

Exploring the association of dairy product intake with the fatty acids C15:0 and C17:0 measured from dried blood spots in a multi-population cohort: findings from the Food4Me study

Article

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Title

Exploring the association of dairy product intake with the fatty acids C15:0 and C17:0 measured from dried blood spots in a multi-population cohort: findings from the Food4Me study

Author Names

Viviana Albani¹, Carlos Celis-Morales¹, Cyril F M Marsaux², Hannah Forster³, Clare B O'Donovan³, Clara Woolhead³, Anna L Macready⁴, Rosalind Fallaize⁴, Santiago Navas-Carretero⁵, Rodrigo San-Cristobal⁵, Silvia Kolossa⁶, Christina Mavrogianni⁷, Christina P Lambrinou⁷, George Moschonis⁷, Magdalena Godlewska⁸, Agnieszka Surwiłło⁸, Thomas E. Gundersen⁹, Siv E Kaland⁹, Yannis Manios⁷, Iwona Traczyk⁸, Christian A Drevon¹⁰, Eileen R Gibney³, Marianne C Walsh³, J Alfredo Martinez⁵, Wim H M Saris³, Hannelore Daniel⁶, Julie A Lovegrove⁴, Michael J Gibney³, Ashley J. Adamson¹, John C Mathers¹, Lorraine Brennan^{1,3} on behalf of the Food4Me Study.

Author Affiliation

¹Human Nutrition Research Centre and Institute for Health and Society, Newcastle University, Newcastle upon Tyne, UK

²Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre + (MUMC+), Maastricht, The Netherlands

³Institute of Food & Health, University College Dublin (UCD), Belfield, Dublin, Ireland

⁴Hugh Sinclair Unit of Human Nutrition and Institute for Cardiovascular and Metabolic Research, University of Reading, Reading, UK.

⁵Department of Nutrition, Food Science and Physiology, University of Navarra; CIBER Fisiopatología Obesidad y Nutrición (CIBERObn), Instituto de Salud Carlos III, Spain (SN-C & JAM)

⁶ZIEL Research Center of Nutrition and Food Sciences, Biochemistry Unit, Technische Universität München, Germany.

⁷Department of Nutrition and Dietetics, Harokopio University, Athens, Greece.

⁸National Food & Nutrition Institute (IZZ), Poland.

⁹Vitas AS, Olso Innovation Park, Gaustadalleen 21, N-0349, Oslo, Norway.

¹⁰Department of Nutrition, Institute of Basic Medical Sciences, Faculty of Medicine, University of Oslo, Oslo, Norway.

Corresponding Author

Prof. Lorraine Brennan

Human Nutrition Research Centre and Institute for Health and Society, Newcastle University, Newcastle upon Tyne, UK and School of Agriculture & Food Science, University College Dublin, Belfield, Dublin 4

Telephone: 00 353 1 7162811

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Email: lorraine.brennan@ucd.ie

Abbreviations List

DBS	dried blood spots
FFQ	Food Frequency Questionnaire
OLS	ordinary least squares
ROC	receiver operating characteristic
AUC	area under the curve
iqr	interquartile range
g/day	grams per day

Keywords

Dairy intake, biomarkers, pentadecanoic acid, heptadecanoic acid, dried blood spots

Abstract

Scope

The use of biomarkers in the objective assessment of dietary intake is a high priority in nutrition research. The aim of this study was to examine pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) as biomarkers of dairy foods intake.

Methods and results

The data used in the present study were obtained as part of the Food4me Study. Estimates of C15:0 and C17:0 from dried blood spots and intakes of dairy from an FFQ were obtained from participants (n=1,180) across 7 countries. Regression analyses were used to explore associations of biomarkers with dairy intake levels and receiver operating characteristic (ROC) analyses were used to evaluate the fatty acids. Significant positive associations were found between C15:0 and total intakes of high-fat dairy products. C15:0 showed good ability to distinguish between low and high consumers of high-fat dairy products.

Conclusion

C15:0 can be used as a biomarker of high-fat dairy intake and of specific high-fat dairy products. Both C15:0 and C17:0 performed poorly for total dairy intake highlighting the need for caution when using these in epidemiological studies.

Graphical abstract:



The study explored the association between C15:0 and C17:0 levels in dried-blood spots and intake of dairy, including high-fat and low-fat dairy products.

Introduction

Adequate measurement of dietary intake is crucial to health-related research as much of the public health burden in both industrialised and developing societies is due to chronic diseases associated with lifestyle factors, notably poor diet quality and excess energy intake [1, 2]. However, research on dietary exposures relies on self-reported methods, such as food frequency questionnaires, food diaries and 24-hour recalls, that are susceptible to random and systematic measurement errors, including recall bias and energy under-reporting [3]. Mis-classification of individuals' dietary intakes resulting from these errors may contribute to inconsistencies in diet-disease outcomes in published nutritional epidemiological studies [4]. Given these limitations, interest has grown in the identification and use of biomarkers as more objective assessment methods of food intake [2]. The development, validation and application of novel biomarkers of food intake are particularly pertinent for those foods where associations with important health outcomes are poorly understood or subject to controversy. One such example is dairy products for which improved measurement of

intake would help to clarify inconsistent findings about the relationships between intake of dairy foods and cardiovascular health [5] and to strengthen the evidence for links between dairy products and the risk of metabolic syndrome and type 2 diabetes [6, 7].

