BACKGROUND AND SCIENTIFIC REVIEW

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TRANS FATTY ACIDS AND CORONARY HEART DISEASE

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On November 12, 1999, the Food and Drug Administration announced its proposal to include the *trans*-fatty acid (*trans* fat) content of foods on the standard food label. At present, only saturated fats are listed. Because many persons will be unfamiliar with *trans* fat and its health effects, we have posted the following review.

- **What are *trans* fatty acids?**

Trans unsaturated fatty acids, or *trans* fats, are solid fats produced artificially by heating liquid vegetable oils in the presence of metal catalysts and hydrogen.[1] This process, partial hydrogenation, causes carbon atoms to bond in a straight configuration and remain in a solid state at room temperature. Naturally-occurring unsaturated fatty acids have carbon atoms that line up in a bent shape, resulting in a liquid state at room temperature.

- **Which foods contain *trans* fatty acids?**

Trans fats are produced commercially in large quantities to harden vegetable oils into shortening and margarine. Food manufacturers also use partial hydrogenation of vegetable oil to destroy some fatty acids, such as linolenic and linoleic acid, which tend to oxidize, causing fat to become rancid with time. The oils used to cook french fries and other fast food are usually this kind of partially hydrogenated oil, containing *trans* fats. Commercial baked goods frequently include

trans fats to protect against spoilage. A small amount of *trans* fat is also produced in the gastrointestinal tract of cattle, so that low levels of these isomers are found in dairy and beef fat.

Commercial production of partially hydrogenated fats began in the early 20th century and increased steadily until about the 1960's as processed vegetable fats displaced animal fats in the diets of the U.S. and other Western countries. Lower cost was the initial motivation, but health benefits were later claimed for margarine as a replacement for butter.

Although the average level of *trans* fat in margarines has declined with the advent of softer versions, per capita consumption of *trans* fatty acids has not changed greatly since the 1960's because of the increased use in commercially-baked products and fast foods.

- **What are the health effects of *trans* fats?**

Concerns have been raised for several decades that consumption of *trans* fatty acids might have contributed to the 20th century epidemic of coronary heart disease.[2]

Metabolic studies have shown that *trans* fats have adverse effects on blood lipid levels—increasing LDL ("bad") cholesterol while decreasing HDL ("good") cholesterol. This combined effect on the ratio of LDL to HDL cholesterol is double that of saturated fatty acids.[3]

Trans fats have also been associated with an increased risk of coronary heart disease in epidemiologic studies.[4]

Based on the available metabolic studies, we estimated in a 1994 report that approximately 30,000 premature coronary heart disease deaths annually could be attributable to consumption of *trans* fatty acids.[4]

In response to these reports, a 1995 review sponsored by the food industry concluded that the evidence was insufficient to take action and that further research was needed.[5] Since that

time many more metabolic studies have been conducted and additional prospective epidemiologic studies have been reported.

Because of the weight of the evidence, the FDA has recently issued a proposal for including *trans* fatty acid content on the food label. One important issue is whether to list *trans* fat as a separate constituent or to combine it with saturated fat.

- **What are the arguments for listing *trans* fat separately from saturated fat?**

The combined results of metabolic and epidemiologic studies strongly support an adverse effect of *trans* fat on risk of CHD. Furthermore, two independent methods of estimation indicate that the adverse effect of *trans* fat is stronger than that of saturated fat. By our most conservative estimate, replacement of partially hydrogenated fat in the U.S. diet with natural unhydrogenated vegetable oils would prevent approximately 30,000 premature coronary deaths per year, and epidemiologic evidence suggests this number is closer to 100,0000 premature deaths annually. These reductions are higher than what could be achieved with realistic reductions in saturated fat intake.

What alternatives exist to *trans* fats?

In Europe, producers have responded rapidly to the evidence on effects of *trans* fats by developing *trans*-free margarines that are also low in saturated fats.[48] More recently, these products have also become available in the U.S., although a large share of the market is still heavily hydrogenated stick margarine.[5]

It is thus evident that *trans*-free products are feasible, and that the technical constraints often invoked by the food industry can be overcome. However, out of the *trans* fatty acids provided by hydrogenated vegetable oil in the U.S., only 25%[5] to 37%[49] comes from margarines,

the remainder comes from baked goods, fast foods and other prepared foods. Replacement of *trans* in such products by healthier fats may be more difficult than in margarines, but can be achieved.

