Do trans fatty acids increase the risk of coronary artery disease? A critique of the epidemiologic evidence1,2

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ABSTRACT On the basis of metabolic and epidemiologic data it has been claimed that trans fatty acid intake causes coronary artery disease (CAD), with ≥30,000 deaths/y in the United States and a considerably greater number of nonfatal cases. The metabolic evidence is still controversial; the epidemiologic evidence is reviewed here. In most studies the likelihood that CAD “caused” margarine use, rather than the reverse, was not excluded. Uncontrolled confounding (particularly! confounding by indication) was ubiquitous. Selection bias conditional on margarine use was common. The projection of 30,000 deaths/y is not justified. If the metabolic evidence, when fully evaluated, is deemed to be suggestive, then the question of whether trans fatty acids are indeed harmful to human populations will be resolved only by means of a randomized controlled trial. Am J Clin Nutr 1997;66(suppl):1011S–7S.

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INTRODUCTION

In 1994 Willett and Ascherio (1) published a commentary in the American Journal of Public Health in which they stated that “the combined results of metabolic and epidemiologic studies provide strong evidence that trans fatty acid [TFA] intake is causally related to risk of coronary disease.” Yet, “like the tobacco industry, the oil processing industry claims that...[causality]...cannot be established without a randomized controlled trial, which of course is ethically and logistically impossible.” They also claimed that, on the most conservative assumptions, “more than 30,000 deaths per year [in the United States] may be due to the consumption of partially hydrogenated vegetable fat.”

TFAs are present in processed vegetable fats (principally margarine, cooking oils, and shortening) and in animal fats such as butter and tallow. However, TFAs from vegetable and animal fats are chemically different, and Willett and Ascherio ad others (1–3) have claimed that it is specifically the ingestion of TFAs derived from vegetable fats that is associated with coronary disease. Indeed, they have gone further to state that “the occasional use of butter or lard will not have any important effect on health” and such use “may not be as unhealthy as generally believed” (1). (In the remainder of this critique, the term TFA is used to refer to derivation of these fatty acids from vegetable fats, unless otherwise stated.)

If true, these claims are of profound public health importance. Not surprisingly, they have attracted considerable publicity in the media and have given rise to confusion and concern (4, 5). The evidence, therefore, calls for rigorous critical scrutiny. The need for scrutiny is made all the more compelling because for at least half a century the public has been advised that margarine (the main source of TFAs) is preferable to butter (6) and that the intake of animal fats should be reduced. That advice has been urged most forcefully on those who already have coronary disease (eg, angina or previous myocardial infarction) or who are thought for any reason to be at increased risk (eg, hypertension or family history). There has been a large rise in the consumption of partially hydrogenated vegetable fats over much of this century, partly as a result of these recommendations (1, 6–8). Willett and Ascherio (1) claimed that evidence to show any benefit has never been produced. Nevertheless, what they now advocate represents no less than a major and fundamental about-face in a long-standing public health policy.

The evidence adduced to implicate TFAs in the etiology of coronary disease is derived from two sources, metabolic and epidemiologic studies. The metabolic studies were reviewed by others at this meeting. Here, by way of background, it is sufficient to note that in some studies the short-term feeding of TFAs under experimentally controlled conditions appears, among other things, to increase low-density-lipoprotein (LDL) cholesterol and to decrease high-density-lipoprotein (HDL) cholesterol relative to feeding mono- and polyunsaturated fatty acids (9–12); the effects are less marked than those induced by feeding saturated fatty acids. The validity of those experiments is under debate because of conflicting results (12–14). If, however, validity is assumed, the adverse effects of TFAs have not been shown to persist in the long term and their relevance to coronary risk can only be established by epidemiologic means.

The epidemiologic evidence is reviewed below. Before doing so, however, some selected principles of direct relevance to some or all of the studies under review are briefly considered.

