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Milk fat biomarkers and cardiometabolic disease

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Purpose of review
Dairy is a major food group with potential impact on cardiometabolic health. Self-reported dairy intake has limitations that can partly be avoided by using biomarkers. This review aims to summarize the evidence of odd-chain saturated fatty acids (OCFAs), that is, pentadecanoic acid (C15 : 0) and heptadecanoic acid (17 : 0), as biomarkers of dairy fat intake. In addition, the associations of OCFA biomarkers with cardiometabolic disease will be overviewed.

Recent findings
Adipose tissue 15 : 0 is the preferred biomarker but also circulating 15 : 0, and to a weaker extent 17 : 0, reflects both habitual and changes in dairy intake. Whereas results from studies assessing cardiovascular outcomes are inconsistent, OCFA biomarkers are overall associated with lower diabetes risk. Residual confounding should however be considered until interventional data and mechanisms are available. Although OCFA biomarkers mainly reflect dairy fat intake, recently proposed endogenous synthesis and metabolism do motivate further research.

Summary
Taking into account the study population diet and limitations of OCFA biomarkers, both adipose and circulating levels of 15 : 0, in particular, are useful for estimating total dairy fat intake. OCFA biomarkers are overall not linked to cardiovascular disease risk, but a possible beneficial role of dairy foods in diabetes prevention warrant further study.

Keywords
biomarkers, dairy food intake, milk fat, odd-chain saturated fatty acids, pentadecanoic acid

INTRODUCTION

Dairy food is a major source of energy and nutrients in many countries. To assess the impact of dairy food on preventable diseases such as type 2 diabetes (T2D) and cardiovascular disease (CVD), objective measures of intake are critical. Biomarkers of dairy food intake can add to self-reported data by e.g. avoiding memory bias, and under- and over-reporting, and food database errors [1]. Odd-chain saturated fatty acids (OCFAs) including pentadecanoic acid (C15 : 0) and heptadecanoic acid (17 : 0) are characteristic of dairy fat because they are synthesized through microbial fermentation in the ruminant gut from where they are absorbed and subsequently excreted in the milk [2]. The mean concentrations of 15 : 0 and 17 : 0 in milk are ~1.2 and 0.54% of total fatty acids, respectively [3,4]. As further discussed, these fatty acids are also present in beef and lamb, and fish, but at lower concentrations. In humans, OCFAs can be measured in adipose tissue [5,6] and blood constituents (e.g., cholesterol esters [6]) and are commonly used as biomarkers of dairy fat intake.

The aim of this review was to provide a scientific update on the use of OCFAs as biomarkers of dairy fat intake. This review focus on the most commonly used specific milk fat biomarkers in the literature, 15 : 0 and 17 : 0, although other biomarkers (e.g., 14 : 0 and trans16 : 1n-7) of dairy fat intake exist [6,7]. In addition, we aimed to overview observational studies that have examined the associations of circulating and tissue 15 : 0 and 17 : 0 with risk of CVD-related outcomes and T2D.

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KEY POINTS

- OCFAs, especially 15 : 0, in adipose tissue and circulating lipids are valid biomarkers of dairy fat intake in many populations as shown in several observational and randomized controlled studies.
- OCFAs are synthesized in ruminants although recent data indicate that some endogenous production in humans may occur, but the latter requires further research.
- Limitations of OCFA biomarkers include inability to discriminate between different dairy foods and impact of nondairy determinants (e.g., alternative dietary sources and endogenous metabolism).
- Associations of OCFAs in circulating lipids with cardiovascular outcomes have been inconsistent, whereas OCFAs are generally associated with lower diabetes risk.

BIOCHEMICAL ASSESSMENT OF 15 : 0 AND 17 : 0

From fasting blood samples (often stored in −70 to −80°C), 15 : 0 and 17 : 0 can be analyzed in most blood compartments and fractions by using thin-layer chromatography, as described previously by Wolk *et al.* [6]. Alternatively, they can be measured in adipose tissue triglycerides [6]. The fatty acids in the serum cholesterol esters and phospholipids as well as in adipose tissue triglycerides are commonly separated by gas-liquid chromatography after transmethylation, as described earlier [8]. The concentrations of the fatty acids are usually presented as percentages of the sum of all fatty acids analyzed, as weight% or mol% [9]. The within-analysis CV of 15 : 0 among studies has usually been 10% or less, which must be regarded as clearly acceptable. The CV may differ somewhat between lipid compartments, for example, it was shown to be ∼13% in cholesterol esters and ∼9% in phospholipids in one study [6].