In spite of this, many products including most baked goods and fried fast foods still are made with partially hydrogenated fat both in Europe and in the U.S. and are high in *trans* fatty acids. It is unlikely that this situation will change without strong federal regulations.

- **How important are label changes?**

Current regulations in the U.S. require food labels to include the amount of saturated fat, but not the amount of *trans*, thereby providing an incentive to manufacturers to increase the *trans* content while decreasing the amount of saturated fat.

Although changes in labeling are extremely important, many products, including fast food, which often contain extremely high levels of *trans* isomers, are exempt from labeling regulations and can carry deceptive labels such as “cholesterol-free” and “cooked in vegetable oil.”

For example, a person eating one doughnut for breakfast (3.2 g)[50] and a large order of french fries for lunch (6.8 g)[50] would ingest 10 g of *trans* fatty acids, or 5 percent of the total energy of an 1,800-calorie diet. Thus, simple labeling changes alone will not be sufficient.

The following is a more detailed review of the scientific studies behind these conclusions:

Effects on plasma lipids

Studies in the 1960's compared the effects of partially hydrogenated fat with those of

unhydrogenated vegetable oils or saturated fats on the concentrations of total serum cholesterol. Overall, these earlier studies suggested that the cholesterol raising effect of hydrogenated fat was somewhat lower than that of saturated fats.[1, 6] Only in 1990 was attention given to the fact that although *trans* fatty acids increase LDL cholesterol to a similar degree as saturated fat, they decrease HDL cholesterol relative to both *cis* unsaturated or saturated fats.[3] In a rigorous metabolic study, Mensink and Katan demonstrated that replacement of 10% of energy from oleic acid (the primary monounsaturated fat in diets) with *trans* 18:1 fatty acids caused a 0.34 mmol/L increase in LDL cholesterol and a 0.17 mmol/L decrease in HDL cholesterol; whereas replacement of oleic acid with saturated fat caused a similar increase in LDL cholesterol, but virtually no change in HDL cholesterol. As a result, the LDL/HDL cholesterol ratio was significantly higher on the *trans* (2.58) than on the saturated (2.34) or oleic (2.02) diets. These findings were soon confirmed in several investigations, including the study by Lichtenstein et al. that appears in this issue of the *Journal*, using lower levels of *trans* fatty acids and different mixtures of *trans* isomers.[7-10] Figure 1 summarizes the randomized trials that allow a direct comparison of *trans* fatty acids with isocaloric amounts of *cis* unsaturated fat;[3, 7-15] also included is the study of Aro et al.[13] that used stearic (which is usually said to have a neutral effect on blood lipids) rather than oleic acid as the control diet. Overall, *trans* fatty acids increased LDL cholesterol similarly to saturated fat, but, unlike saturated fat, they also decreased HDL cholesterol. As a result, the net effect of *trans* fat on the LDL/HDL cholesterol ratio is approximately double that of saturated fat. The difference between the effect of *trans* fat and that of saturated fat on the LDL/HDL ratio was significant in each of the six studies that allowed a direct comparison. The corresponding P values were: <0.0001,[3] <0.001,[7] <0.001,[8] <0.009,[9]

<0.01,[10] <0.05.[14] Thus, the probability that these results were due to chance is vanishingly low; taken together, these studies provide definitive evidence that *trans* fats raise the LDL/HDL ratio more than saturated fats. Moreover, these effects of *trans* fat on the LDL/HDL cholesterol ratio are remarkably constant across studies. The only somewhat discordant result was obtained by Sundram et al. among 27 men and women who were staff of the Palm Oil Research Institute in Malaysia.[14] In that study, the effect of *trans* fat on the LDL/HDL cholesterol ratio was considerably stronger than in the other investigations (see Figure 1), whereas little effect was seen for saturated fat (palmitic acid). The more marked response has been attributed to the habitual Malaysian diet consumed by participants at baseline, which is lower in total fat (26% energy) than typical western diets.[14] Although the possibility that the adverse effects of *trans* fat are more marked in populations with a lower percent of energy from fat cannot be excluded, we have conservatively excluded the Malaysian study in estimating the regression lines in Figure 1. Its inclusion would make the association between *trans* fat and LDL/HDL ratio even stronger. Also not included was the study by Almendingen at al. that compared the effects of diets containing partially hydrogenated fish oil (8.4% energy from *trans* fat), partially hydrogenated soy bean oil (6.6% energy from *trans* fat), or butter. The LDL/HDL cholesterol ratio was significantly higher on the partially hydrogenated fish oil diet (4.20) compared to both the partially hydrogenated soy bean oil diet (3.65) or the butter diet (3.85), that were not significantly different from each other. Thus, the Almendingen Study did not find an increase in the LDL/HDL ratio on partially hydrogenated soybean oil compared with butter. The lack of a high oleic or polyunsaturated diet prevented its inclusion in Figure 1, but the inclusion of these data does not materially change the estimated effect of *trans* fatty acids on the LDL/HDL ratio.

