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Editorial

trans-Fatty acids, insulin resistance/diabetes, and cardiovascular disease risk: should policy decisions be based on observational cohort studies, or should we be waiting for results from randomized placebo-controlled trials?

Cardiovascular disease remains the leading cause of morbidity and mortality in the United States [1,2]. Although the number of deaths from cardiovascular disease appears to be decreasing, primarily because of more aggressive interventional approaches, the prevalence of many of the risk factors associated with and leading to cardiovascular morbidity has been increasing constantly [2]. Of special interest is the dramatic increase in the prevalence of obesity [3] and diabetes mellitus (DM) [4].

These and many other risk factors for cardiovascular disease have, among others, been traditionally associated with diet quantity and quality. Among components of macronutrients, *trans*-fatty acids (TFAs) have been most strongly associated with cardiovascular morbidity and mortality [5]. *trans*-Fatty acids are unsaturated fatty acids in which the double bond between 2 consecutive carbon atoms is in the *trans* configuration. They can be monounsaturated (MUFA) or polyunsaturated (PUFA) depending on the number of double bonds. In contrast, saturated fatty acids (SFAs) do not have any double bonds on their carbon chain, whereas *cis*-MUFAs and *cis*-PUFAS do have one or more double bonds, respectively, but exist in the *cis* configuration. Dietary TFAs are derived from 2 sources: dairy and meat products from ruminant animals (which represent an extremely small percentage of the total TFA intake in the Western world) and from industrial partial hydrogenation of vegetable oils [6]. The latter represents the vast majority of sources of TFA and is found in foods such as bakery products, deep-fried and frozen food, snacks, margarines, crackers, and soup powders [5].

The official reported average consumption of industrial TFA in the United States is around 2% of total energy intake (2%E), which, for an average person, is equivalent to 4 g/d [7]. More recent studies support the notion that, on an individual basis, it is possible that a person in the United States could consume up to 50 g of TFA from a single high-fat meal and snacks [8]. The American Heart Association recommends that TFA intake should be less than 1%E [9,10], that is, suggesting to reduce the current intake by at least 50%. This recommendation, along with other similar recommendations and the public health implications that are associated with them, has been primarily based on observational evidence (see below) and have led to significant public policy changes. New York City has banned use of artificial *trans*-fat in the city's food establishments. Thirteen local governments, 1 state, and Puerto Rico have now followed suit to implement a TFA ban; and many states have considered adopting this measure [11,12]. Furthermore, as of January 2006, the Food and Drug Administration required *trans*-fat content to be listed on the nutrition facts panel of foods and dietary supplements to heighten consumer awareness [13]. The implementation of the above is translated into huge expenses for taxpayers, food companies, and small business owners.

Importantly, the evidence that has been accumulated on the association between TFA consumption and disease has been mostly originating from observational cohort studies. The association of TFA and cardiometabolic risk has predominantly been examined at 5 levels: (*a*) induction of dyslipidemia (increase low-density lipoprotein [LDL] and triglycerides, decrease LDL particle size and high-density lipoprotein [HDL] levels); (*b*) activation of systemic inflammation and endothelial dysfunction; (*c*) increase of visceral adiposity, insulin resistance, and metabolic syndrome; (*d*) onset of DM; and (*e*) hard cardiovascular end points (myocardial infarction and coronary heart disease [CHD] deaths) [5].

Animal studies do not consistently demonstrate effects of TFAs above and beyond other factors inducing atherosclerosis. For example, experiments using atherosclerosis-prone mice show that TFAs do not stimulate further effects beyond the strongly atherogenic effects of dietary cholesterol (2% of diet for 14 weeks) [14]. Although observational epidemiologic data describing the effect of TFAs on serum lipid profile are largely missing, much of our knowledge is derived from short-term metabolic studies. These studies have demonstrated that increased TFA consumption is associated with an increase in triglycerides, LDL, and total cholesterol, and a significant decrease in HDL and apolipoprotein (Apo) A-1 levels [15-17]. In addition to this, TFA consumption increases circulating Apo-B particles and decreases Apo-A particles [18].

Furthermore, in a meta-analysis of 60 randomized placebo-controlled trials, with a total of 1672 volunteers and 159 data points, Mensink et al [19] demonstrate that TFA was the macronutrient that increased the total to HDL cholesterol ratio the most (substituting 1% of the energy derived from carbohydrates with TFA, total to HDL cholesterol ratio increased by 0.022). Moreover, in a different meta-analysis of 13 randomized controlled trials with duration of 17 to 70 days, Mozaffarian et al [20] demonstrated that for each 1% isocaloric energy exchange of TFAs with SFAs, *cis*-MUFAs or, *cis*-PUFAs, the triglyceride levels, total to HDL cholesterol ratio, and Apo-B/Apo-A ratio decreased significantly by 0.004 to 0.015 mmol/L, 0.031 to 0.067, and 0.007-0.011, respectively. Lipoprotein (a) was also found to be decreased (by 1.11-3.76 mg/L) [20]. At this point, it is worth mentioning that 3 independent studies examining the effect of TFAs on lipid profile demonstrated that their effect is proportional to the amount of TFA consumption, whereas their origin (ruminant vs industrial) seems to have no effect [21-23].

