 can also be formed by hydrogenation and deodorization.

High levels of TFA in the diet have repeatably been shown to affect serum lipids/lipoproteins adversely (3). Higher intake results in higher serum LDL cholesterol, the so-called bad cholesterol, and lower serum HDL cholesterol, the so-called good cholesterol. Several population studies have examined the relationship between TFA intake and risk of coronary heart disease (4). Except for one (5), all these studies have found a positive association attributable to the intake of TFA. A recent study showed that *trans* linoleic acid may have significant effects on the occurrence of coronary heart disease (6). Consequently, recommendations to reduce the content of TFA in margarine products to less than 5% of FA, the level found in dairy products, have been advanced by the Danish Nutrition Council (7), and follow-up data have demonstrated that the industry has complied with these recommendations (8,9), although shortenings still contain high levels of TFA.

With the purpose of establishing the changes in the FA pattern and TFA content of Danish margarines and shortenings from 1992 and 1995, a follow-up investigation was carried out in 1999. The results are presented in this report.

EXPERIMENTAL PROCEDURES

Sample description. All Danish margarine brands available on the retail market and all shortening brands intended for use in the baking industry were sampled in May/June 1999. Of each brand, one package representing 500 g was taken at random directly from the production line from the two margarine- and shortening-producing factories in Denmark, namely, Vejle Margarinefabrik (Vejle, Denmark), and Dragsbæk Margarinefabrik (Thisted, Denmark), and from the stock of the Unilever company Van den Bergh Foods, which now has its products made in Sweden. A total of 73 brands were drawn, consisting of 34 brands of margarines and 39 brands of shortenings. This sampling covers the entire Danish market for the retail outlet and the baking industry.

Sample preparation. Margarines and shortenings were considered homogeneous and consequently not homogenized further. A subsample of about 50 g was kept frozen until analysis for fat and FA.

Methods of analysis. The fat was extracted with a mixture of diethyl ether and petroleum ether. For the fat determination, an aliquot was evaporated to dryness and the remaining fat weighed after drying to constant weight in a drying oven at 103°C. The FA were determined in another aliquot by boiling the fat in methanolic potassium hydroxide, methylation by boiling with methanol and boron trifluoride, and extraction of the FAME with isooctane, followed by GLC on a 60-m capillary column, CP Sil 88, 0.25 mm i.d., 0.2 μm film thickness (Chrompack International, Middelburg, the Netherlands) with a temperature program, FID, and *n*-C17 as internal standard for quantification of the FA as described earlier (8,9). As reported by others (10), a complete separation of all *trans* and *cis* C18:1 isomers is not possible; however, with the temperature program and column used, good separation was obtained, especially for *trans* C18:1 amounts below 10% of the FA (11).

Analytical quality assurance. Nu-Chek-Prep Standards GLC 68 and 17A′ (Nu-Chek-Prep, Inc. Elysian, MN) were used for calculation of response factors. The samples were

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analyzed with single determinations in a series of 16 samples including double determinations on one sample, an in-house reference material of margarine, and on a certified BCR reference material, CRM 162 Soya-maize oil (Community Bureau of Reference, Brussels, Belgium). The double determinations were used to make the so-called R-charts to monitor the precision of the following FA: palmitic (C16:0), stearic (C18:0), oleic (*cis* C18:1n-9), and linoleic (*cis* C18:2n-6). The relative SD (RSD) was found to be between 2.4 and 2.7%. The reference materials were used to construct X-charts to monitor the trueness of the analysis.

Statistics and data presentation. Results for contents of single FA are given as mean and range (wt% of total FA) and for summation of FA as mean ± SD (g per 100 g sample). A *t*-test for comparison of group means or one-way ANOVA was used, followed by Duncan's multiple range test if $P < 0.05$, which was the chosen level for significant differences (The SAS System for Windows V8, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

The results for the individual FA are given in Table 1 for margarines and Table 2 for shortenings. For practical reasons, the margarines and shortenings are divided into categories based on their content of linoleic acid (LA), and the results have been compared for the years 1992, 1995, and 1999 for margarines and for the years 1995 and 1999 for shortenings.