In addition to the studies summarized above, other dietary trials have compared the effects of butter and margarine on blood lipids. Because margarines are usually higher in *cis* polyunsaturated fat than butter, the specific effects of *trans* fat cannot be accurately estimated from these trials. A meta-analysis of these investigations, however, showed that replacement of butter with hard stick margarines -- that typically have contained 20-25% *trans* fat -- does not affect the total/HDL cholesterol ratio, whereas a reduction was obtained with low-*trans* tub margarine.[16] These results confirm the deleterious effects of *trans* fat on blood lipids and indicate that these may offset the beneficial effects of polyunsaturated fat. Thus individuals who are replacing butter with margarine high in *trans* fat to reduce their risks of coronary disease may obtain no benefit or -- if *trans* fat has deleterious effects beyond those on LDL and HDL -- may even increase their risk.

In addition to increasing the LDL/HDL cholesterol ratio, *trans* fatty acids increase Lp(a) when substituted for saturated fat. A significant increase was reported in nine[3, 8, 10, 12-15, 17] of ten trials. The null result was from an investigation that included only 14 subjects who consumed a diet with 3.7% energy from *trans* fat; the power of this study may have been to low to demonstrate an effect.[11] High blood levels of Lp(a) have been associated in some studies with increased risk of CHD, independently of LDL or HDL cholesterol concentrations. However diet-induced variations in blood concentrations of Lp(a) are modest relative to the genetic differences, and their quantitative impact on risk of CHD remains to be established.

Yet another effect of *trans* fatty acids on blood lipids is that on fasting triglyceride levels. Already in 1961, the group of Ancel Keys noted that hydrogenated corn oil resulted in higher triglyceride levels than natural oils or butter.[6] A triglyceride-raising effect was also consistently

seen in seven recent studies that directly compared *trans* fatty acids with *cis*-unsaturated fatty acids;[3, 7-9, 11, 14, 15] the increases ranged from 0.5 to 12 mg/dL, with an average of 1.5 mg/dL per 1% of energy intake. The effect on triglyceride levels of substituting saturated fatty acids for *cis*-unsaturated fatty acids is about zero.[18] Thus, *trans* fatty acids increase triglyceride levels when compared with other fatty acids. Eliminating 2% of energy *trans* fatty acid from the diet would lower triglyceride levels by about 3 mg/dL; the relation between triglycerides and risk of CHD is still uncertain, but the resulting benefit is probably modest.

Potential effects of *trans* fat on LDL oxidation[8, 19] and coagulation and fibrinolytic factors[20-22] have also been investigated, but so far there is no conclusive evidence of adverse effects.

Epidemiological studies

One of the most influential studies on diet and CHD was the work of Keys[23] who related the incidence of heart disease in 16 defined populations in seven countries to their intake of fat and cholesterol. The clear association that he found between percent of energy as saturated fat and CHD incidence and mortality has often been quoted as strong evidence that saturated fat increases the risk of CHD. The original investigations have now been complemented by collection and analyses of food composites representing the average intake of each cohort at baseline, so that the relation of CHD incidence and mortality to intake of *trans* fat and other specific nutrients could be examined.[24] Whereas saturated fat intake was strongly correlated to CHD mortality ($r=0.88$; $P < 0.0001$), confirming the original results, a similar positive

correlation was found between CHD mortality and *trans* fat intake (r=0.78; P < 0.0001). Interpretation of such comparisons of populations with widely different lifestyles is hazardous, but at the very least these data leave room for a major effect of *trans* fat on CHD risk.