The odd-chain length saturated fatty acids pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) have been proposed as potential biomarkers of dairy intake. The available evidence from observational studies suggests that C15:0 may be a good biomarker of long-term dairy fat intake [8, 10-12]. Relationships between dairy fat intake and C15:0 levels in adipose tissue and blood lipid fractions have been similar with correlations ranging from 0.74 to 0.45 for adipose tissue samples and from 0.50 to 0.45 in serum or plasma lipid fractions [12, 13]. Levels of C15:0 in plasma and adipose tissue are also significantly associated with reported consumption of dairy and dairy products, such as whole milk, butter and ice cream; with correlations ranging from 0.28 to 0.22 for total dairy products between 0.21 and 0.13 for butter, and between 0.27 and 0.17 for cheese [6, 11, 13-15]. Levels of C17:0 in adipose tissue, serum cholesterol esters and phospholipids have also been reported to be associated with dairy fat intake, but less so with dairy product intake. Reported correlations between dairy fat intake and C17:0 in adipose tissue, serum cholesterol esters and phospholipids are in the range of 0.30 to 0.16 [12]; between 0.13 and 0.11 for dairy fat and plasma lipid fractions [15]; and between 0.24 and 0.16 for dairy product intake and adipose tissue samples [10, 16]. More recently, a randomized controlled trial conducted with a free-living Canadian population found a significant increase in plasma levels of C15:0 and of C17:0 following a 4-week intervention with 3 servings of dairy products daily (low fat milk -1% fat, low fat yoghurt -1.5% fat, and cheese - 34% fat) [17].

Levels of C15:0 and C17:0 in plasma phospholipids, cholesteryl esters, total plasma lipids, erythrocytes, triacylglycerol, and subcutaneous adipose tissue from the buttock or abdomen have been explored as biomarkers of dairy foods intake [11-13, 15, 19]. In addition, Baylin et al. [20] found that whole blood levels of C15:0 and C17:0 reflected dietary intake of these fatty acids. Given that

the collection of whole blood samples as dried blood spots (DBS) is a technically relatively easy and cost efficient method, there is interest in assessing their use for the measurement of dietary intake biomarkers, including biomarkers of dairy products intake.

The objective of this study is to examine blood levels of C15:0 and C17:0 measured in DBS as potential biomarkers of dairy product intake in a large multicultural cohort of European adults.

Materials and methods

Study sample

Data for the study were sourced from the Food4Me Proof of Principle study, a randomized controlled trial of personalised nutrition among European adults. The study ran from August 2012 to February 2014 and included participants from Germany, Greece, Ireland, the Netherlands, Poland, Spain and the UK. Participants were recruited through local advertising directing them to the project website (<http://www.food4me.org/>). Local recruitment sites were University College Dublin (Ireland); Maastricht University (the Netherlands); University of Navarra (Spain); Harokopio University (Greece); University of Reading (United Kingdom; UK); National Food and Nutrition Institute (Poland); Technische Universität München (Germany). A screening questionnaire excluded interested individuals who reported being pregnant, having food intolerances or allergies, having chronic disease (e.g. diabetes), or being on a therapeutic diet. A full description of the study protocol and screened participants has been previously published [21, 22]. The Research Ethics Committees at each University or Research Centre delivering the intervention granted ethical approval for the study. The Food4Me trial was additionally registered as a Randomized Clinical Trial (NCT01530139) at Clinicaltrials.gov. All participants provided informed consent.

Of 1607 individuals randomised to the intervention, 1,471 observations on blood fatty acid levels and dairy foods intake at baseline ("Month 0") were initially included in this study. After exclusion of participants (n=262) with under- or over-reporting of energy intake (see below), and accounting for missing values for C15:0 and C17:0 levels (n=29), the final sample consisted of 1,180 observations.

Measurements

Participants provided finger-prick blood samples through two DBS cards (Vitas Ltd, Oslo, Norway; DSM N.V., Heerlen, Netherlands) providing approximately 5 drops of blood (150µl) per card [21]. Participants were instructed to let the blood spots dry at room temperature for at least 2 hours but for no more than 4 hours. One of the DBS samples was sent by post using the collection pack provided to the corresponding recruitment centre, and from there to Vitas (Vitas Ltd, Norway) for measurement of whole blood fatty acids. Very briefly, punches of dried human whole blood were removed from the DBS sample and directly methylated with 0.5 M sodium methoxide. After incubation at 50 °C for 20 minutes, the formed fatty acid methyl esters (FAMES) were extracted into hexane and analysed on a Agilent Technologies GC-FID. The fatty acid methyl esters were separated on a Supelco SP2380 (30m x 250µm x 0.2µm) column and reported as g/100 gram FAME. Theoretical response factors were used for all fatty acids except for EPA, DPA and DHA. For these PUFAs, a USP FAME mixture was used to calculate empirical response factors within each sequence. Whole blood samples stored as DBS were used as quality control samples (QC). Two QCs were run in each sequence, one at the beginning and one at the end. CV% for the estimation of C15:0 and C17:0 were 4.3% and 4.9%, respectively. BMI was estimated as kg/m² using participants' online self-reported body weight and height.