Weak associations

In causal research based on nonexperimental methods, a fundamental question is whether, for weak associations (de-
fined here as relative risk estimates ≤ 2.0), the epidemiologic
microscope has the resolving power to distinguish between bias
and confounding, as against causation. No study is perfect.
However, when strong associations are identified in reasonably
well-conducted studies (eg, heavy smoking and lung cancer,
with increases in the risk of the order of 10-fold or more),
unrealistically large sources of bias or uncontrolled confound-
ing would have to be present to explain them. The imperfec-
tions may be judged to be acceptable. By contrast, when there
are weak associations, minor sources of bias and uncontrolled
confounding can readily account for them. Statistical significa-
ce does not resolve the difficulty, because for any associa-
tion, whether causal or not, “significance” can be attained
simply by increasing the sample size sufficiently. For these
reasons, in the absence of randomization it is virtually impos-
tible to confidently interpret weak associations as evidence of
causality.

**Precision**

A related matter concerns precision and in nutritional stud-
ies, in particular, the precision of dietary data obtained by
interview or questionnaire (15, 16). To check validity, the usual
procedure is to correlate dietary intake as measured by a study
instrument with a selected gold standard (for example, dietary
recall in a food-frequency questionnaire with prospectively
recorded food diary data). A coefficient of 1.0 indicates exact
correlation and a value of 0.0 indicates no correlation. In most
dietary studies the coefficients, although they may be statisti-
cally stable, seldom exceed a value of 0.5. The usual argument
advanced, or implied, to justify validity is that such coefficients
indicate that the data show some degree of agreement with
more exact measurements, albeit with a fair amount of mis-
classification. However, provided the misclassification is ran-
dom, its effect is to underestimate the magnitude of any given
association: the true magnitude would be greater if the data
were more precise. That is, the effect of randomly imprecise
measurement is to produce conservative estimates of risk.

There are several fallacies to this line of reasoning, the most
obvious being that it is intuitively absurd to invoke the con-
servative effect of random imprecision as a justification for
placing a causal interpretation on any association, especially a
small one. It is a moot question as to when imprecision merges
with an unacceptable amount of random noise, and random
noise sometimes produces random associations. What is far
more important, however, is that the greater the imprecision,
the greater the opportunity for the occurrence of bias; the
misclassification may not be random at all, but systematic (17).

It is also important to appreciate that the problems of mis-
classification apply not only to the exposure under study but to
the confounding variables as well. To the degree that the latter
are misclassified, confounding is incompletely controlled. Not
only do the correlation coefficients for the intake of substances
such as saturated fats, ununsaturated fats, and carbohydrates
seldom exceed 0.5, but there is close correlation (direct or
inverse) among them. Thus, for example, when it is claimed
that in the evaluation of TFA intake the intake of other fatty
acids has been allowed for, that allowance is invariably
incomplete.

A certain amount of misclassification is inevitable in any
study, and the findings may nevertheless be valid. However,
that is more likely to be the case if the errors are minor and if
a given relative risk is large to begin with; even then major
imprecision would still cast doubt on the findings.

**Confounding by indication**

Confounding by indication exists when any given factor is
the reason for the exposure at issue and, independently, a
determinant of the disease under study (18, 19). For example,
patients with hypertension may tend to use margarine and,
independently, be at increased risk of coronary disease. In
addition, the more severe the hypertension, the more strong
may be the preference for margarine. The underlying severity
of the hypertension cannot be adequately measured, and thus
its confounding effect cannot be fully controlled by any means
other than randomization.

**Timing sequence of the exposure and the outcome**

An absolute requirement for inferring causation is that the
exposure at issue must precede the onset of the disease. Yet
that requirement, although obvious, is commonly ignored.
Thus, for example, if angina induces patients to switch from
butter to margarine, the coronary disease “caused” the expo-
sure and the association is not valid. Timing is particularly a
problem in cross-sectional studies.

**Selection bias**

Selection bias exists if the exposure, or a determinant of the
exposure, is also a determinant of selection of the study sub-
jects. This would commonly apply, for example, to cases of
coronary artery disease (CAD) identified by angiography. Cor-
onary angiography is commonly an elective procedure and the
decision to perform an angiogram may be influenced by factors
such as anxiety about hypercholesterolemia. The same factors
may influence patients to use margarine. In this example it is
worth noting that selection bias would be largely averted if the
endpoint were myocardial infarction rather than angiographi-
cally diagnosed coronary disease because the former diagnosis
is for the most part obligatory and independent of anxiety. It
should also be noted that if anxiety about angina is the reason
for the angiogram, this is a matter not only of selection bias but
also of violation of the time sequence between the exposure
and the onset of the disease.

