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Concerns about the use of 15:0, 17:0, and *trans*-16:1n–7 as biomarkers of dairy fat intake in recent observational studies that suggest beneficial effects of dairy food on incidence of diabetes and stroke

Dear Editor:

It is with interest that I read the articles by Santaren et al. (1) and Yakoob et al. (2) about the relation of circulating concentrations of pentadecanoic acid (15:0), heptadecanoic acid (17:0), and *trans*-palmitoleic acid (*trans*-16:1n–7) with the incidence of diabetes and stroke. Santaren et al. (1) reported that serum concentrations of pentadecanoic acid are associated with insulin sensitivity and β cell function, as well as a 27% decreased risk of type 2 diabetes. Yakoob et al. (2) reported no significant associations of total plasma or red blood cell pentadecanoic acid, heptadecanoic acid, and *trans*-palmitoleic acid with risk of stroke. Because several previous studies implicated pentadecanoic acid, heptadecanoic acid, and *trans*-palmitoleic acid in serum, plasma, red blood cells, and adipose tissue as valid biomarkers for dairy intake (3–9), Santaren et al. (1) suggested that their findings may contribute to future recommendations regarding the benefits of dairy products on type 2 diabetes, and Yakoob et al. (2) concluded that circulating biomarkers of dairy fat are not significantly associated with stroke. A commentary written by Arne Astrup in the same issue of the Journal (10) stated that "there is no evidence left to support the existing public health advice to limit consumption of dairy to prevent CVD [cardiovascular disease] and type 2 diabetes."

I am concerned with the use of pentadecanoic acid, heptadecanoic acid, and *trans*-palmitoleic acid as biomarkers of dairy fat intake. It is true that these are present in dairy fat, although at very low amounts (pentadecanoic acid at 1.0%, heptadecanoic acid at 0.6%, and *trans*-palmitoleic acid at 0.3%) (11). These 3 fatty acids, however, are not limited to dairy fat. In particular, fat from beef, veal, lamb, and mutton also contains all of these fatty acids at amounts similar to those found in dairy fat (12, 13). The presence of pentadecanoic acid and heptadecanoic acid, at amounts comparable to dairy fat, has also been reported in many other common dietary fats and foods, including chicken and lard (13), marine and freshwater fish (14), marine oils (15), some vegetables (cabbage and cucumber) (16), and seaweeds (17). Several common vegetable oils also contain small amounts of heptadecanoic acid (18). Rapeseed (canola) oil contains both pentadecanoic acid and heptadecanoic acid (19). These data suggest that pentadecanoic acid and heptadecanoic acid are widely distributed in nature and present in many common foods, including dietary fats, albeit in small amounts. Unfortunately, this information is not commonly available because many scientific publications on fatty acid composition of dietary fats and foods focus only on the major and nutritionally important fatty acids and do not show data for pentadecanoic acid and heptadecanoic acid because these fatty acids are minor components and have no known nutritional or biological significance.

Another factor that needs to be considered in choosing a fatty acid as a biomarker is that it should not be endogenously synthesized. Many previous studies made the assumption that circulating *trans*-palmitoleic acid is solely derived from the consumption of dairy fat (9). However, it was recently found that circulating *trans*-palmitoleic acid is not exclusively diet derived but may also be endogenously produced by the partial β-oxidation of dietary vaccenic acid (*trans*-18:1n–7) (20). Vaccenic acid is the major *trans* fatty acid isomer in dairy fats but is also present in partially hydrogenated oils. In Canadian dairy products, vaccenic acid accounts for 22–43% of total *trans*-18:1 isomers (21). Partially hydrogenated vegetable oils also contain considerable amounts of vaccenic acid: proportions ranging from 15% to 24% of total *trans*-18:1 isomers have been found in partially hydrogenated canola and soybean oil samples (22). Trace amounts of pentadecanoic acid and heptadecanoic acid are synthesized in leaves (23) and are present in common vegetables as noted above (16). It is not known whether animals and humans have the capability to synthesize pentadecanoic acid and heptadecanoic acid, but this should not be ruled out until it has been examined.