Several case-control or cross-sectional studies have also been conducted. In a case-control study in the Boston area, we found a strong and significant positive association between *trans* fat intake assessed using a FFQ and risk of acute myocardial infarction.[25] The relative risk comparing the highest to the lowest quintile of *trans* fat intake was 2.4 (P for trend <0.0001); this association was entirely explained by *trans* intake from hydrogenated vegetable oil. Previously, Bolton-Smith et al. examined cross-sectionally the association between *trans* intake and undiagnosed CHD among participants in the Scottish Heart Study.[26] Subjects were considered to have CHD if they had angina or possible MI according to the Rose chest pain questionnaire, or an electrocardiogram recording indication of ischemia.[26] *Trans* intake, estimated by a FFQ, was positively correlated with the (LDL+VLDL)/HDL cholesterol ratio. The odds ratio for risk of CHD comparing the highest versus the lowest quintile of intake were elevated but not statistically significant (1.26 in women, and 1.08 in men). Positive associations between consumption of margarine and risk of acute myocardial infarction were found in a case-control study in Italy[27] and one in Greece.[28] Investigations in which tissue or plasma fatty acids composition was used as a biomarker of *trans* intake[29-36] gave conflicting results. With one exception,[33] however, these investigations were small, and the power too low to reliably detect an association. The only large study, the EURAMIC study,[33] included 671 men with acute myocardial infarction in eight European countries. The overall analyses revealed no association

between *trans* intake and risk of myocardial infarction (multivariate odds ratio comparing the top vs. the bottom quartile was 0.97). The two centers in Spain, where CHD rates are very low, however, had extremely low *trans* levels and little between-person variation compared to those from other countries, and thus provided little or no information on the relation between *trans* and CHD. After appropriately excluding these centers, the odds ratio in the third and fourth quartile increased to 1.53 (95% confidence interval: 1.02, 2.28) and 1.44 (0.94, 2.20) respectively. In addition, there was significant heterogeneity in the odds ratios between countries, from 0.2 in Spain and Moscow, to 5.0 in Finland and 5.4 in Norway. This heterogeneity may be due to different amounts, and sources of *trans* -- and therefore different isomers -- in different countries (for example in Spain, unlike in the other countries, most dietary *trans* fatty acids are from animal sources), or, more likely, to interaction with other dietary factors, or confounding by unmeasured or poorly measured covariates. Interpretation of the EURAMIC results is controversial, but in any case they do not provide strong evidence against the hypothesis that *trans* fatty acids increase the risk of CHD, and if anything add weight to the existence of a positive association.

The strongest epidemiological evidence relating dietary factors to risk of CHD is provided by prospective investigations. The relation between *trans* fatty acids intake and risk of coronary disease has now been reported from three large cohort studies, the Health Professionals Follow-up Study (HPFS),[37] the Alpha-Tocopherol Beta-Carotene study (ATBC)[38] and the Nurses' Health Study (NHS)[39]. In these studies, *trans* fat consumption was assessed using detailed food frequency questionnaires (FFQ) that were validated by comparison with adipose

composition[40, 41] or several days of diet records.[42] In addition, the relation between margarine intake and risk of CHD has been reported from the Framingham cohort.[43] The results of each of these investigations support an adverse effect of *trans* fatty acids. The relative risk of coronary heart disease for a 2% increase in *trans* fatty acids intake was 1.36 (95% confidence interval: 1.03, 1.81) in the HPFS, 1.14 (0.96, 1.35) in the ATBC, and 1.93 (1.43, 2.61) in the NHS. The higher relative risk in the NHS may be related to the fact that this investigation took advantage of up to four repeated dietary measurements during the follow-up, thereby reducing the error in assessing *trans* consumption; in analyses using only the baseline dietary measure, the corresponding relative risk was 1.62. In all cohorts, these relative risks were considerably higher than those for saturated fat. For example, in the NHS replacing 5 percent of energy from saturated fat with energy from unsaturated fat was associated with a 42 percent lower risk, whereas replacing 2 percent of energy from *trans* unsaturated fat with energy from unhydrogenated, unsaturated fats was associated with a 53 percent lower risk. These studies have been criticized on the grounds that measurements of *trans* intake were unreliable;[5] however, errors in measuring *trans* fatty acids intake can only have led to underestimation of the association with CHD risk.[44] Also, it has been suggested that the observed associations resulted from a shift from butter to margarine among subjects at high risk of CHD.[45] If so, the association between *trans* intake and risk of CHD should be weaker among subjects with stable margarine consumption, and should be stronger during the first few years of follow-up. In fact, the opposite was true in the Nurses' Health Study,[46] where exclusion of women who changed their diet before the beginning of the study strengthened the association,[46] and in the Framingham cohort, where the positive association between margarine consumption and CHD risk was strengthened after