**Information bias**

Information bias exists when the exposure is recorded dif-
ferently in the compared groups. Sometimes the direction of
the bias may not be predictable. For example, patients who
know they are at increased risk (eg, because of diabetes) may
overestimate their consumption of margarine if they conceive it
to be a healthier behavior. Alternatively, they may overestimate
their butter consumption if they believe the perceived un-
healthy behavior caused their illness. Many other factors may
also influence the direction of information bias; however, if
biases are plausible in either direction, as is commonly the case
in nutritional studies (15, 16), the data are suspect.

**EPIDEMIOLOGIC STUDIES OF TFAs AND CORONARY
DISEASE**

The relation of TFAs to coronary risk or to indicators of
coronary risk has been examined in eight published epidem-
ologic studies (2, 3, 20–25). Thomas (20) compared 136 case subjects who died of ischemic heart disease with 95 control subjects who died of other causes. There was a significantly higher concentration of TFAs in the adipose tissue of the case subjects. The main source of TFAs was marine oils, commonly used at the time (in Britain) in the manufacture of margarine.

Apart from data on age and sex there was no information on confounding in this study. The possibility, and indeed the likelihood, that well-established risk factors such as a family history, diabetes, obesity, or hypertension could have been associated with TFA intake was not considered. If factors such as angina or previous myocardial infarction induced the case subjects to use margarine, the disease caused the exposure. The association of ischemic heart disease with TFAs derived principally from animal fats is also incompatible with the hypothesis that it is only those TFAs derived from hydrogenated vegetable fats that increase risk (1–3). It is compatible, however, with a preference for margarine by persons already known to be at increased risk.

In a cross-sectional study, Troisi et al (21) administered a semiquantitative food-frequency questionnaire to 748 men aged 43–75 y who were participants in a longitudinal study (26). Serum lipid concentrations were estimated after multivariate adjustment for age, body mass index, waist-to-hip ratio, current and former smoking, alcohol intake, physical activity, total energy, and dietary cholesterol intake. Additional multivariate models made allowance for serum cholesterol status and intake of other fatty acids at the last examination. Confounding was also partly controlled by excluding men who were taking diuretics or medications for hypoglycemia or hypolipidemia. Increased TFA intake was associated with increased total cholesterol (P = 0.04), LDL cholesterol (P = 0.01), ratio of LDL to HDL cholesterol (P = 0.001), and ratio of total to HDL cholesterol (P = 0.002), and with decreased HDL cholesterol (P = 0.03).

The authors acknowledged that “the limitations of this study relate to its cross-sectional nature and the health consciousness of its subjects” who “may have increased their intake of margarine...resulting in a spurious relation.” However, they considered that explanation to be unlikely because the results were the same for those who were told that they had high serum cholesterol and those who were not. The latter comparison does not eliminate the possibility that prior knowledge of an increased risk could have induced the use of margarine in preference to butter. In addition, potential confounding due to factors such as a family history of coronary disease, diabetes, or hypertension that could have influenced the subjects to prefer margarine was not controlled. Allowance for the intake of fatty acids other than TFAs was incomplete. There was no information on angina or other antecedent coronary disease, factors that could readily have caused margarine use rather than the reverse.

In a cross-sectional study, Siguel and Lerman (22) measured plasma TFA concentrations in 47 patients with angiographically documented major coronary artery obstruction or stenosis. The patients were compared with a previously reported control group of 56 nonobese subjects of whom 24 were healthy volunteers and 32 were randomly selected from the Framingham Cardiovascular Offspring Study (27). Individual and total plasma TFA concentrations were significantly higher in the patients with documented obstruction or stenosis.