Much less evidence exists with respect to the effect of TFA on systemic inflammation and endothelial dysfunction. Cross-sectional studies have reported that increased TFA intake is associated with increased circulating inflammatory markers such as sTNFR1 and 2, sICAM1, sVCAM1, CRP, and IL-6 [24,25]. To date, there is only one published randomized controlled study that investigates the effects of TFA consumption on systemic inflammation markers in the context of a crossover design involving the administration of 6 different diets (viz, carbohydrate control diet, diet enriched with oleic acid, diet enriched with TFA, diet enriched with TFA and stearic acid, diet enriched with stearic acid alone, and diet enriched with 12:0-26:0 SFAs) for 5 weeks to 50 subjects [26]. This study reports that only E-selectin was higher (by 14.4%) after high-TFA diet when compared with diet enriched with SFA, whereas the levels of IL-6, CRP, and fibrinogen were not significantly different. When comparing the high-TFA diet with the control carbohydrate diet, CRP and E-selectin were higher by 242% and 12.2%, respectively, whereas there was no significant difference in the levels of IL-6 and fibrinogen [26]. However, there was no statistically significant difference in CRP levels between the TFA + stearic acid diet and the carbohydrate control diet or the SFA diet. Thus, the evidence that TFA consumption increases inflammatory markers arises mostly from observational studies, whereas the only available randomized controlled trial challenges results from these observational studies.

With regard to adiposity, insulin resistance, and diabetes, things are even less clear. One study in nonhuman primates has demonstrated that increased TFA intake leads to higher weight gain, mainly through increases of visceral fat, when compared with isocaloric diet where the TFA had been replaced by *cis*-MUFA [27]. In humans, these findings have been confirmed only by one observational cohort study in male subjects (n = 16 587) [28] and one in female subjects (n = 41 518) [29]. No other cohort study examining this issue is available to date. In a randomized crossover study, Christiansen et al [30] demonstrate an increase in postprandial insulin levels, but no change in the lipid profile, in subjects consuming high-TFA diet for 6 weeks when compared with a diet where TFAs were substituted with *cis*-MUFAs or SFAs. In addition to this, a study with similar design demonstrated that high TFA consumption led to 20% to 24% higher fasting insulin levels, a 12% to 14% increase at the LDL cholesterol levels, and a 25% to 28% increase in the homeostasis model assessment of insulin resistance index [31]. However, these studies were conducted in obese subjects with type 2 DM and hyperlipidemic, overweight individuals, respectively; were short in duration (5-6 weeks); and had a small sample size (n = 16 and n = 15, accordingly). Importantly, TFA intakes in these studies (20%E and 4.2%E, respectively) were much higher than the officially reported average in the US diet [30,31]. On the other hand, 2 crossover controlled studies, conducted in healthy, euglycemic, normal-weighted individuals, with similar sample size and duration with the aforementioned studies, failed to show any change in insulin sensitivity with 5%E to 9%E TFA consumption vs *cis*-MUFA or SFA [32,33]. No studies have evaluated the effect of regularly consumed amounts of TFA vs no TFA in the diet at all.

Our knowledge on the effect of high-TFA diet on the incidence of overt DM is at an even more infantile stage. In this case, all available evidence emerges from 3 observational prospective cohort studies with conflicting results. The Nurses' Health Study (85 095 female subjects, 8 years of follow-up) reports that for every 2%E increase in TFA consumption, there is an expected increase in the relative risk for development of type 2 DM by 1.39 [34]. Contrary to this, the Iowa Women's Health Study [35] (35 988 female subjects, 11 years of follow-up) and the Health Professionals Follow-up study [36] (42 504 male subjects, 12 years of follow-up) report no significant change in the relative risk of DM across the TFA intake quintiles. No randomized placebo-controlled studies exist to date examining whether reduction of TFA intake could result in reduction of the incidence of DM.

The level of evidence is still limited to observational cohort studies when it comes to hard cardiovascular outcomes, that is, acute coronary events and deaths from CHD. The largest study among them, the Nurses' Health Study, demonstrates that the CHD relative risk was significantly higher by 1.33 (95% confidence interval [CI], 1.07-1.66) in the subgroup that was consuming more than 2%E TFA [37]. A meta-analysis of all available cohort studies (Nurses' Health Study, Health Professional Study, Finnish ATBC Study, and Zutphen Elderly study) confirms that the relative risk of CHD is 1.23 (95% CI, 1.11-1.37) higher in the cohort with average TFA intake greater than 2%E compared with the less than 2%E TFA cohort [20]. Population-based case-control studies have linked endogenous red blood cell membrane fatty acids with sudden cardiac arrest. Palmitic acid (16:0) and hexadecanoic acid (16:1n9) were associated with a 38% and an 88% higher risk of sudden cardiac arrest, respectively [38]. This same group also reported an association between α-linolenic acid and cardiac arrest after adjusting for matching factors and controlling for smoking, diabetes, hypertension, and other risk factors [39]. However, according to the US Preventive Services Task Force *Guide to Clinical Preventive Services* (August 1989, DIANE Publishing, pp 24; Appendix A), well-designed cohort studies constitute level of evidence II-2; and any recommendation that would arise from them should be classified as level B, which means that the

evidence is not enough to permit implementation of public health policies.

