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EDITORIAL

Natural *trans* fat, dairy fat, partially hydrogenated oils, and cardiometabolic health: the Ludwigshafen Risk and Cardiovascular Health Study

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This editorial refers to '*Trans*-fatty acids and mortality in patients referred for coronary angiography: the Ludwigshafen Risk and Cardiovascular Health Study'†, by M.E. Kleber *et al.*, on page 1072.

Kleber and colleagues have investigated levels of *trans*-fatty acids (TFAs) in erythrocyte membranes and risk of total mortality, cardiovascular mortality, and sudden cardiac death (SCD) in a convenience cohort of German patients hospitalized for angiography.[1] After multivariable adjustment, they found that higher levels of total TFAs, and specifically C16 : 1n-7t, were associated with lower risk of SCD (for C16:1n-7t, ~37% lower risk in the top tertile, compared with the bottom). No other significant associations were identified, including for total TFAs or C16:1n-7t and total or cardiovascular mortality, or for C18:1t or C18:2t and total mortality, cardiovascular mortality, or SCD.

How should these findings be interpreted, and what are their implications? In any research study—whether *in vitro* science, animal experiment, metabolic study, clinical trial, or observational epidemiology—the devil is in the details. Such crucial details are frequently overlooked in our modern age of compressed news, internet, and social media reporting.

In this case, the first important detail is the population studied. TFA levels were not measured in a general community, but in patients with chest pain or a positive non-invasive cardiac stress test who were hospitalized for coronary angiography between 1997 and 2000 at a tertiary care centre. Accordingly, these were not generally healthy individuals: for instance, at baseline, 73% had high blood pressure, 50% were taking lipid-lowering medication, and 40% had diabetes. Such patients would often be conscious of their symptoms and health prior to hospitalization, and consequently have altered their dietary behaviours and other lifestyle factors in response to their concern.

Such a population produces a classic set-up for 'reverse causation': underlying poor health leading to changed behaviours and avoidance of TFA-containing foods (e.g. processed foods, whole-fat dairy), rather than TFA levels predicting future poor health. Is there evidence for such reverse causation in the present study? Yes: those with the lowest TFA levels, i.e. those eating the fewest processed foods and whole-fat dairy, were generally much sicker: they had a higher body mass index, higher blood pressure, more smoking, and substantially more diabetes and worse glucose–insulin homeostasis. TFAs are also known to increase LDL-cholesterol and decrease HDL-cholesterol.[2] Yet, in these patients, lower TFA levels were associated with lower HDL-cholesterol and more lipid-lowering therapy—further suggesting reverse causation. This directly contrasts with more general, community-based cohorts (in which reverse causation from pre-existing disease would be less likely), in which higher erythrocyte or phospholipid TFA levels are associated with higher LDL-cholesterol and lower HDL-cholesterol,[3,4] as expected.

When substantial reverse causation may be present, fully accounting for its effects can be challenging. Thus, the second important detail is the multivariable adjustment that was—and was not—performed. Given the multiple strong associations of lower TFA levels with poor health markers in this population, the first model—only adjusted for age and gender—is not very informative, almost surely burdened by substantial confounding. The second model ('Model 2') included many relevant variables. However, it only roughly accounted for significant observed differences in metabolic dysfunction (diabetes, yes/no; hypertension, yes/no), rather than

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using more discriminative data on levels of systolic and diastolic blood pressure, haemoglobin A_{1c}, and HOMA (homeostatic model assessment) insulin resistance. Not including these produces residual confounding by these factors. Consistent with this, when the authors further adjusted for haemoglobin A_{1c}, antihypertensive medication use, and alcohol, the inverse association of C16:1n-7t with SCD was further attenuated (P for trend = 0.035; Supplementary table S9). It seems plausible that, with further adjustment for blood pressure levels and HOMA insulin resistance, this borderline association would no longer have been statistically significant.

The authors also adjusted for levels of blood cholesterol—a major pathway whereby TFAs would influence cardiovascular risk.[2] Such adjustment for potential 'mediating' variables would attenuate true associations toward the null, making it harder to detect potential harmful effects. Finally, none of the models adjusted for other dietary habits, which were not assessed in these patients. Differences in other diet behaviours across the tertiles of TFAs could appreciably alter the findings, in unpredictable directions.

Based on these challenges, i.e. the multiple (12) different TFA–endpoint pairs evaluated, the evidence suggesting reverse causation, the statistically borderline association, and absence of optimal adjustment for potential confounding variables, it is difficult to interpret the published findings. In new analyses shared with me, the authors reported that, after removing adjustment for blood lipids and lipid-lowering medication and adding self-reported exercise, systolic blood pressure, antihypertensive medication, haemoglobin A_{1c}, HOMA index, and all individual TFA species simultaneously, the inverse association between C16:1n-7t and SCD was unchanged [across tertiles, relative risk (RR) 0.60, 95% confidence interval (CI) 0.42–0.85; P for trend = 0.01], while C18:1t was now associated with higher risk of SCD (across tertiles, RR 1.29, 95% CI 0.92–1.81; P for trend = 0.02) (Clemens von Schacky personal communication, 6 October 2015). While reverse causation cannot be fully excluded, these new results provide some assurance that the modelling at least partly accounts for such confounding.