excluding the first ten years of follow-up.[43] Moreover, high consumption of *trans* (or margarine) was not related to other dietary behaviors perceived as healthy for the heart, such as a preference for skim rather than whole milk[43] and high-*trans* foods that are hardly perceived as healthy, such as cookies, were also positively associated with risk of CHD in the Nurses' Health Study.[46] Thus there appear to be no likely alternative to the hypothesis that high *trans* intake increases the risk of CHD. Although confounding by unmeasured or poorly measured risk factors cannot be excluded, as is usually the case in observational studies, we lack a credible hypothesis of what such confounder(s) could be, as these associations were controlled for an extensive number of other dietary and lifestyle risk factors. In the Health Professionals Follow-up Study, adjustment for dietary fiber attenuated the relation of *trans* to risk of CHD, however no attenuation occurred in the other two cohorts[38] (Hu, personal communication). In summary, prospective studies provide strong evidence that *trans* fatty acids consumption increases substantially the risk of CHD.

Quantitative estimates of risk

Independent estimates of the effect of *trans* fat can be obtained by combining the effects of *trans* on blood lipids and the relationship between lipids and coronary heart disease risk, or from the results of cohort studies. We have used these different methods to estimate the number of deaths that may be due to consumption of *trans* fatty acids from partially hydrogenated fat in the amount of 2% energy (approximately the U.S. average).

The first approach uses only the effect of *trans* fats on blood lipids, and ignores the

associations observed in epidemiological studies. As shown above (Figure 1), replacing 2% energy from *cis* unsaturated fat with an isocaloric amount of *trans* fat causes a 0.13 increase in the LDL/HDL cholesterol ratio (we used this because the effect of *trans* on the total/HDL cholesterol ratio that we used in the past was not provided in one of the new studies). Since a change in one unit in the total/HDL cholesterol ratio has been associated with a 53% change in the CHD risk,[47] we estimated a relative risk of 1.07 (1 + 0.53 x 0.13) for a 2% increase in *trans* fat (the use of LDL/HDL ratio instead of total/HDL cholesterol ratio has trivial effects), corresponding to an attributable risk of 6.5% (0.07 divided by 1.07). The effect of saturated fat on the LDL/HDL ratio is about half that of *trans* fat, so that the same attributable risk would be estimated for a 4% of energy increase from saturated fat. These are likely to be underestimates of the true effect, because the lipid-CHD relation that we used has not been corrected for the attenuation caused by within-person variation in lipid measurements. Also, the estimate for *trans* fat does not take into account adverse effects on triglycerides or Lp(a). In the metabolic studies, *cis* unsaturated fats replaced *trans* fat as would be the case if the original oils were simply not partially hydrogenated. Because unsaturated fats themselves have beneficial effects on blood lipids, the benefits of eliminating *trans* or saturated fats would be less if they were replaced by carbohydrate.

The second approach calculates risk directly from the strength of the association between *trans* fat and CHD as observed in epidemiological studies. A pooled estimate of the results reported in the prospective studies (ATBC, HPFS, and NHS) gives a relative risk of 1.31 (1.15, 1.49) for an increase in *trans* consumption of 2% of energy. Assuming that this relation is causal, the attributable risk would be 24%, or over 100,000 coronary deaths per year. Moreover,

according to the results of the HPFS and NHS, it would require a 10% of energy reduction in saturated fat intake to obtain a benefit comparable to that of eliminating *trans* fat from the U.S. diet. No benefit of reducing saturated fat intake would be predicted by the results of the ATBC.

Our first approach, using data from metabolic studies obtained above, assumes that the adverse effects of *trans* are entirely mediated by their effects on blood levels of LDL and HDL; whereas, the second approach, using results of epidemiological studies, suggests that the increase in risk of CHD caused by *trans* fat is higher than predicted by effects on blood lipids alone. Ignoring this possibility could cause a substantial underestimation of the adverse effects of *trans* fat.

Conclusion

Five years ago evidence was strong that *trans* fat had deleterious impacts on blood lipids; ensuing studies have confirmed these metabolic findings and strengthened epidemiologic support for an important adverse effect on risk of coronary heart disease. These data highlight the need for rapid implementation of labeling requirements that include fast foods. Because partially hydrogenated fats can be eliminated from the food supply by changes in processing that do not require major efforts in education and behavioral modification, these changes would be an extremely efficient and rapid method for substantially reducing rates of coronary disease.

Acknowledgment

We would like to thank Jill Arnold for her expert assistance with this review.

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