In the current issue of *Metabolism*, Bendsen et al [40] report the results of a dietary intervention in 52 overweight but otherwise healthy postmenopausal women who were randomized to receive either partially hydrogenated soybean oil (15 g/d TFA) or a control oil (mainly oleic and palmitic acid) for 16 weeks. Results showed that the controlled dietary intervention did not significantly alter insulin sensitivity, β-cell function, or the metabolic clearance rate of insulin; also unchanged was the ability of insulin to suppress plasma nonesterified fatty acids and glycerol during oral glucose ingestion. This study is in agreement with a prior study in premenopausal overweight female subjects where 4 weeks of high TFA intake (both ruminant and industrial TFA, 4.86-5.58 g/d) had no effect on insulin sensitivity compared with control diet (0.54 g/d TFA) [41]. Furthermore, the strength of this study is that the authors have approached the association of TFA consumption and glycemic control by asking whether substitution of TFA with oleic and palmitic acid, in a randomized controlled trial, could have an impact on glycemic control. The adequate sample size (n = 52) and the long follow-up period (16 weeks) in addition to the randomized control design of the study make the derived results extremely strong and informative. This study adds some turbulence in the water by further highlighting the necessity of verifying observational data with randomized controlled studies of adequate sample size, power, and long follow-up period. Bendsen et al [40] recommend that unrealistically high levels of TFA consumption and/or a longer duration of exposure might be required to influence insulin sensitivity.

To summarize, on the basis of observational studies, there is clear evidence that increased TFA consumption is associated with increased triglycerides, LDL cholesterol, total to HDL cholesterol ratio, Apo-B/Apo-A ratio, and cardiovascular mortality and morbidity. There is less clear or even conflicting evidence from observational studies regarding the effect of TFA on systemic inflammation, endothelial function, insulin resistance, and DM; and there is practically no evidence regarding central adiposity and metabolic syndrome. Observational studies do not prove causality, however, because there is also the possibility that their results are due to uncontrolled confounding.

Thus, one would need appropriately designed randomized controlled trials to prove or disprove whether TFA consumption may have an effect on either disease-specific or overall morbidity or mortality. Existing trials provide limited evidence on whether reduction of TFA intake could decrease systemic inflammation, improve endothelial function and insulin sensitivity, and reduce the incidence of DM. In addition to this, there is no evidence to indicate whether reduction of TFA intake could reduce visceral adiposity and the incidence of metabolic syndrome. Moreover, there is absolutely no evidence to date demonstrating that complete elimination of TFA could lead to improvement in terms of hard cardiovascular end points, systemic inflammatory status, endothelial function, visceral adiposity, insulin resistance, and overt DM vs continuing on the average 2%E TFA American diet. Ideally, such trials, if positive, would need to be followed by appropriately designed studies on implementation, adoption, and cost-effectiveness of any changes to be made. Nonetheless, there are no studies investigating the cost-effectiveness of completely removing TFA from the commercially available goods. The latter is essential, given the fact that the cost of implementing such a measure is huge. We strongly recommend and encourage the design and performance of randomized controlled trials that will compare the effect of 0%E TFA diet with the average 2%E, at all the previously described levels, especially focusing on hard clinical outcomes such as CHD deaths, overall cardiovascular mortality, and onset of overt DM. Only the results of such studies could provide enough evidence to support drastic policy decisions such as the ones that have already started to be taken. Until data from such studies are in, quitting smoking, increasing exercise, decreasing total caloric intake, increasing fruit and vegetable consumption [42], and following a Mediterranean-type diet [43-45] seem to be clearer, better documented, and more cost-effective methods to decrease mortality from cardiovascular disease [46-49].

Konstantinos N. Aronis
Division of Endocrinology, Diabetes, and Metabolism
Beth Israel Deaconess Medical Center, Boston
MA 02215, USA
E-mail: karonis@bidmc.harvard.edu

Richard J. Joseph
George L. Blackburn
Department of Surgery, Center for the Study of Nutrition Medicine
Beth Israel Deaconess Medical Center, Boston, MA 02215
E-mails: rjoseph1@bidmc.harvard.edu
gblackbu@bidmc.harvard.edu

Christos Mantzoros
Division of Endocrinology, Diabetes, and Metabolism
Beth Israel Deaconess Medical Center, Boston
MA 02215, USA
Boston VA Healthcare System, Section of Endocrinology, USA
Department of Environmental Health
Harvard School of Public Health, USA
E-mail: cmantzor@bidmc.harvard.edu

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