Assessment of dietary intake

Habitual food intake over the previous month was assessed using an on-line Food Frequency Questionnaire (FFQ) with 157 food items specifically developed for the Food4Me intervention and validated against the printed EPIC-Norfolk FFQ (version CAMB/PQ/6/1205) [23, 24]. Bland Altman

analysis showed good agreement between these two FFQs for intake of dairy food groups.

Correlations analysis also revealed good agreement with coefficients varying from 0.55 (milk) to 0.77 (yogurts). Validity of the FFQ against a 4-day weighed food record (n=49) showed good agreement for dairy food groups with correlation coefficients ranging from 0.46 (cheese) to 0.73 (yogurt). [25].

Under-reporting of energy intake was determined from the comparison of total reported energy intake against the lowest estimated energy requirement (1.1 times the predicted basal metabolic rate [26]). Over-reporting of total energy intake was defined as values exceeding 18.84 MJ/day.

Statistical analyses

Normality was explored through Shapiro-Wilk tests of normality and quantile-quantile plots. Means, medians, standard deviations, and inter-quartile ranges were calculated to explore demographic characteristics of the participants, variation in dairy product intake and blood levels of fatty acids, in the whole cohort and also according to country of recruitment. Differences between countries were explored using Kruskal-Wallis equality-of-populations rank tests with post-hoc pairwise comparisons for continuous variables, and logistic regression analyses for categorical variables (overall mean as reference group). Differences by sex in the pooled sample and within countries were tested using the Wilcoxon rank-sum non-parametric test for two independent samples.

Separate ordinary least square (OLS) regression analyses were used to examine the independent association between each dairy product intake category (total dairy, high-fat dairy, low-fat dairy, cheese) as the dependent variable, and the log-transformed C15:0 or C17:0 levels as the independent variable. For variables with large proportions of reported zero consumption (milk: 12%, cream: 48%, yoghurt: 12%, butter: 42%, and zero-fat milk: 69%), associations were analysed using a corner-solution Tobit model with left-censoring at zero [27: 595]. For outcomes that tend to pile up at specific values (e.g. zero), this type of model provides more appropriate estimates of the conditional mean of the outcome variable because it accounts for the fact that, under such conditions, there is not a linear relationship with the explanatory variables [27: 668]. All regressions

included total energy intake as an independent covariate. Four specifications were explored: an “unadjusted” model including only the fatty acid and total energy; a “basic” model, including age, age squared and sex; an “adjusted” model additionally including smoking status and standardized BMI, and a “full” model incorporating all of the previous covariates and a set of individual country variables with value 1 if an observation was from a particular country and 0 otherwise.

Robust standard errors (Huber-White) were used to address heteroskedasticity in the data.

Estimated coefficients of interest included the β values for the OLS regressions, and the marginal effects for Tobit models estimated at the sample mean of the fatty acid independent variable.

Calculated this way, Tobit marginal effects have a similar interpretation to OLS coefficients [27: 599].

ROC curves were used to assess the sensitivity and specificity of blood levels of C15:0 and C17:0 as biomarkers of intake of dairy products. Parameters from a ROC analysis give an indication of the biomarker’s ability to correctly classify subjects given an observed binary outcome (e.g. positive vs. negative). The area under the curve (AUC) estimates the probability that a randomly selected positive outcome will have a higher “classifier” or biomarker value than a randomly selected negative outcome [28] and can be used to evaluate the “classification performance” of biomarkers [29]. According to the AUC value, the discriminating ability of biomarkers can be broadly classified as excellent ($AUC > 0.90$), good ($0.8 < AUC < 0.9$), fair ($0.7 < AUC < 0.8$) and poor ($AUC < 0.70$) [28].

ROC analyses compared the ability of the log-transformed C15:0 and C17:0 levels to discriminate individuals in the 10th percentile of intake and above the 90th percentile of intake. Details of the cut-offs of intake for each dairy product category analysed are given in the Supporting Information (Table A3). The curve tester facility of the online ROC Curve Explorer and Tester tool (ROCCET, <http://www.rocet.ca>) [28] was used for the ROC curve analyses. STATA Software version 13.0 [30] was used for all other analyses. *p*-values (two-sided) of ≤ 0.05 were considered evidence of statistical significance.

Results

Data from 1,180 participants (39% males) with an average ($\pm$ sd) age of 40 ± 13 years (18 to 73 years) were analysed. The average BMI was 25.2 ± 4.8 kg/m² and waist circumference was 84.8 ± 13.3 cm (**Table 1**). The mean total energy intake was 10.6 MJ/day $\pm$ 6.4 MJ/day, with dairy products supplying on average 12.5 ± 6.4 % of this energy. Along with Germany, Poland had one of the highest values for % energy intake from dairy (13.7 ± 7.4 %; 14.1 ± 6.7 %, respectively) and % of total fat from dairy (18.8 ± 9 %). Other differences were observed for proportion of smokers in the sample, in mean total energy intake, and in average total fat intake (**Table 1**).

Dairy products intake

The median ($\pm$ interquartile range) intake of dairy products was 304.4 ± 279.6 g/day (**Table 2**). The median intake of low-fat dairy products was almost 14-fold higher than that of high-fat dairy products (251 g/day vs. 18.6 g/day). Across product categories, milk had the highest median intake per day (177.7 ± 266.6 g/day), followed by yoghurt (53.6 ± 94.9 g/day) and cheese (24.4 ± 36.3 g/day).