Trans C18:2 and conjugated C18:2 are not present in Danish margarines and shortenings, in any year, in any significant amounts (<0.1% of total FA). There was a significant decrease from 1992 to 1999 in *trans* C18:1 in margarines with LA <10%, 10–20%, and 20–40%, whereas in 1999 *trans* C18:1 was found only in a few samples destined for use in the catering sector. Margarines with LA >40% already had a low *trans* C18:1 content in 1992, and in 1999 *trans* C18:1 was entirely absent. From 1992 to 1999 *cis* C18:1 increased significantly in margarines with LA <10% and 10–20%, but remained constant in the other categories. Other significant differences seem nonsystematic.

In shortenings there was no reduction in *trans* C18:1 from 1995 to 1999; however, in 1995 only three brands were free from TFA as opposed to eight in 1999. There was a significant increase in *cis* C18:1 in both the LA <10% and 10–20% categories. There was a significant decrease in the group with other FA from 1995 to 1999 in the category with LA <10%, and a significant increase in the category with LA 10–20%, however with a reduction in the range. Other significant differences seem nonsystematic. Also for the margarines, there was a trend to reduction of other FA and especially a narrowing of the range.

In 1995, thirteen margarines and shortenings coming from hydrogenated fish oil contained long-chain TFA, and these FA, especially *trans* C20:1 and C22:1, were placed in the group "Other." These long-chain TFA were entirely absent in the 1999 margarines and shortenings, which explains the trend toward a reduction of the FA in the group of "Other"

TABLE 1
FA Composition (wt% of total FA, mean; range or ± SD) of Danish Margarines in 1992, 1995, and 1999[a]