By definition, a large proportion of the patients had symptomatic long-standing coronary disease, which is why they came to angiography. Once again, coronary disease could have caused margarine use, rather than the reverse. In addition, among those subjects who did not have documented antecedent coronary disease, there were selection biases that affected both the patients with coronary disease and the control subjects. Among the patients with coronary disease the decision to perform angiograms could have been influenced by factors such as health consciousness or anxiety about diabetes or hypercholesterolemia. Health consciousness or anxiety could also have been associated with a preference for margarine. As for the control subjects, the multiple biases introduced by the selection of nonobese subjects (of whom 42% were healthy volunteers) enrolled in another study, at another place, and at another time, entirely disqualify the findings. Confounding was uncontrolled: allowance was not made even for body mass index, a factor for which the patients with coronary disease and control subjects were selected to be different.

In a hospital-based case-control study carried out in Greece, Tzonue et al (23) compared 329 case subjects with coronary disease (181 with a first myocardial infarction; 174 with a first positive coronary arteriogram) with control subjects admitted to the same hospital for conditions thought to be unrelated to diet. Dietary histories were obtained by interview. Multivariate adjustment was made in the analysis for interviewer, sex, age, years of schooling, duration of siesta, body mass index, regular exercise, smoking, alcohol intake, coffee intake, and total energy intake. For those who used margarine as the principal cooking fat, the relative risk was 1.87 (95% CI: 0.82, 4.28).

Angiographically diagnosed coronary disease accounted for 49% of the case subjects; those with antecedent angina or other coronary disease manifestations, such as an abnormal resting electrocardiogram or effort tests (presumably the majority, if not all of the case subjects), were improperly included because preexisting coronary disease could have caused margarine use. In addition, if health-conscious or anxious case subjects were more inclined to use margarine and to be subjected to angiography, there was a selection bias. A first myocardial infarction accounted for 52% of the case subjects. Among these subjects there would presumably have been an appreciable prevalence of antecedent angina, which could have induced the subjects to use margarine. It is only in case subjects with infarction that was not preceded by angina that a valid estimate of the effect of TFA exposure could have been obtained. However, such an estimate would not have been robust because not even the overall association was significant.

In a follow-up study of nurses enrolled in 1978, Willett et al (2) administered a semiquantitative food-frequency questionnaire; the subjects were followed until 1988. After the exclusion of women with previous diagnoses of angina, myocardial infarction, stroke, high serum cholesterol, or diabetes, the base population comprised 85 095 women. The endpoint was the incidence of CAD, defined as nonfatal myocardial infarction or death from CAD. Follow-up for mortality was > 98% complete. To assess the validity of the measure of TFA intake, the intake, calculated from a version of the questionnaire administered to 115 control subjects in a case-control study of breast cancer (28), was compared with proportion of TFA contained in aspirates of adipose tissue. The correlation was 0.51.
During 661,996 person-years of follow-up there were 431 incident cases of CAD. With multivariate adjustment for age, smoking, body mass index, hypertension, alcohol intake, menopausal status, postmenopausal estrogen use, energy intake, family history of myocardial infarction before age 60 y, use of multivitamins, and intake of saturated fat, monounsaturated fat, and linoleic acid, the relative risk estimates (and their 95% CIs) across quintiles of TFA intake were as follows: 1.0 (reference), 1.1 (0.8, 1.6), 1.0 (0.7, 1.4), 1.2 (0.8, 1.7), and 1.5 (1.0, 2.2) (P for trend: 0.006). When women who reported in 1980 that their margarine intake had greatly changed in the past 10 y were excluded, the corresponding estimates for TFA intake derived from vegetable fats were 1.0 (reference), 1.4 (1.0, 2.0), 1.1 (0.7, 1.7), 1.4 (0.9, 2.1), and 1.8 (1.1, 2.8) (P for trend: 0.009). By contrast, for TFA intake derived from animal fats, the estimates were 1.0 (reference), 0.8 (0.5, 1.1), 0.7 (0.4, 1.1), 0.6 (0.3, 1.0), and 0.6 (0.3, 1.2).

A strength of this study is that women with a previous history of angina, myocardial infarction, stroke, diabetes, or known hypercholesterolemia were excluded. Those exclusions reduced the possibility of identifying spurious associations due to improper timing of exposure in relation to disease onset and of confounding from several sources. However, they by no means eliminated them. A preference for margarine could have been associated with a wide range of factors, some of them indicative of preexisting coronary disease and some of them confounders, such as the following: nonspecific electrocardiographic changes; awareness of chest pain, either typical or atypical, but not yet given the formal diagnosis of angina; arrhythmias; extensive family history of coronary disease; familial hypercholesterolemia; or high blood pressure readings not yet labeled as hypertension.