Countries differed in their levels of dairy intake (**Table 2**). Median ($\pm$ interquartile range) intake of high-fat dairy products was highest in Germany (35.7 ± 30.8 g/day) and lowest in Spain (11.1 ± 13.7 g/day), while Greece had the lowest intake of both low-fat (197.2 ± 230 g/day) and total dairy products (237.8 ± 285.3 g/day) across all countries. Approximately half of the Spanish and Greek participants reported no consumption of butter and cream, while Germans and Polish participants had median intakes of 6.4 ± 13.3 g/day and 3.9 ± 11.5 g/day respectively. The median intakes of the other categories of dairy products are reported in **Table 2**.

Within countries, Greek and Irish women had significantly ($p < 0.05$) lower intakes of butter than did men (**Table A.1**, Supporting Information), whereas Irish and UK women had higher median intakes of

yoghurt than did men (53.6 g/day vs. 17.9 g/day; and 53.6 g/day vs. 24.5 g/day, respectively).

Women also reported higher consumption than men of cream in Germany (4.3 g/day vs. 3.0 g/day).

C17:0 and C15:0 levels in dried blood spots

Across the entire sample, the average ($\pm$ sd) level (expressed as % composition total fatty acids) of C17:0 was 0.32 ± 0.06 %, and that of C15:0 was 0.20 ± 0.06 % (**Table 3**). These values are in good agreement with previously reported values for adipose tissue and serum [31]. Overall, the Pearson correlation coefficient between C15:0 and C17:0 was 0.57 (0.55 for the logged variables).

The lowest mean levels of C15:0 were in Spain (0.16 ± 0.04 %, $p < 0.05$) and Greece (0.19 ± 0.05 %, $p < 0.05$), and the highest in Germany (0.24 ± 0.06 %, $p < 0.05$). The highest levels of C17:0 were in Germany and Poland (0.33%, $p < 0.05$), but were otherwise similar across countries. Men and women differed significantly in their average levels of C15:0 (0.19% vs. 0.20%, $p < 0.05$) and of C17:0 (0.32% vs. 0.31%, $p < 0.05$) (**Table A.2**, Supporting Information). By country, levels of C15:0 between men and women were significantly different only in Poland, where women had higher levels than men (0.23% vs 0.19%, $p < 0.05$). In contrast, there were more sex differences in C17:0 levels within countries (lower for women in Ireland, Germany and the Netherlands, and higher in Poland) (**Table A.2**, Supporting Information).

Associations of blood levels of C15:0 and C17:0 with dairy intake

Levels of C15:0 and of C17:0 were positively and significantly associated ($p < 0.001$) with intakes of total dairy, high-fat dairy, and low-fat dairy (**Table 4**). These associations remained significant after adjusting for age, sex, smoking status, BMI and country (full model). C15:0 had stronger associations with intakes across all the different dairy product categories compared to C17:0 (full model, standardized coefficients). The largest difference was found for high-fat dairy, with the coefficient for C15:0 almost double the value of that of C17:0. In contrast, levels of C17:0 showed no association with intake of zero-fat milk, and levels of C15:0 showed a negative association with consumption of zero-fat milk that was not significant after adjusting for the full set of controls (**Table 4**).

Unadjusted regressions showed strongly significant ($p < 0.001$) positive associations between C15:0 and intake levels of cream, yoghurt, cheese and butter; and between C17:0 levels and intake of cheese and butter. Unadjusted regressions also showed significant positive associations of C17:0 levels with milk ($p = 0.003$) and with yoghurt ($p = 0.01$), and of C15:0 levels with total milk consumption ($p = 0.007$). This pattern of associations remained after controlling for age, sex, BMI, smoking status and country of origin. The largest independent associations (adjusting for all factors) were mostly observed for C15:0, particularly for butter (standardized beta = 0.27) and cheese (standardized beta = 0.19). These higher-fat products also showed the largest difference between C15:0 and C17:0 coefficient values (**Table 5**). Robustness checks adjusting for alcohol, ruminant meat and fish showed no change in results.

ROC analyses showed that C15:0 levels provided acceptable predictability for discriminating between low and high consumers of high-fat dairy (AUC=0.84; 95% CI: 0.79, 0.89); while levels of C17:0 had low AUC values for total, high-fat and low-fat dairy products (AUCs < 0.69) (**Figure 1**). The combined C15:0 and C17:0 model did not improve the AUCs over the results obtained for C15:0 alone.

By product category, C15:0, but not C17:0, showed good to fair discriminating ability when comparing low consumers and high consumers of butter (AUC=0.80; 95% CI: 0.76, 0.83), low and high consumers of cheese (AUC=0.72, 95% CI: 0.66, 0.78), and low consumers and high consumers of cream (AUC=0.66, 95% CI: 0.61, 0.71) (**Figure 2**). The joint model showed little improvement from the individual C15:0 models. In contrast, the AUC values for the food products with lower dairy fat content of yoghurt and milk showed very low predictability for both C15:0 and C17:0 (AUCs < 0.60) (**Figure A.1**, Supporting Information).

Discussion

The present study demonstrated the potential utility of C15:0 and C17:0 levels measured in DBS as biomarkers of intakes of dairy products in European adults across 7 countries. C15:0 levels were significantly associated with intakes of high-fat dairy products. However, both fatty acids performed poorly as biomarkers of total dairy intake highlighting that caution should be exercised when using such markers as measures of total dairy intake in epidemiological studies.