	<10% linoleic acid			10–20% linoleic acid			20–40% linoleic acid			>40% linoleic acid		
FA	1992 (*n* = 4)	1995 (*n* = 4)	1999 (*n* = 4)	1992 (*n* = 5)	1995 (*n* = 22)	1999 (*n* = 16)	1992 (*n* = 18)	1995 (*n* = 11)	1999 (*n* = 10)	1992 (*n* = 14)	1995 (*n* = 7)	1999 (*n* = 4)
C8:0	—	—	0.2; 0.0–0.7	—	0.4; 0.0–2.1	0.6; 0.0–2.6	0.3; 0.0–2.4	0.4; 0.0–1.5	0.2; 0.0–0.9	—[a]	0.8; 0.0–2.2[b]	—[a]
C10:0	—	—	—	—	0.2; 0.0–1.4	0.3; 0.0–1.9	0.1; 0.0–1.6	0.3; 0.0–1.0	0.2; 0.0–0.8	—[a]	0.5; 0.0–1.4[b]	—[a]
C12:0	0.1; 0.0–0.2	0.4; 0.0–1.4	3.3; 0.0–7.1	0.5; 0.0–1.4[a]	2.1; 0.0–8.8[a]	5.5; 0.0–13.9[b]	1.5; 0.0–13.4	3.4; 0.0–10.7	3.8; 0.0–10.3	0.6; 0.0–2.9[a]	4.4; 0.0–9.6[b]	2.8; 0.0–5.1[a,b]
C14:0	3.9; 1.7–6.2[a]	7.0; 4.1–8.2[b]	2.2; 0.8–2.9[a]	3.3; 1.7–6.2[a]	1.8; 0.0–5.1[b]	2.3; 0.0–5.5[a,b]	0.8; 0.0–4.9	1.5; 0.0–3.5	1.5; 0.0–3.5	0.3; 0.0–1.1[a]	1.9; 0.6–3.9[b]	1.1; 0.0–1.8[a,b]
C16:0	26.0; 19.6–33.9	25.1; 20.5–32.0	31.9; 27.5–36.4	24.4; 20.2–34.6	21.8; 10.5–33.7	20.8; 4.8–34.2	16.7; 7.2–23.4	15.4; 5.4–21.1	13.6; 5.2–21.7	12.8; 9.0–19.0	13.0; 10.9–18.0	9.7; 6.5–15.1
C16:1n-7	0.3; 0.0–0.6[a]	1.2; 0.6–2.0[b]	—[a]	0.5; 0.0–1.9[a]	0.2; 0.0–1.0[a,b]	—[b]	0.1; 0.0–2.1	—	—	—	—	—
C18:0	8.0; 7.6–8.3	8.1; 7.6–8.8	9.0; 6.3–10.7	9.7; 5.8–16.5[a]	6.2; 3.5–9.4[b]	5.5; 2.5–10.5[b]	6.1; 3.6–11.0	7.0; 3.9–20.9	6.7; 3.5–20.4	6.5; 5.4–8.4	7.2; 5.4–10.1	6.7; 3.6–9.4
***trans* C18:1**	**5.3; 1.5–8.0[a]**	**6.2; 3.1–7.9[a]**	**1.0; 0.0–4.0[b]**	**6.5; 2.0–12.5[a]**	**4.6; 0.0–8.2[a]**	**0.7; 0.0–5.8[b]**	**10.4; 0.0–22.7[a]**	**3.6; 0.0–10.6[b]**	**1.0; 0.0–9.7[b]**	**2.8; 0.0–8.5**	**1.1; 0.0–5.5**	—
cis C18:1	29.1; 20.9–37.5[a,b]	22.7; 17.9–31.2[a]	35.7; 33.4–40.3[b]	28.1; 11.9–38.0[a]	40.3; 20.6–52.3[b]	42.8; 34.8–58.8[b]	32.0; 16.8–42.4	34.0; 15.2–48.9	33.2; 18.2–48.4	21.2; 17.1–24.1	20.5; 15.1–27.9	23.9; 21.3–27.2
C18:2n-6	8.8; 7.2–10.5[a]	6.2; 4.8–8.8[b]	9.6; 9.2–9.9[a]	14.1; 9.9–16.8	15.6; 9.5–19.8	13.2; 10.1–19.4	26.4; 11.2–50.7[a]	29.5; 20.8–39.3[a,b]	34.1; 25.9–39.0[b]	54.1; 41.9–60.9	49.6; 41.4–56.1	51.9; 44.9–60.5
C18:3n-3	3.4; 2.7–4.0	3.1; 2.3–3.4	2.6; 1.8–3.0	2.6; 1.2–3.3[a]	4.3; 1.5–7.3[b]	5.2; 3.0–9.4[b]	3.0; 0.0–6.1	4.0; 0.9–6.5	3.4; 0.8–7.5	0.3; 0.0–1.5[a]	0.8; 0.0–1.5[a,b]	1.0; 0.0–1.7[b]
C20:0	2.1; 1.2–3.0[a]	3.1; 2.6–4.0[a]	0.5; 0.0–1.9[b]	1.5; 0.1–2.8[a]	0.7; 0.0–2.6[b]	0.1; 0.0–0.8[b]	0.1; 0.0–0.8[a]	0.4; 0.0–0.9[b]	0.1; 0.0–0.8[a]	—	—	—
C20:1n-9	1.2; 0.4–2.1[a,b]	1.6; 1.0–2.2[a]	0.3; 0.0–1.3[b]	0.7; 0.0–1.6	0.7; 0.0–1.7	0.7; 0.0–1.9	0.3; 0.0–2.5	0.4; 0.0–0.9	0.3; 0.0–1.1	—	—	—
C22:0	2.0; 1.0–3.1[a,b]	2.4; 1.5–4.0[a]	0.5; 0.0–2.0[b]	1.4; 0.0–2.9[a]	0.3; 0.0–1.8[b]	0.2; 0.0–1.3[b]	0.1; 0.0–0.7	0.3; 0.0–2.2	0.2; 0.0–2.2	0.5; 0.0–1.1	0.5; 0.0–1.1	0.9; 0.0–2.0
Other[b]	8.6; 1.8–17.5	13.0; 5.0–19.2	3.3; 1.5–7.2	6.9; 1.5–19.2[a]	1.1; 0.0–8.9[b]	2.0; 0.4–3.8[b]	1.9; 0.4–9.5[a]	0.1; 0.0–0.4[b]	1.9; 0.6–3.3[a]	0.8; 0.2–2.2[a]	0.1; 0.0–0.4[b]	2.1; 1.7–2.3[c]
TFA	**5.3 ± 3.3[a]**	**6.2 ± 2.2[a]**	**1.0 ± 1.9[b]**	**6.5 ± 4.1[a]**	**4.6 ± 2.7[a]**	**0.7 ± 2.0[b]**	**10.4 ± 6.6[a]**	**3.6 ± 4.0[b]**	**1.0 ± 3.0[b]**	**2.8 ± 2.8**	**1.1 ± 2.1**	—
SFA	42.1 ± 2.8	46.0 ± 1.6	45.8 ± 6.8	40.7 ± 3.9	33.6 ± 10.7	35.0 ± 12.3	25.8 ± 5.2	28.6 ± 8.8	25.9 ± 10.6	20.8 ± 2.2[a]	28.3 ± 8.0[b]	21.5 ± 6.1[a]
MUFA	30.6 ± 8.1	25.5 ± 5.0	34.7 ± 2.8	29.3 ± 9.5[a]	41.2 ± 8.8[b]	43.2 ± 7.9[b]	32.5 ± 9.0	34.5 ± 8.8	33.2 ± 9.0	21.2 ± 2.1	20.5 ± 4.9	24.2 ± 2.2
PUFA	12.2 ± 1.0[a]	9.3 ± 1.3[b]	11.7 ± 0.8[a]	16.7 ± 3.5	20.0 ± 4.0	18.2 ± 4.8	29.5 ± 7.6[a]	33.5 ± 6.2[a,b]	37.2 ± 4.2[b]	54.4 ± 5.6	50.5 ± 5.7	53.4 ± 4.8