A further concern is the uncertainty of correct identification of pentadecanoic acid, heptadecanoic acid, and *trans*-palmitoleic acid in the gas chromatography (GC) analysis of fatty acid mixtures. These fatty acids are always found in very low concentrations in blood samples and dietary fats and very often coelute with other fatty acids in GC analysis. For example, pentadecanoic acid overlaps with 9-*cis*-tetradecenoic acid (9c-14:1), *trans*-palmitoleic acid overlaps with *iso*-heptadecanoic acid and 3-*trans*-hexadecenoic acid (3t-16:1; a common *trans* fatty acid in plants), and heptadecanoic acid elutes close to 11-*cis*-hexadecenoic acid (11c-16:1) and 13-*cis*-hexadecenoic acid (13c-16:1) (24). Thus, if the GC conditions are not optimized, it is possible that the concentrations of pentadecanoic acid, *trans*-palmitoleic acid, and heptadecanoic acid may be exaggerated due to inclusion of the overlapping components. Because the fatty acid analytic methods used were not described by Santaren et al. (1) and Yakoob et al. (2), it is not known whether they have encountered any such fatty acid analysis problems.

Considering these possible uncertainties of the dietary origin and the analysis of pentadecanoic acid, heptadecanoic acid, and *trans*-palmitoleic acid, we should be cautious in making conclusions about the role of dairy fats in diabetes and stroke.

The author had no financial or conflicts of interest to declare.

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Note: Yakoob et al. chose not to submit a reply.

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Reply to M Lankinen and U Schwab and WMN Ratnayake

Dear Editor:

We thank Lankinen, Schwab, and Ratnayake for their interest in our article. Their letters express concern with regard to the use of the fatty acid pentadecanoic acid (15:0) as a valid biomarker for dairy intake, because low amounts of this fatty acid are also present in other foods such as fish, beef, veal, and lamb. Although doubts regarding the overall validity of pentadecanoic acid as a biomarker for dairy intake have been previously raised by other researchers (1, 2), the majority of observational studies have documented a significant correlation of pentadecanoic acid with dairy intake (3–5). In line with these previous reports, we found that serum pentadecanoic acid was correlated with total dairy intake ($r = 0.20$, $P < 0.0001$) as well as with total milk ($r = 0.13$, $P = 0.0006$) and total cheese ($r = 0.16$, $P < 0.0001$) intakes in the Insulin Resistance Atherosclerosis Study (IRAS) cohort (6). In contrast, we did not find pentadecanoic acid to be correlated with total fish intake ($r = -0.04$, $P = 0.31$) or oily fish intake ($r = -0.02$, $P = 0.68$), nor was pentadecanoic acid correlated with serum EPA ($r = 0.05$, $P = 0.19$) or serum DHA ($r = -0.05$, $P = 0.24$) in IRAS participants. As we previously reported, fish intake in this cohort is very low, with a median consumption of 1.1 servings/wk; and oily fish, specifically, was consumed at only 0.38 servings/wk on average (7), indicating that fish intake in this cohort was unlikely to be a major contributor to pentadecanoic acid in serum. Nevertheless, we agree that it is possible that in other populations with higher intakes of fish, pentadecanoic acid may be associated with the consumption of foods other than dairy products. Furthermore, we did not find pentadecanoic acid to be correlated with intakes of beef ($r = 0.07$, $P = 0.06$), cabbage ($r = -0.01$, $P = 0.80$), or chicken ($r = -0.04$, $P = 0.33$). The IRAS food-frequency questionnaire did not include individual questions regarding veal, lamb, mutton, lard, cucumber, or seaweed intake. Despite accumulating evidence suggesting that pentadecanoic acid may be a reliable biomarker for dairy intake, the true relation between dairy intake and circulating pentadecanoic acid can only ascertained by using controlled feeding studies in which the quantity and quality of the dairy products are known (2).

Ratnayake also raised a technical concern regarding the identification of pentadecanoic acid peaks by using gas chromatography (GC) analysis. We agree that accurate peak identification of fatty acids using GC is a concern due to the potential for overlapping peaks of fatty acids that coelute. To overcome this issue in our study, peaks identified by using GC-FID (flame-ionization detector), as previously described (8), were confirmed with the use of mass spectrometry, as well as various internal quality assurance and quality-control procedures, including the following: testing new methods against a library of compounds to ensure chromatographic separation, comparing results against the historical average and range of each metabolite, manual peak reviews, and testing across many types of columns.

In summary, although the existing literature is not in full agreement regarding the utility of pentadecanoic acid as a marker of dairy intake (9), the majority of existing studies support it as a biomarker for dairy consumption. Importantly, correlations in our cohort suggest that this is the case for IRAS participants as well.

The authors had no conflicts of interest.

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