The correlation coefficient for the questionnaire-ascertained TFA intake, as against measured TFAs in adipose tissue, was 0.51. Such a low correlation augments the likelihood of bias and confounding. Bias could have arisen, for example, if knowledge of a strongly positive family history of myocardial infarction beyond age 60 y or of covariates such as diabetes selectively induced women at risk to overestimate their reported intake of margarine. Confounding was inadequately controlled, both because the intake of other substances, such as saturated fatty acids, was to an appreciable degree misclassified and because such intake may have been reported differently by women who were and were not at risk.

To reduce the possibility that women may have altered their dietary habits because of awareness of an increased risk, the analysis was repeated after excluding women who reported in 1980 that their margarine intake had greatly changed in the past 10 y. That maneuver was inadequate: the meaning of greatly changed is ambiguous: women could have changed their intake > 10 y previously, and women who did not report major changes could still in the aggregate have preferred margarine if they already had coronary disease or thought they were otherwise at increased risk. The data suggest that this was the case: with the lowest quintile of intake as the reference for TFAs derived from vegetable fats, the relative risk estimates for each of the remaining four quintiles exceeded unity. By contrast, the corresponding estimates for TFAs derived from animal fats were all below unity; indeed, for the fourth and fifth quintiles of intake the relative risk estimates were both 0.6 and for the fourth quintile risk estimates were significant (upper 95% confidence limit: 1.0).

Willett et al (2) interpreted these divergent findings as an indication that the "positive association... was due to the intake of partially hydrogenated vegetable fats rather than [TFA] isomers from ruminant sources." If so, the relative risk estimates for TFAs derived from animal fats should have approximated unity and not been reduced, unless it is proposed in addition that the intake of TFAs derived from animal fats prevents CAD. Such a proposition is far-fetched. The most parsimonious explanation, and one that accounts for all the facts, is that women already at increased risk tended to avoid animal fats and to ingest partially hydrogenated vegetable fats instead.

Willett et al (2) argued that the significant dose-response gradient across quintiles of TFA intake constituted evidence to support causality. However, there was no evidence of a monotonic gradient and indeed little opportunity to observe one given that the highest overall relative risk estimate in the uppermost quintile was 1.5. Finally, this study serves to illustrate the impossibility of confidently inferring causation on the basis of a relative risk estimate of 1.5. Of all the studies reviewed in this critique, this study was one of the more rigorous, but it was not possible to make it rigorous enough. Despite attempts to keep bias and confounding to an absolute minimum, there were plausible noncausal explanations for the findings.

In a case-control study reported by Ascherio et al (3), 450 case subjects with first myocardial infarction were identified, of whom 366 (81%) were enrolled in the study. The control group was selected from town residential lists; 741 control subjects matched for age, sex, and residence were selected at random. Of these, 423 (57%) were successfully enrolled. Subjects with a history of previous myocardial infarction, angina, diabetes, or hypercholesterolemia were excluded. The final study population comprised 239 case subjects with myocardial infarctions and 282 control subjects. A semiquantitative food-frequency questionnaire was used to determine the intake of TFAs. The correlation coefficients for the comparison between the questionnaire data and measured TFAs in adipose tissue were 0.51 in women (28) and 0.34 in men (29) (P < 0.001 for both).

Across quintiles of energy-adjusted TFA intake the relative risks (and their 95% CIs), adjusted for age, sex, smoking, history of hypertension, body mass index, alcohol intake, family history of ischemic heart disease, physical activity, and intake of saturated fat, monounsaturated fat, linoleic acid, and cholesterol were as follows: 1.0 (reference), 0.8 (0.4, 1.6), 0.4 (0.2, 0.8), 0.7 (0.4, 1.5), and 2.0 (1.0, 4.2) (P for trend: 0.001). The corresponding estimates for TFAs from vegetable sources were 1.0 (reference), 0.7 (0.4, 1.5), 0.4 (0.2, 0.9), 0.6 (0.3, 1.3), and 1.9 (0.9, 4.0); for TFAs derived from animal sources, the estimates were 1.0 (reference), 1.2 (0.6, 2.3), 1.1 (0.5, 2.3), 1.0 (0.5, 2.2), and 1.0 (0.4, 2.4).