Previous studies looking into biomarkers of dairy have found correlations of plasma C15:0 with measures of intake of total dairy, high-fat dairy, low-fat dairy, cheese, butter and full-fat milk [6, 13-15]. This study also found associations of C15:0 with high-fat dairy, low-fat dairy, butter and cheese; and additionally with total cream, total yoghurt and total milk consumption. Moreover, after controlling for potential confounding factors, including country of origin, the largest associations were observed for high-fat dairy, butter, and cheese. Additional analyses using ROC curves showed that levels of C15:0 did not distinguish well between low consumers and high consumers of total and low-fat dairy. In contrast, levels of C15:0 classified low and high consumers of high-fat dairy, butter, cheese and cream reasonably well; corroborating findings from the regression analyses and providing further evidence of the potential role of C15:0 as a biomarker for high-fat dairy intake.

Results also demonstrated positive associations for blood levels of C17:0 and dairy intake. With the exception of total cream, significant associations were found for all three dairy product categories (total, high-fat and low-fat dairy) and all the individual product groups examined here. Previous studies evaluating the levels of C17:0 in blood lipid fractions have also found positive correlations of C17:0 with total dairy intake levels, but generally of a lower magnitude than those reported for C15:0 [15, 18, 32]. Observed associations of C17:0 with dairy intake levels were also lower than those for C15:0 in this study, across all the dairy categories. ROC curves also mostly reflected the weaker relationships of C17:0 with dairy intake levels. Lower associations for blood levels of C17:0

may be reflecting other metabolic processes associated with this fatty acid in addition to dietary sources [16].

Results across the linear regressions and the ROC curves showed that C15:0 and C17:0 performed better for high-fat dairy products compared to lower-fat dairy products (e.g. milk, yoghurt). Different levels of saturated fatty acids per unit of dairy product may explain the relatively stronger associations of blood C15:0 and C17:0 levels with high-fat dairy. For example, dietary estimates of C15:0 in Norway indicated much lower relative levels of this fatty acid in skimmed milk compared with full fat milk [19]. The strong results for high-fat dairy in this study may also reflect the fact that milk was not included in this category during analyses, given previous findings of a lack of association of plasma C15:0 with milk intake, and accompanying suggestions that this lack of relationship is owing to the fact that milk fat may be metabolized differently than fats from other dairy products [19].

Strengths of this study include the use of a large sample of dietary patterns and blood lipid levels across seven European countries, allowing more information to be extracted from the data than would be the case with typical small-sample biomarker studies [33]. The use of ROC analyses to explore the ability of C15:0 and C17:0 to correctly classify high and low consumers of dairy provided greater insight into the performance of these fatty acids as biomarkers of intake compared to more conventional correlation techniques [32], and is another important contribution of this study. Finally this study highlights the use of DBS for collection of samples and analysis of diet related biomarkers: use of this approach in large longitudinal cohort studies could represent an inexpensive method for assessing diet related parameters. Work from others has demonstrated the stability of the markers and the use of this DBS approach in measuring a range of other fatty acids including n-3 poly-unsaturated fatty acids which are related to oily fish consumption [34, 35]. Limitations to consider when evaluating the results of the study are the possibility that, although data used in this study was

from a purposely-designed and validated FFQ questionnaire [24, 25], the strength of observed associations may have been attenuated by the use of this instrument for dietary data collection [19].

In conclusion, C15:0 measured in DBS performed well as a biomarker of high-fat dairy intake only. Overall findings highlight the utility of this biomarker for dietary assessment of high-fat dairy and, ultimately, for improved understanding through epidemiological studies of the role of diets high in dairy fat and in specific dairy products in the development of cardiovascular disease [36]. Moreover, recent findings of a negative association of high blood levels of C15:0 and C17:0 with type 2 diabetes [37, 38] indicate the potential of these fatty acids to act as biomarkers when studying diet-disease relationships. However, caution needs to be exerted when interpreting these biomarkers as indicators of food intake: the present data clearly demonstrates stronger relationships with high fat dairy products. Finally, the results extend the evidence base for the use of DBS in measuring dietary biomarkers and their potential application in large-scale epidemiological studies of nutrition and health.

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

The authors have declared no conflict of interest

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Tables

Table 1. Sample demographic and anthropometric characteristics from 1,180 respondents across Germany, Greece, Ireland, the Netherlands, Poland, Spain and the UK.