Even with its limitations, the report has interesting potential implications, when considered in the context of prior work. As pointed out by the authors, total TFA levels in this population (mean: 0.96%) were appreciably lower than in US cohorts in the 1990s (1.66%[3], 2.58%[5]). This is consistent with dietary estimates from this time period, which suggest low total TFA consumption in Germany due to very low consumption of industrial TFAs (i.e. processed foods containing partially hydrogenated oils), so that nearly all TFAs (~80%) came from natural (e.g. dairy) sources.[6]

In this light, the observed beneficial association with SCD of C16:1n-7t is interesting. C16:1n-7t, the most specific TFA marker of dairy fat, has also been associated with lower incidence of diabetes,[7,8] while other biomarkers of dairy fat (e.g. odd-chain saturated fats) are associated with lower risk of diabetes[9] and cardiovascular disease.[10] These findings, utilizing objective biomarkers, suggest that some aspect of dairy fat or dairy fat-rich foods is cardiometabolically protective. Self-reported consumption of high-fat dairy foods, such as cheese, has been shown to have beneficial or neutral associations with diabetes,[11–13] while dairy fat intake and dairy fat biomarkers correlate with improved hepatic and systemic insulin sensitivity and lower hepatic steatosis.[14,15] Because dairy fat is often consumed in hidden amounts in numerous mixed foods, dishes, and recipes, rather than just as whole foods (e.g. milk, cheese, yogurt, and butter), circulating biomarkers such as C16:1n-7t can provide more accurate assessments of total exposure to dairy fat than self-reported consumption. Furthermore, while one small study (n = 12) suggested that minor amounts of C16:1n-7t could derive from partial beta-oxidation of C18:1t,[16] large genome-wide association studies have not identified any significant genetic determinants of C16:1n-7t levels,[17] suggesting that strong endogenous influences are not present.

Together, these findings suggest that something in dairy fat (e.g. branched-chain fatty acids, medium-chain saturated fats, or C16:1n-7t itself) or some other aspect of dairy fat-rich foods (e.g. fermentation of cheese) meaningfully improves cardiometabolic health (*Table 1*). The report by Kleber and colleagues adds further supportive evidence. Dairy foods represent one of the largest categories of calories in many nations. It is astounding, and unforgiveable, that nearly all our dietary recommendations about dairy foods (including the conventional emphasis on low-fat dairy[18]) are derived from theories about isolated nutrient contents (e.g. calcium, vitamin D, and saturated fat) rather than direct empiric evidence on health effects. It is time for major funding agencies, the food industry, and foundations to support additional well-designed prospective cohort studies, especially those utilizing biomarkers (e.g. circulating fatty acids, metabolomics), and large clinical trials to determine the full effects of different dairy products on human health. Until then, the current evidence provides little support to promote low-fat dairy, and suggests that whole-fat products, in particular yogurt and cheese, may be beneficial.

Outside of natural ruminant TFAs from dairy and meats, the other major source of TFAs is processed foods containing partially hydrogenated oils. Based on consistent evidence for their harm, the US Food and Drug Administration has ruled that partially hydrogenated oils are no longer 'generally regarded as safe' (GRAS).[19] The harm of partially hydrogenated oils may not be due to the major TFA isomer, elaidic acid (C18:1n-9t), but to other adverse bioactive compounds induced by the industrial hydrogenation process.[20] Regardless of the mechanism of harm of partially hydrogenated oils, it is clear that no safe level of consumption exists. The present findings by Kleber and colleagues provide no evidence to the contrary. Nearly all the TFA exposure in this study appears to derive from dairy and other ruminant sources, not partially hydrogenated oils, providing little

Table 1 Potential pathways for cardiometabolic benefits of whole-fat dairy foods

- Benefits of specific fatty acids in dairy fat
  - Branch-chain fatty acids
  - Medium-chain saturated fats
  - Trace ruminant *trans* fats, e.g. C16:1n-7t
- Benefits of fermentation (e.g. cheese)
  - Vitamin K2
  - Other microbiome interactions
- Benefits of probiotics (e.g. in yogurt)
- Replacement of calories from unhealthful foods, especially refined complex carbohydrates, starches, and sugars
- Other, unknown trace bioactives

inference on health effects of the latter. Also, as described above, C18:1t was linked to higher risk of SCD after appropriate multivariable adjustment.

In sum, what have we learned from this study of TFAs in the Ludwigshafen Risk and Cardiovascular Health Study? Understand, and appreciate, the crucial methodological details of any research investigation. Continue to consider and evaluate the potential benefits of dairy fat for cardiometabolic health. And, don't let partially hydrogenated oils off the hook.

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