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## Reply to H Pareja-Galeano et al.

Dear Editor:

We appreciate the interest from Pareja-Galeano et al. in our study and acknowledge that they considered our study well powered and clinically relevant. Similar to others (1), our results suggested that the greatest reduction in the hazard of mortality was found between the “inactive” and the “moderately inactive” group. This reduction was observed across general and abdominal obesity groups, suggesting health benefits from increasing physical activity (PA) regardless of adiposity. On the basis of our validation study (2), we estimated that the difference between the “inactive” and “moderately inactive” group was equivalent to ~20 min of brisk walking each day. This equates to 140 min/wk, which is almost in line with current PA recommendations for public health (3–5).

We do not dispute the health benefits associated with higher levels of PA, which Pareja-Galeano et al. have highlighted, but the key message from our study was that there would be substantial public health benefits from people in the inactive group engaging in even a small amount of PA each day.

None of the authors had a conflict of interest to declare.

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## Biomarkers of dairy fat

Dear Editor:

There were 2 interesting articles in a recent issue of the Journal in which odd-chain fatty acids (15:0 and 17:0) were used as circulating biomarkers of dairy fat (1, 2). In one of the studies (1), serum pentadecanoic acid (15:0) was shown to be inversely associated with incident type 2 diabetes, and in the other study (2) the association of pentadecanoic acid and heptadecanoic acid (17:0) with the risk of incident stroke was studied and no significant association was found. These odd-chain fatty acids are considered to be validated biomarkers for dairy fat and they correlated with dairy consumption in many studies (1–4). However, the association between the intake of dairy fat and the relative serum content of heptadecanoic acid has not been clear in all studies (5, 6). In a large cohort study [EPIC (European Prospective Investigation into Cancer and Nutrition)], there was a strong positive ecologic correlation ( $r = 0.8$ ,  $P \leq 0.01$ ) between the total intake of fish and plasma concentration of heptadecanoic acid, whereas there was no correlation between heptadecanoic acid or pentadecanoic acid and dairy products (6). Accordingly, we have seen in our studies (MA Lankinen et al., 2015) a positive correlation between pentadecanoic and heptadecanoic acids with DHA in plasma phospholipids. The fatty acid heptadecanoic acid is present in the fat of fish (0.31–2.0% depending on fish species) (7, 8). Salmon contains ~40 mg heptadecanoic acid and 20 mg pentadecanoic acid per 100 g (9). Therefore, we are a bit concerned if these odd-chain fatty acids are considered to be a valid biomarker for dairy fat intake in populations who consume considerable amounts of fish. In populations with a high consumption of dairy fat and a low consumption of fish, odd-chain fatty acids are probably valid biomarkers for dairy fat intake. In populations who consume fish, the presence of odd-chain fatty acids in fish should be taken into account to avoid misleading conclusions.

Neither of the authors had a conflict of interest.

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

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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  $\beta$  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  $\beta$ -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.

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

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