BIOMARKERS OF DAIRY INTAKE: OBSERVATIONAL STUDIES

It was shown in Swedish women that adipose tissue OCFAs are valid biomarkers of long-term dairy fat intake [5]. The correlations were weaker when food intake was assessed by food frequency questionnaire (FFQ) compared with repeated food records [5]. Notably, dairy intake was more strongly correlated with adipose 15 : 0 than 17 : 0 [5,6,10,11], showing a clear dose–response relationships between intake and 15 : 0 [11]. In adipose tissue, the mean or median proportions of 15 : 0 range from 0.19 (Costa Rica) to 0.39 (Norway) and for 17 : 0 from 0.21 to 0.34 [5,6,10,12–14]. It was also elegantly shown by Wolk *et al.* [6] that 15 : 0 and 17 : 0 in serum and in adipose tissue among men were closely associated with dairy fat intake assessed by 24-h recalls. If adipose tissue is not available, 15 : 0 in serum cholesterol esters may be the best choice, because it has shown strong correlations ($r = 0.58$) with adipose tissue [10]. However, correlation coefficients between total dairy intake and biomarker 15 : 0 were comparable in phospholipids ($r = 0.53$) and cholesterol esters ($r = 0.47$) when assessed in the same population [6]. In addition, there is a strong correlation between 15 : 0 in cholesterol esters and phospholipids ($r = 0.67$) [15], and thus either of these compartments seems appropriate. When comparing 15 : 0 in total plasma lipids with that in erythrocytes, the former compartment was more strongly correlated to dairy fat intake assessed by FFQ [16,17].

Overall, the proportions of both 15 : 0 and 17 : 0 in human plasma, serum and erythrocytes correlate with intake of dairy fat [6,9,10,13,15,17–22], but in some populations (erythrocytes used) only weakly or not at all [17]. Even in young children, 15 : 0 in plasma phospholipids was recently shown to reflect dairy fat and dairy food intake [23▪], confirming a previous randomized study (intervention replaced regular dairy with low-fat dairy) in children where serum 15 : 0 (compared with 14 : 0 and 17 : 0) best reflected baseline total dairy fat intake as well as detecting changes in dairy fat intake [24].

However, OCFAs may not be good biomarkers of dairy fat intake when assessed in populations with low intake of dairy intake and concomitantly higher intake of beef or fish [5]. Thus, the background diet of the study population needs to be considered before interpreting the results from OCFA biomarkers.

BIOMARKERS OF DAIRY INTAKE: INTERVENTION STUDIES

Although the fairly strong correlations between biomarker 15 : 0 and dairy intake suggest the use of OCFAs as biomarkers of habitual dairy fat intake in various populations, it is perhaps even more important to also note that a number of randomized controlled studies have demonstrated that a change in dairy food intake is reflected by changes in 15 : 0 and 17 : 0, in various circulating and tissue lipid fractions [25–29]. Thus, such studies suggest a direct link between changes in dairy fat intake and change in OCFAs, also during weight-stable conditions. These strictly controlled studies have either provided all or some food to the participants, which presumably improve compliance and thus simplifies the interpretation of the data. For example, when butter

and other dairy products are iso-calorically replaced with vegetable fats, there is a reduction of 15 : 0 and 17 : 0 in circulating lipids [25–29]. In addition, in mixed diets, which either increase or decrease of dairy fats, such intake variations are reflected by changes in circulating OCFAs [30–32]. Although these results support the use of 15 : 0 as dairy fat biomarker, the precise dose–response association between dairy fat intake and circulating 15 : 0 can only be obtained by strictly controlled feeding studies in which the quantity and quality of the dairy products are monitored and known [33].

NONDAIRY SOURCES OF 15 : 0 AND 17 : 0

In addition to dairy and ruminant meats, fish also contains 15 : 0 and 17 : 0 [34–37,38▪]. The proportions are clearly higher for 17 : 0 than 15 : 0 and varies dependent on fish type; the content range of 17 : 0 is ~0.30 to 2% [39]. In a recent study that analyzed 27 freshwater fish species captured in the northeastern USA, mean content of 17 : 0 was 0.6% [38▪]. As the absolute intake of fish is overall considerably lower than total dairy intake in most Western populations, the contribution of 15 : 0 through fish is likely very limited. However, 17 : 0 may also reflect high fish intake in some populations including the USA [38▪], and especially in those where dairy intake is moderate or low. Some concern has recently been raised about the use of OCFA biomarkers of dairy fat intake in populations with high intake of fish [40]. Although correlations between plasma OCFAs and very long-chain n-3 fatty acids observed in some populations [15], the recent large EPIC-Interact case-cohort study found no associations between plasma OCFAs and fish intake, either lean or fatty fish [41]. In at least most Western populations, the levels of OCFAs in blood components and adipose tissue seemingly mainly reflect dairy fat intake, rather than fish or beef. For example, the significant associations between adipose tissue proportions of 15 : 0 and 17 : 0 and dairy intake were not noticeably affected by adjustment for either fish intake or meat intake in a population of Swedish men [19]. Importantly, randomized trials in Nordic populations (where diets are usually high in both fish and dairy) have suggested negligible effects of fish consumption on plasma OCFAs. For example, interventional prudent diets high in fish and low in high-fat dairy, cause significant relative reductions of plasma 15 : 0 and concurrent increases of EPA and DHA [30,31]. Overall 15 : 0 seems to be a solid dairy fat biomarker despite its content in fish, especially in populations with moderate and high dairy intake.