In the total series of 521 subjects, 75 (15%) had greatly increased their margarine intake over the past 10 y, 27 (5%) had decreased their intake, and 100 were consuming a special diet, giving a total of 202 (39%) (my calculation) who were currently on a diet or who had recently changed their diets. When these subjects were excluded, the relative risk for the highest quintile of TFA intake among the remaining 344 sub-
projects was 2.5 (95% CI: 1.0, 6.2). Individual food items were also examined. Relative to the intake of less than one pat of margarine per day, the relative risk for the intake of > 2.5 pats was 3.2 (95% CI: 1.6, 6.4) (P for trend: 0.002).

A strength of this study is that myocardial infarction is an endpoint that is relatively free of diagnostic bias conditional on TFA use. Otherwise, virtually the same criticisms as those mentioned in connection with the study of Willett et al (2) apply here, with the exception that the relative risk estimates for TFAs from animal sources were not reduced.

Additional criticisms are as follows. Only 57% of the eligible control subjects were enrolled; dietary intake of TFAs, in particular, could well have been substantially different among the 43% who were not. Such a high attrition rate raises the possibility of selection bias. It could easily explain, for example, why reduced relative risks were not observed for TFAs derived from animal fats, as in Willett et al’s study (2), if control subjects who consumed animal fats selectively elected not to participate. Because this was an interview-based case-control study, there could also have been information bias, both in the reporting of the exposures and the confounders.

The data suggested that there was bias. For example, the relative risk estimates for TFA intake were reduced in four of the five quintiles of TFA intake, in the third quintile significantly so; the risk was significantly elevated only in the top quintile. No explanation was offered for that anomalous pattern, which was incompatible with a monotonic, dose-response gradient.

There was additional evidence to suggest bias: the relative risk estimate for the consumption of > 2.5 pats of margarine/d was 3.2. That finding is not credible: 2.5 pats would barely cover two slices of bread. A clue as to how such an implausible finding could have emerged was present in the data: fully 39% of the study population were currently on a diet or had recently changed their diet. The reasons for dieting and for preferring margarine could well have included underlying coronary disease or known predisposition to myocardial infarction. The rates of current or recent dietary changes were not given separately for the case and control subjects, but the remarkably high overall rate of 39% raises questions as to whether the data can be interpreted at all.

In an international multicenter study conducted in eight European countries and Israel, Aro et al (24) compared 642 men with a first episode of myocardial infarction with 717 control subjects without a history of infarction who were series-matched for age. In some centers the control subjects were drawn from population registries or other community sources (e.g., general practitioners’ lists) but when this was not feasible, hospital control subjects with diagnoses not known to be associated with dietary factors were selected. Subjects were excluded if, among other things, they had changed their diets for health reasons or had lost > 5 kg in the past year. Adipose tissue samples were obtained by needle aspiration biopsy and assayed for TFAs. Success rates in enrolling subjects and then obtaining biopsies varied by center and were higher for the case subjects with myocardial infarction than for the control subjects (my calculations). Among eligible case subjects with myocardial infarction the median biopsy rate was 87% (range: 43–98%); among eligible control subjects it was 59% (range: 23–96%). In the analysis, multivariate methods were used to adjust for age, center, smoking, and body mass index (socioeconomic status did not affect the relative risk estimates).

Overall there were no differences in the proportions of adipose tissue 18:1 TFA between case and control subjects. However, TFA proportions in adipose tissue were much lower in subjects from Granada and Malaga (Spain). When Spanish subjects were excluded, there was a nonsignificant tendency toward an increased risk in the upper quartile of 18:1 TFA. There was marked variability in the data according to study center. With the first quartile of 18:1 TFA as the reference, the range of relative risks (and their 95% CIs) for the topmost quartile was 5.4 (1.5, 13.1) in Sarpsborg (Norway) and 5.0 (1.3, 19.6) in Helsinki (Finland); at the other extreme, the estimates were 0.3 (0.1, 1.0) in Malaga (Spain), 0.2 (0.1, 0.7) in Moscow (Russia), and 0.2 (0.0, 0.6) in Granada (Spain). In the remaining centers (Berlin; Edinburgh; Zurich, Switzerland; Jerusalem; and Zeist, Netherlands) the relative risk estimates ranged from 0.8 to 1.8 and were not significant.