[a]Different roman superscript letters inside the same linoleic acid category indicate significantly different results ($P < 0.05$) between years. Boldface type highlights *trans*-FA. An em dash (—) indicates "not found" (<0.1%).
[b]Can contain long-chain *trans*-FA (C20:1 and C22:1).

TABLE 2
FA Composition (wt% of total FA, mean; range) of Danish Shortenings in 1995 and 1999[a]

	<10% linoleic acid		10–20% linoleic acid	
FA	1995 (n = 17)	1999 (n = 15)	1995 (n = 36)	1999 (n = 24)
C8:0	0.2; 0.0–1.8[a]	0.8; 0.0–3.0[b]	0.2; 0.0–2.2	0.1; 0.0–1.5
C10:0	0.1; 0.0–1.3[a]	0.6; 0.0–2.2[b]	0.1; 0.0–1.5	0.0; 0.0–1.1
C12:0	0.8; 0.0–9.1[a]	4.7; 0.0–17.0[b]	1.4; 0.0–9.9	0.4; 0.0–8.0
C14:0	4.5; 0.8–7.3[a]	2.5; 0.7–7.0[b]	1.5; 0.0–6.8[a]	0.4; 0.0–3.4[b]
C16:0	24.1; 18.4–33.2[a]	28.0; 19.8–36.5[b]	26.0; 19.1–36.4	24.4; 5.4–42.6
C16:1	0.8; 0.0–2.1[a]	0.1; 0.0–1.5[b]	0.1; 0.0–1.7[a]	—[b]
C18:0	7.6; 5.8–15.1	6.9; 3.8–14.9	6.0; 3.4–9.2[a]	4.9; 2.7–9.0[b]
***trans* C18:1**	**8.0; 4.5–14.2**	**7.0; 3.8–14.9**	**6.5; 0.0–13.4**	**5.6; 0.0–20.1**
cis C18:1	30.3; 20.4–41.2[a]	35.6; 30.2–41.1[b]	39.8; 21.1–48.0[a]	43.6; 31.0–58.3[b]
C18:2	7.7; 4.7–9.6	8.5; 7.5–9.8	13.0; 9.9–19.7	12.8; 10.1–19.0
C18:3	3.3; 1.8–4.2[a]	2.4; 1.5–3.0[b]	3.6; 1.6–5.3	4.2; 0.0–8.2
C20:0	1.8; 0.0–3.8[a]	0.2; 0.0–1.1[b]	0.5; 0.0–2.4[a]	0.1; 0.0–0.8[b]
C20:1	1.1; 0.0–2.0[a]	0.2; 0.0–0.7[b]	0.6; 0.0–1.7[a]	0.8; 0.0–1.8[b]
C22:0	1.6; 0.0–3.8[a]	0.1; 0.0–1.0[b]	0.2; 0.0–2.1	0.1; 0.0–1.3
Other[b]	8.1; 0.0–17.2[a]	2.4; 0.9–4.1[b]	0.9; 0.0–11.0[a]	2.6; 1.2–3.7[b]
TFA	**8.0 ± 3.4**	**6.8 ± 4.4**	**6.5 ± 3.3**	**5.5 ± 4.5**
SFA	40.8 ± 3.5	42.8 ± 5.9	36.0 ± 6.1[a]	29.9 ± 9.9[b]
MUFA	32.2 ± 5.9	35.0 ± 3.1	40.6 ± 6.2[a]	43.6 ± 5.4[b]
PUFA	11.0 ± 1.9	10.6 ± 1.1	16.6 ± 2.7	16.7 ± 3.5

[a]Different superscript letters inside the same linoleic acid category indicate significantly different results ($P < 0.05$) between years. Boldface type highlights *trans*-FA. An em dash (—) indicates "not found" (<0.1%).
[b]Can contain long-chain *trans*-FA (C20:1 and C22:1).