	Total (n=1180)	Germany (n=172)	Greece (n=154)	Ireland (n=187)	Nether. (n=183)	Poland (n=157)	Spain (n=173)	UK (n=154)
Men (%)	39.3	44.8	35.1	38.0	49.2	27.4	46.8	31.2
Age (years)	40.3 (13.0)	43.8 (13.5)	38.5 (11.2)	39.2 (12.7)	43.6 (14.5)	35.8 (12.2)	42.2 (10.6)	37.7 (13.3)
Smoking (%)	12.1	5.8	35.7	6.4	5.5	9.6	19.1	5.2
BMI (kg/m ²)	25.2 (4.8)	24.5 (3.9)	26.9 (6.5)	25.3 (4.8)	24.6 (4.4)	24.5 (4.9)	26.1 (4.1)	24.8 (4.4)
Waist circumference (cm)	84.8 (13.3)	85.0 (13.5)	88.3 (14.7)	84.8 (13.4)	85.3 (12.6)	80.9 (13.7)	86.9 (12.3)	82.1 (11.5)
Total energy intake (MJ/day)	10.6 (3.1)	10.6 (2.6)	9.6 (3.2)	11.1 (2.8)	11.4 (3.0)	10.3 (3.4)	10.9 (3.1)	10.4 (3.1)
Energy intake from dairy (%)	12.5 (6.4)	14.1 (6.7)	12.3 (6.8)	10.9 (5.1)	12.9 (6.4)	13.7 (7.4)	11.6 (6.6)	11.8 (5.1)
Total energy from fat (%)	35.8 (5.8)	37.1 (5.1)	37.6 (6.6)	36.4 (5.2)	34.5 (6.0)	34.8 (5.3)	35.5 (5.9)	35.1 (6.0)
Total fat intake from dairy (%)	16.5 (8.7)	18.8 (9.0)	16.8 (9.2)	14.6 (7.1)	18.8 (9.1)	17.9 (9.5)	14.0 (8.2)	14.7 (6.6)

Nether. = the Netherlands. Values are means with standard deviations in parentheses except for smoking status, which represents proportion of the sample that smokes.

Table 2. Mean, median and standard deviation of dairy intake (g/day) by product categories and country

	Total (n=1180)	Germany (n=172)	Greece (n=154)	Ireland (n=187)	Nether. (n=183)	Poland (n=157)	Spain (n=173)	UK (n=154)	p^1
Total dairy									
Mean	360.9	334.7	290.7	336.9	383.4	394.8	409.4	373.4	0.0003
sd	269.4	216.5	266.5	204.1	295.4	312.3	318.8	238.5	
Median	304.4	285.7	237.8	310.4	318.0	301.1	334.6	345.6	
iqr	279.6	285.3	225.0	240.4	305.4	326.0	336.5	264.3	
High-fat dairy									
Mean	27.0	42.9	25.5	26.3	27.7	27.8	15.8	22.5	0.0001
sd	27.2	33.3	25.8	23.5	28.8	25.1	21.2	23.1	
Median	18.6	35.7	17.3	18.7	17.9	21.2	11.1	15.6	
iqr	30.9	30.8	29.4	26.1	34.8	33.9	13.7	25.0	
Low-fat dairy									
Mean	310.4	283.0	253.3	293.8	321.2	342.6	353.6	323.9	0.0013
sd	256.4	210.2	264.0	194.4	271.9	304.3	295.3	230.2	
Median	251.8	240.8	197.2	280.1	262.5	241.1	272.4	305.6	
iqr	271.5	290.5	230.0	212.4	321.2	300.6	294.9	259.3	
Milk									
Mean	213.7	185.1	187.5	223.9	191.5	202.2	252.1	254.2	0.0001
sd	227.6	184.6	249.1	169.2	229.2	262.8	266.9	213.1	
Median	177.7	135.1	133.3	189.0	108.0	133.3	189.0	196.7	
iqr	266.6	228.6	239.6	214.0	297.5	225.0	234.8	242.5	
Cream									
Mean	2.6	6.4	1.7	1.1	2.0	4.0	1.4	1.8	0.0001
sd	6.0	12.5	2.5	2.1	3.2	6.2	2.2	2.7	
Median	1.1	4.3	0.0	0.0	1.1	2.1	0.0	0.0	
iqr	3.2	4.6	2.7	2.1	2.1	4.3	2.1	3.0	
Yoghurt									
Mean	80.3	85.4	48.7	60.5	113.7	106.1	84.8	59.5	0.0001
sd	99.9	86.8	69.9	80.1	143.7	108.1	103.7	57.2	
Median	53.6	62.5	24.4	40.1	71.4	80.1	53.6	44.6	
iqr	94.9	98.2	44.6	89.3	129.5	89.3	107.1	98.1	
Cheese									
Mean	35.0	38.3	40.1	25.9	38.2	48.0	30.1	25.8	0.0001
sd	34.7	30.9	39.3	27.0	33.3	44.1	35.7	24.3	
Median	24.4	30.1	26.6	17.3	28.3	34.4	17.3	19.1	
iqr	36.3	31.8	40.9	25.3	42.0	45.4	27.9	29.3	
Butter									
Mean	5.8	11.3	0.7	8.7	3.3	10.1	1.2	5.0	0.0001

	Total (n=1180)	Germany (n=172)	Greece (n=154)	Ireland (n=187)	Nether. (n=183)	Poland (n=157)	Spain (n=173)	UK (n=154)	p^1
sd	11.2	14.6	2.4	13.2	7.8	15.6	2.8	8.2	
Median	1.1	6.4	0.0	2.1	0.6	3.9	0.0	1.1	
iqr	6.4	13.3	0.0	11.5	2.1	11.5	1.1	6.4	

¹ p -value of test of differences across countries (Kruskal-Wallis test of difference across k independent samples). sd= standard deviation, iqr= interquartile range: difference between the third and first quartiles. High fat dairy includes double cream, high fat cheese, medium fat cheese and butter. Low fat dairy includes full fat or whole milk, low-fat or semi-skimmed milk, zero-fat or skimmed milk, single or sour cream, fruit yoghurt or fruit mousse, full fat Greek yoghurt, low-fat natural yoghurt, low-fat cheese, and very low-fat cheese. Milk: full-fat, semi-skimmed and zero-fat milk. Cream: double/clotted cream and single cream. Yoghurt: full-fat/Greek yoghurt, low-fat yoghurt and fruit yoghurt/mousse. Cheese: high-fat (e.g. Stilton, Cheddar, Brie), medium-fat (e.g. Goats, Camembert, Feta, Emmental), low-fat (e.g. Fresh Mozzarella, Cream Cheese, Katiki) and very low-fat cheeses (e.g. Cottage Cheese, Quark). Nether. = the Netherlands.