The findings are open to question on several counts. First, the relative risk estimates according to study center ranged from 0.2 to 5.4. It is not justifiable to combine such heterogeneous data. Second, it is not justifiable to exclude selected centers simply because the TFA concentrations were lower than elsewhere. Under causal assumptions, TFA concentrations should be higher in case subjects with myocardial infarction than in control subjects in all participating centers, regardless of baseline TFA concentrations, unless it is proposed that there is a threshold below which no effect can be observed. In that case, however, that hypothesis should be specified in advance, not post hoc. In any case, there is the further difficulty of having to propose one or more hypotheses that also explain apparent causal effects in some other centers.

Third, in most of the centers the recruitment rates for adipose tissue biopsy were exceedingly low for the control subjects and much lower than for the case subjects with myocardial infarction. If potential control subjects who refused to participate differed in TFA intake from those who did not refuse, there could have been selection bias. That bias could have been substantial and in different directions in the different centers.

Fourth, preexisting coronary disease other than a previous myocardial infarction was not excluded. Persons with angina, for example, could have preferred margarine to butter and the tendency to do so could have varied among the centers. Fifth, potential confounding from sources such as a positive family history, diabetes, or known hypercholesterolemia or hypertension was not controlled. Once again, the influence of such factors on TFA intake could have varied among the study centers.

Roberts et al (25) compared 66 cases of sudden cardiac death due to CAD with 286 healthy age- and sex-matched control subjects. Adipose tissue was obtained at necropsy from the case subjects; control subjects were drawn from the same general practitioner lists in which the case subjects were listed, and adipose tissue was obtained by biopsy. Tissue samples were obtained from 79% of the eligible case subjects (my calculation) and 89% of the control subjects. Subjects with any antecedent history of coronary disease were excluded. For case subjects, information on occupation and medical history was obtained by interviewing spouses, or when this was not possible, from coroners’ or necropsy reports. For control subjects.
the analogous information was obtained from the spouse or, when there was none, directly from the subjects themselves.

With multivariate adjustment for age, reported smoking, history of hypertension and diabetes, and consumption of oleic and linoleic acids, there was no overall association between TFA concentration and sudden cardiac death. With the first quintile of TFA as the reference, the relative risk for the fifth quintile was 0.6 (95% CI: 0.2, 1.8). The corresponding estimates for 18:1 and 18:2 TFAs were 0.6 (0.2, 1.8) and 1.0 (0.4, 2.8).

This study had certain unique strengths. In particular, the determination of TFA concentrations, and hence the inferred antecedent intake of TFAs, was entirely free of information bias and the measurements were subject to minimal misclassification. The exclusion of subjects with known preexisting coronary disease reduced the likelihood of identifying a spurious positive association due to antecedent coronary disease that may have induced case subjects to prefer margarine. Selection bias was unlikely because this was a population-based study and the enrollment rates for both case and control subjects were high. Potential confounding due to a range of important risk factors was controlled.

There were also certain weaknesses. The sample size was small and statistical power was limited. The information on preexisting coronary disease and on coronary risk factors may have been incomplete, as well as recorded differently for case and control subjects, and thus incompletely allowed for; certain risk factors, such as family history or known hypercholesterolemia, were not allowed for, and control of confounding was incomplete. On balance, however, the strengths of this study, particularly the minimal misclassification of TFA concentrations, appear to outweigh the weaknesses. The data do not suggest that TFAs increase the risk of coronary disease.

**DISCUSSION**

It is a common fallacy to argue that borderline evidence perhaps compatible with causation should be interpreted as definitive evidence because the subject matter, although complicated and difficult to unravel, is of major public health importance. That argument is commonly buttressed by the reasoning that identified small risks would become larger if only the data could be recorded more precisely. We have done our best, the argument goes; we judge that there is an increased risk, and we should act as if there were one. It becomes necessary to restate that difficulty and complexity are not criteria of scientific validity and that a given question, although important, may sometimes not be answerable.