FA. There was a great variation between brands in the same LA category as can be seen from the range values.

Apart from the decrease in TFA content, very few changes in the mean values for saturated FA (SFA), monounsaturated FA (MUFA), and PUFA have been found (see Tables 1 and 2).

In a recent update of their report from 1994 (12), the Danish Nutrition Council concluded that the contribution of a high intake of TFA to the risk of coronary heart disease has gained further support, and a suspicion of a deleterious effect on the human fetus and newborn still exists. Besides, the effect on cancer risk has not yet been settled one way or the other. It is therefore recommended to remove all industrially produced TFA from foods at once. This has inspired the Danish Veterinary and Food Administration to propose legal action, and at the present time legislation has been submitted for notification in the European Economic Community with a proposal of reducing the industrially produced TFA to below 1% of the fat. From a public health perspective, it would not be appropriate to substitute the *trans* content in margarines and shortenings with fats with a high content of SFA. However, this does not seem to be the case for Danish margarines.

With TFA practically out of all the LA categories of margarines, the reduction in TFA was not big enough to be correlated in any systematic way with changes in SFA, MUFA, or PUFA content. Some fluctuations were found. The MUFA seemed to decrease in margarines with LA <10% from 1992 to 1995 and increase again from 1995 to 1999, but there were only four products in this category and the differences were not significant. In margarines with LA 10–20% there was an increase in MUFA from 1992 to 1999 and a general reduction in SFA.

Thus, the Danish margarine industry has seemingly succeeded in reducing the content of TFA without changing the other classes of FA and even with a tendency to increase in MUFA and decrease in SFA, thus improving the "cardiac risk profile" of margarine from 1992 to 1999. It appears that earlier claims about not being able to remove TFA without damage to the consistency of the margarines were unfounded, as no noticeable difference in consistency over the years was observed. However, the manufacturers have other ways to adjust the consistency, among others the m.p. of the oils used in the manufacture of margarines.

In shortenings TFA remained the same in 1995 and 1999, but in the category with LA 10–20% there was a significant decrease in SFA and a significant although small increase in MUFA, also indicating a small improvement in the "cardiac risk profile" for shortenings.

Margarine manufacturers still claim that it is necessary to use hydrogenated oils with a high *trans* content to give satisfactory mouthfeel and texture, especially in Danish pastry but also in other products such as cakes and biscuits, and it will take them 2–3 yr to develop the necessary alternatives. However, such claims are very difficult to substantiate.

Trans monoenoic FA with chain lengths longer than 18 carbon atoms are now completely absent in Danish margarines and shortenings compared to 13 products in 1995 containing long-chain TFA, which means that partially hydrogenated fish oils no longer are used by the Danish margarine industry.

Based on sales data from the three manufacturers selling margarines and shortenings in Denmark, a weighted average

FA composition for each LA category has been calculated and used to calculate the intake of TFA. The calculation assumes that all the margarines and shortenings sold were consumed by the 5.5 million inhabitants in Denmark. The calculation indicated that the per capita intake per day of margarines and shortenings was 23.2 g in 1999 compared to 30 g in 1995, with 7.5 g shortening and 15.7 g margarine in 1999 compared to 10 g shortening and 20 g margarine in 1995. The average TFA intake was 0.35 g per person per day, with 0.16 g from shortening with LA <10% corresponding to 2.74 g shortening and 0.19 g TFA from shortening with 10–20% LA corresponding to 4.74 g shortening. This was a significant reduction from the 2.2 g daily in 1992 and 1.5 g daily in 1995. However, these are mean values and special dietary habits could give a much higher intake of TFA, so a reduction or a removal of TFA in shortenings is still important.

Compared to the national mean SFA intake of 40 g/d in 1995 (13), the intake of TFA from margarines and shortenings was very low in 1995, and ruminant fat and frying fat are now the dominant contributors to the intake of TFA as well as SFA. Thus, following the recommendation to reduce the intake of SFA will automatically also reduce the intake of TFA.

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