Table 3. Mean and standard deviation of C15:0 and C17:0 blood fatty acid levels

	Total (n=1180)	German y (n=172)	Greece (n=154)	Ireland (n=187)	Nether . (n=183)	Poland (n=157)	Spain (n=173)	UK (n=154)	p^1
Pentadecanoic (C15:0)									
Mean	0.20	0.24	0.19	0.21	0.21	0.22	0.16	0.20	0.0001
sd	0.06	0.06	0.05	0.05	0.05	0.07	0.04	0.05	
Median	0.20	0.23	0.19	0.20	0.20	0.21	0.15	0.20	
iqr	0.07	0.08	0.05	0.06	0.06	0.07	0.05	0.06	
Heptadecanoic (C17:0)									
Mean	0.32	0.33	0.32	0.32	0.32	0.33	0.31	0.31	0.0001
sd	0.06	0.07	0.05	0.05	0.05	0.06	0.05	0.05	
Median	0.32	0.32	0.32	0.32	0.32	0.33	0.30	0.31	
iqr	0.07	0.08	0.07	0.06	0.06	0.07	0.06	0.06	

¹ p -value of Kruskal-Wallis test of difference across k independent samples. sd= standard deviation, iqr= interquartile range: difference between the third and first quartiles. C15:0 and C17:0 blood fatty acid levels as percentage of total blood fatty acids. Nether. = the Netherlands.

Table 4. Associations of blood C15:0 and C17:0 levels and total dairy, high-fat dairy, low-fat dairy and zero-fat milk intake (g/day)

	Total dairy			High-fat dairy			Low-fat dairy			Zero-fat Milk ^a		
	β^c	se	<i>p</i> -value	β	se	<i>p</i> -value	β	se	<i>p</i> -value	β	se	<i>p</i> -value
<i>No adjustment</i>												
Pentadecanoic (C15:0) ^b	115.04	(27.63)	<i>p</i> < 0.001	31.99	(3.00)	<i>p</i> < 0.001	101.63	(27.11)	<i>p</i> < 0.001	-45.88	(12.60)	<i>p</i> < 0.001
	[0.12]			[0.32]			[0.11]			[-0.14]		
Heptadecanoic (C17:0) ^b	186.00	(43.78)	<i>p</i> < 0.001	22.31	(5.14)	<i>p</i> < 0.001	173.16	(42.46)	<i>p</i> < 0.001	-13.92	(18.10)	0.443
	[0.12]			[0.14]			[0.11]			[-0.03]		
<i>Basic model</i>												
Pentadecanoic (C15:0)	111.31	(27.67)	<i>p</i> < 0.001	31.75	(3.00)	<i>p</i> < 0.001	98.74	(27.22)	<i>p</i> < 0.001	-49.48	(12.48)	<i>p</i> < 0.001
	[0.11]			[0.32]			[0.11]			[-0.15]		
Heptadecanoic (C17:0)	201.05	(43.26)	<i>p</i> < 0.001	22.89	(5.15)	<i>p</i> < 0.001	183.74	(42.45)	<i>p</i> < 0.001	-8.14	(17.84)	0.649
	[0.13]			[0.14]			[0.12]			[-0.02]		
<i>Adjusted model</i>												
Pentadecanoic (C15:0)	104.89	(28.53)	<i>p</i> < 0.001	31.94	(2.91)	<i>p</i> < 0.001	94.08	(28.08)	0.001	-50.94	(12.67)	<i>p</i> < 0.001
	[0.11]			[0.32]			[0.10]			[-0.16]		
Heptadecanoic (C17:0)	194.06	(45.18)	<i>p</i> < 0.001	22.14	(5.10)	<i>p</i> < 0.001	179.86	(44.35)	<i>p</i> < 0.001	-6.66	(18.03)	0.712
	[0.12]			[0.14]			[0.12]			[-0.01]		
<i>Full model</i>												
Pentadecanoic	167.3	(30.4)	<i>p</i> <	25.3	(2.92)	<i>p</i> <	151.2	(30.0)	<i>p</i> <	-8.91	(12.9)	0.49

ic (C15:0)	1	7)	0.00	5	)	0.00	0	5)	0.00		7)	4
	[0.17]		1	[0.26		1	[0.16]		1	[-		
				]						0.03]		
Heptadecan	211.4	(45.9	p <	18.0	(4.95	p <	192.3	(45.3	p <	20.2	(18.5	0.27
oic (C17:0)	6	6)	0.00	8	)	0.00	5	5)	0.00	9	7)	3
	[0.13]		1	[0.11		1	[0.13]		1	[0.04		
				]						]		

^a Marginal effects from a Tobit model for corner solutions. ^b Variable in logarithm. Standard errors in parentheses. ^c Regression coefficients. Standardized coefficients in brackets. Standardized coefficients show the standard deviation change in the dependent variable when the independent variable is changed by one standard deviation. Basic model: adjusted for age, age squared and sex. Adjusted model: variables in basic model and smoking status, standardized BMI. Full model: adjusted model covariates plus country indicator variables. All models adjusted for total energy intake (kcal/day). High fat dairy includes double cream, high fat cheese, medium fat cheese and butter. Low fat dairy includes full fat or whole milk, low-fat or semi-skimmed milk, zero-fat or skimmed milk, single or sour cream, fruit yoghurt or fruit mousse, full fat Greek yoghurt, low-fat natural yoghurt, low-fat cheese, and very low-fat cheese.