ENDOGENOUS SYNTHESIS OF ODD-CHAIN SATURATED FATTY ACIDS

Although OCFAs clearly reflect habitual dairy food intake, as well as capturing changes (both increases and decreases) caused by changes in dairy fat intake, emerging data indicate that 15 : 0 and 17 : 0 can also be endogenously synthesized in humans [42,43▪]. The potential evidence and hypotheses behind endogenous formation and metabolism of OCFAs are out of the scope of this review and have recently been described in detail elsewhere [42,43▪]. In brief, observations in vegans indicate plasma lipid levels of 15 : 0 and 17 : 0 comparable to those of dairy consumers [43▪,44], and 17 : 0 occurs in higher concentrations than 15 : 0 in human plasma despite higher intake of the latter [42]. This suggests that some metabolic regulation may occur, and that nondairy consumer could potentially have enhanced endogenous synthesis or metabolism of OCFAs. For example, it is believed that α-oxidation is one possible mechanism behind an endogenous production of OCFAs [42]. Indeed, α-oxidation may not only occur on branched chain fatty acids (e.g. phytanic acid) [45], but also on straight chain fatty acids to generate OCFAs [42]. In this context it can be noted that the branched-chain fatty acid phytanic acid, synthesized in ruminants from chlorophyll, has been correlated to dairy fat intake [46]. Taken together, one cannot exclude that circulating OCFAs, at least to a minor extent, are influenced by OCFAs' metabolism [42,43▪]. Such influence, however, needs further investigation, and if evident, it will probably not have any major impact on the performance of OCFAs as valid biomarkers of dairy fat intake, given the correlations between intake and biomarker observed in diverse populations and randomized trials.

DAIRY FAT BIOMARKERS AND CARDIOMETABOLIC DISEASE

Several observational studies have been published on 15 : 0 and 17 : 0 with regard to risk of CVD or related health outcomes. The sum of 15 : 0 and 17 : 0 in phospholipids was associated with lower risk of incident coronary heart disease (CHD) in a case–control study nested in the British cohort EPIC-Norfolk [47]. Similarly, plasma phospholipid 15 : 0 was inversely associated with incident CVD and CHD in the Multi-ethnic Study on Atherosclerosis (MESA) [48], while both 15 : 0 and 17 : 0 in plasma phospholipids (but not in cholesterol esters) were associated with lower risk of heart failure in the Atherosclerosis Risk in Communities Study (ARIC) [49]. Further, the sum of 15 : 0 and 17 : 0 in phospholipids was associated with lower risk of myocardial infarction (especially among women [50])

in two Swedish nested case–control studies [50,51]. In contrast, plasma (but not erythrocyte) 15 : 0 was associated with higher risk of ischemic heart disease in a nested case–control study of the Nurses' Health Study (NHS) [16] and in the US Women Health Initiative Observational Study, no association of plasma phospholipid 15 : 0 with CHD risk was evident [52]. Another proposed biomarker of dairy fat consumption, trans-palmitoleic acid (trans16 : 1n-7) measured in erythrocytes, was linked to lower risk of cardiovascular mortality in a German clinical cohort (the Ludwigshafen Risk and Cardiovascular Health Study) [53], but plasma phospholipid trans16 : 1n-7 was not associated with incident CVD or CHD in MESA [48].

In regard to stroke risk, 17 : 0 and the sum of 15 : 0 and 17 : 0 in plasma phospholipids were associated with lower stroke risk in a case–control study nested in two Swedish cohorts, Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) and Västerbotten Intervention Programme (VIP) [54]. In contrast, plasma and erythrocyte biomarkers of dairy fat intake were not associated with incident stroke in NHS and the Health Professional Follow-up Study (HPFS) [17]. Likewise, 15 : 0 in plasma cholesterol esters and phospholipids was not associated with risk of ischemic stroke in ARIC [55].

So far, only one cohort study has evaluated associations of OCFAs in adipose tissue with risk of a CVD-related outcome; in a Swedish cohort study on elderly men, 15 : 0 or 17 : 0 in adipose tissue were not associated with cardiovascular death [56▪]. Two studies have evaluated OCFAs measured in fat biopsies collected after a first myocardial infarction, but the results were inconsistent; 15 : 0 in adipose tissue was inversely associated with myocardial infarction among Norwegians [10] but not in Costa Ricans [11].