These considerations certainly apply to the epidemiologic literature on TFA intake and risk of CAD. The multiple problems of bias, misclassification, improper time sequences, and confounding (especially confounding by indication) make it virtually impossible to document a causal relation by nonexperimental means. Of the studies reviewed here, the most rigorous were those of Willett et al (2) and Roberts et al (25). One was a follow-up and one a case-control study. Despite strenuous efforts to eliminate bias, however, neither could fully accomplish that objective, and it is unlikely that it could have been accomplished even with the most optimal nonexperimental study design.

Despite such considerations, Willett and Ascherio (1) have interpreted their own findings (2, 3) and those of others (20, 27) as supporting causation. They have also argued that the total body of epidemiologic and metabolic evidence constitutes additional justification for that inference, although individual studies may be inadequate. As a matter of logic it is fallacious to argue that a series of inadequate studies taken together cancel out their inadequacies. That matter aside, however, the assembled evidence does not come close to justifying a causal inference and still less the assumption that 30,000 coronary deaths may be due to consumption of TFAs. Moreover, the extensive publicity given to those claims can only do damage to the credibility of public health advocacy.

It may be that TFAs do increase the risk of coronary disease, but I agree with Roberts et al (25) that their "study and other epidemiological evidence indicate that the relation between [TFA] intake and increased risk of [CAD] remains unproved." In addition, in the absence of a randomized controlled trial, it is likely to remain unproven. Willett and Ascherio (1) argue that a trial would be neither ethical nor feasible. They offer no justification for their ethical position, but as to feasibility, more difficult studies, such as the Women's Health Initiative (30), are currently in progress. Certainly a controlled trial would be difficult, but if a consensus is eventually achieved that the metabolic evidence is suggestive of an increased risk, there is no reason a trial could not be done.

Finally, one of the studies reviewed here is instructive. Had the study of Aro et al (24) been performed only in Norway and Finland, the relative risk estimates of 5.4 and 5.0 would almost certainly have been interpreted by some as persuasive evidence of causality, the biases notwithstanding. On the other hand, had the study been performed only in two centers in Spain and in Russia, the relative risk estimates of 0.3, 0.2, and 0.2 could equally well have been interpreted as evidence of protection. In the remaining centers one could have concluded that TFAs appear to not affect the risk of coronary disease. These contradictory findings from a single study should have a sobering effect on those who are inclined to overinterpret associations identified in epidemiologic research.

**ADDENDUM**

After the presentation of this critique, three further publications have appeared (31–33). An expert panel conducted a critical review of the scientific data on TFAs and CAD risk (31). The panel concluded that "data supporting a relation between trans fatty acids intake and [CAD] risk are equivocal compared with extensive data... linking saturated fats to [CAD]." A major limitation to the published data was that the independent effects of TFAs had not been shown and further research was needed. The present critique and that of the expert panel are in broad concordance.

In a randomized trial (32), 90 men referred for coronary angiography because of preexisting ischemic heart disease were assigned to a lipid-lowering diet, diet plus cholestyramine, or usual care. Progression, as measured by angiography, was correlated with intakes of palmitic, stearic (18:0), and elaidic acid (t-18:1). There was "... a potentially independent association between progression of [CAD] and trans-unsaturated fatty acids derived chiefly from animal sources." These
findings do not support the hypothesis that TFAs of vegetable origin increase the risk of ischemic heart disease. In addition, random assignment to a general lipid-lowering diet, rather than specifically to high compared with low intakes of TFAs, made it virtually impossible to determine whether there was an independent effect of TFAs distinct from that of saturated fats or cholesterol.

In a correlational evaluation of average dietary intake of saturated fats and TFAs and cholesterol among 16 cohorts of men assembled in seven countries (33), significant associations with 25-y death rates were observed for the average intake of saturated fatty acids, TFA, elaidic acid, and dietary cholesterol. The limitation of the analysis to intercohort comparisons of average intake, the absence of comparisons of exposure at the level of individual exposures, the high correlation of the variables considered, and the long durations of follow-up after recording the dietary information all limit the informativeness of the findings.

REFERENCES