Table 5. Associations of blood C15:0 and C17:0 levels and dairy product intake (g/day)

	Milk ^a			Crea m ^a			Yogh urt ^a			Che ese			Butt er ^a		
	β^c	se	p- val ue	β	se	p- val ue	β	se	p- val ue	β	se	p- val ue	β	se	p- val ue
<i>No adjustment</i>															
Pentadecanoic (C15:0) ^b	54.91	(20.49)	0.007	3.06	(0.76)	p < 0.001	35.77	(8.72)	p < 0.001	24.14	(3.66)	p < 0.001	12.00	(1.17)	p < 0.001
	[0.08]			[0.20]			[0.12]			[0.18]			[0.39]		
Heptadecanoic (C17:0) ^b	97.06	(32.71)	0.003	2.93	(1.60)	0.067	35.57	(13.87)	0.010	21.10	(5.63)	p < 0.001	7.99	(1.65)	p < 0.001
	[0.08]			[0.12]			[0.07]			[0.10]			[0.16]		
<i>Basic model</i>															
Pentadecanoic (C15:0)	54.44	(20.67)	0.008	3.02	(0.76)	p < 0.001	34.06	(8.60)	p < 0.001	23.28	(3.62)	p < 0.001	11.97	(1.17)	p < 0.001
	[0.08]			[0.20]			[0.11]			[0.18]			[0.39]		

Heptadecanoic (C17:0)	102.48	(32.78)	0.002	3.01	(1.57)	0.056	39.12	(13.83)	0.005	23.66	(5.69)	$p < 0.001$	7.97	(1.62)	$p < 0.001$
	[0.09]			[0.12]			[0.08]			[0.12]			[0.16]		
<i>Adjusted model</i>															
Pentadecanoic (C15:0)	54.60	(21.59)	0.011	2.92	(0.71)	$p < 0.001$	29.58	(8.71)	0.001	23.94	(3.52)	$p < 0.001$	11.77	(1.15)	$p < 0.001$
	[0.08]			[0.19]			[0.10]			[0.19]			[0.38]		
Heptadecanoic (C17:0)	104.41	(34.54)	0.003	2.76	(1.46)	0.059	32.57	(13.57)	0.016	24.19	(5.73)	$p < 0.001$	7.31	(1.65)	$p < 0.001$
	[0.09]			[0.11]			[0.07]			[0.12]			[0.15]		
<i>Full model</i>															
Pentadecanoic (C15:0)	96.56	(22.50)	$p < 0.001$	1.46	(0.63)	0.020	35.12	(9.34)	$p < 0.001$	24.06	(3.69)	$p < 0.001$	8.47	(1.11)	$p < 0.001$
	[0.13]			[0.10]			[0.12]			[0.19]			[0.27]		
Heptadecanoic (C17:0)	108.56	(34.57)	0.002	2.11	(1.37)	0.125	38.11	(14.13)	0.007	21.39	(5.56)	$p < 0.001$	5.14	(1.52)	0.001
	[0.09]			[0.09]			[0.08]			[0.10]			[0.10]		

^a Marginal effects from a Tobit model for corner solutions. ^b Variable in logarithm. Standard errors in parentheses. ^c Regression coefficients. Standardized coefficients in brackets. Standardized coefficients show the standard deviation change in the dependent variable when the independent variable is changed by one standard deviation. Basic model adjusted for sex, age and age squared. Adjusted model expanded to include smoking status, and standardized BMI. Full model: adjusted model covariates plus country indicator variables. All models adjusted for total energy intake (kcal/day).

Figure legends

Figure 1. Receiver operating characteristic (ROC) curves for the discriminating power of C15:0, C17:0 by total dairy, high-fat dairy and low-fat dairy categories produced using single fatty acid models (top and middle panels) and a model containing both fatty acids simultaneously (bottom panel). The low and high consumers were selected using the 10th and 90th percentile. Details of the intake level cut-offs for low and high consumption are in Table A.3. The x axis represents the false positive rate (1- specificity), the y axis represents

the true positive rate (sensitivity). The red dot represents the mathematically optimal cut-off point for classifying subjects into “positive” (high consumption) or “negative” (low consumption) outcomes.

Figure 2. Receiver operating characteristic (ROC) curves for the discriminating power of C15:0, C17:0 by dairy product produced using single fatty acid models (top and middle panels) and a model containing both fatty acids simultaneously (bottom panel). The low and high consumers were selected using the 10th and 90th percentile. Details of the intake level cut-offs for low and high consumption are in Table A.3. The x axis represents the false positive rate (1- specificity), the y axis represents the true positive rate (sensitivity). The red dot represents the mathematically optimal cut-off point for classifying subjects into “positive” (high consumption) or “negative” (low consumption) outcomes.