Findings from observational studies assessing associations of OCFA biomarkers with CVD-related outcomes are inconclusive which partly may be due to methodological differences between studies (e.g., study design and populations, outcome definitions, statistical models, biomarker compartment, and underlying diets). The inconsistencies between studies may also reflect that CVD risks associated with substitution of dairy fats with other dietary fats can differ substantially depending on the type of fat or food that replace the dairy fat; in NHS and HPFS, isocaloric replacement of dairy fat with vegetable fat was associated with lower risk of CVD whereas similar substitution of dairy fat with animal fat was linked with higher CVD risk [57]. The lack of clear relationship of OCFA biomarkers with CVD is in line with recent meta-analyses based on self-reported data, that show little or weak evidence for inverse dose–response relationships between total dairy fat and CHD, CVD or stroke in fully adjusted models [58,59▪].

Associations of circulating OCFAs with incident T2D have been assessed in several studies. In the large prospective case-cohort study EPIC-Interact ($n = 12\,132$ cases), plasma phospholipid 15 : 0 and 17 : 0 were inversely associated with T2D risk [41]. Looking at individual studies of different EPIC centers, similar results were obtained in the Swedish VIP study [60], but not in the German EPIC-Potsdam [61] or in a small nested case-control study within the British EPIC-Norfolk [22]. In the Melbourne Consecutive Cohort Study, 15 : 0 in plasma phospholipids was associated with lower risk of T2D [62]. OCFAs in plasma phospholipids were not associated with T2D risk in the two American prospective cohorts Cardiovascular Health Study (CHS) [7] or MESA [63]. However, trans16 : 1n-7 was linked to lower risk of incident diabetes in both these cohorts [7,63]. In a pooled analysis of NHS and HPFS, plasma and erythrocyte levels of trans16 : 1n7 and 17 : 0 were associated with lower diabetes risk, while inverse associations of 15 : 0 were only observed in plasma [64▪]. Serum levels of 15 : 0 (but not 16 : 1n7t) were inversely associated with incident diabetes in the American cohort study Insulin Resistance and Atherosclerosis Study [65].

These results accord with cohort studies using self-reported intake data, showing weak but significant inverse associations between total dairy intake and T2D [66▪]. Importantly to note, however, is that reported intake of particularly yoghurt shows strong inverse association [66▪]. Yoghurt consumption (low-fat yoghurt, <3.9%) was also inversely associated with diabetes incidence in the EPIC-Norfolk study [67]. Furthermore, previous meta-analysis showed that low-fat dairy in particular was inversely linked with diabetes risk [68]. Thus a low-fat dairy food pattern may not be captured by OCFA biomarkers which presumably more reflect high-fat dairy. To date, there is no evidence for a direct role of OCFAs in improving glucose metabolism. Cause and effect needs to be confirmed by randomized trials, and the mechanisms behind a possible diabetes preventive effect of dairy food is unknown [69]. Several components in dairy foods may however be of interest e.g. short-chain fatty acids, fermentation (probiotic effect), high-quality proteins, calcium, and specific phospholipids.

BIOMARKERS OF HEALTHY LIFESTYLE?

It is possible that the inverse relationships between OCFAs and cardiometabolic disease, as

observed in some studies, are explained by residual confounding of various lifestyle factors. Notably, alcohol intake has been strongly inversely related to both serum phospholipid and adipose tissue OCFAs in several populations [19,70], whereas intakes of fruit and vegetables [41] and fiber [19] have been correlated with OCFA biomarkers. Physical active vs. inactive Swedish men had ~5% higher proportions of 15:0 and 17:0 in phospholipids and adipose tissue, although not statistically significant [19]. Adjustment for physical activity did however not affect the association between serum 15:0 and dairy intake, or with clinical characteristics [15]. In contrast, intake of various foods such as meat, vegetables, and beer, did influence an inverse association between serum 15:0 and various metabolic risk factors in men, suggesting potential confounding [15]. In support of this, the large pan-European EPIC-Interact study showed that apart from a strong correlation between OCFAs in plasma phospholipids and dairy food intake, OCFAs were simultaneously correlated with food groups that are typically included in prudent dietary patterns, for example fruits, vegetable and nuts, but inversely to red and processed meat [41].

CONCLUSION

Although not entirely specific for dairy foods, OCFAs and 15:0 in particular, are useful biomarkers of total dairy fat intake in many populations, for example, in Europe and the USA. In addition, they reflect changes in dairy fat intake and are thus important for monitoring dietary compliance in intervention studies. However, considering possible uncertainties of the dietary origin, endogenous metabolism, and intake of dairy in the population under study, caution is warranted in the interpretation of links between these biomarkers and disease risk. Regarding reported inverse relationships between OCFAs and diabetes, but not CVD, confounding lifestyle factors must be strongly considered until interventional data are available.

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Conflicts of interest

There are no conflicts of interest.

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