Within-person reproducibility and sensitivity to dietary change of C15:0 and C17:0 levels in dried blood spots: data from the European Food4Me Study

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Abbreviations List

DBS	dried blood spots
FFQ	food frequency questionnaire
iqr	interquartile range
g/day	grams per day
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
ICC	intra-class correlation coefficient
CV	coefficient of variation
WCV	within-subject coefficient of variation
CI	confidence interval
GC-FID	gas chromatography-flame-ionization detector
MLE	maximum likelihood estimator

Abstract

Scope

Previous work highlighted the potential of odd-chain length saturated fatty acids as potential markers of dairy intake. The aim of this study was to assess the reproducibility of these biomarkers and their sensitivity to changes in dairy intake.

Methods and Results

Fatty acid profiles and dietary intakes from food frequency questionnaires (FFQs) were measured three times over six months in the Food4Me Study. Reproducibility was explored through intra-class correlation coefficients (ICCs) and within-subject coefficients of variation (WCV). Sensitivity to changes in diet was examined using regression analysis. C15:0 blood levels showed high correlation over time (ICC: 0.62, 95% CI: 0.57, 0.68), however, the ICC for C17:0 was much lower (ICC: 0.32, 95% CI: 0.28, 0.46). The WCV for C15:0 was 16.6% and that for C17:0 was 14.6%. There were significant associations between changes in intakes of total dairy, high-fat dairy, cheese and butter and C15:0; and change in intakes of high-fat dairy and cream and C17:0.

Conclusions

Results provide evidence of reproducibility of C15:0 levels over time and sensitivity to change in intake of high-fat dairy products with results comparable to the well-established biomarker of fish intake (EPA+DHA).

1 Introduction

Pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) are linear odd-chained fatty acids found mainly in the milk and meat fat of ruminants such as cattle and sheep [1]. These saturated fatty acids originate both from bacterial synthesis in the reticulo-rumen of these animals, as well as from synthesis in their mammary glands [2]. In humans, these fatty acids have been proposed as potential biomarkers of intake of high-fat dairy foods [3-6], and as such are of interest to researchers studying the effects of dairy fats on health conditions like cardiovascular disease and type-2 diabetes. In a study of 1595 Coronary Heart Disease (CHD) cases and 2246 controls, the concentrations of C15:0 and C17:0 in the phospholipid fraction of human blood were inversely related to CHD incidence [7]. Similarly, a systematic review and meta-analysis of four fatty acid biomarkers and coronary risk showed a negative association of coronary risk with C17:0, and with C17:0 + C15:0 [8]. Furthermore, a recent study demonstrated that plasma phospholipid C15:0 and C17:0 concentrations had strong inverse associations with type 2 diabetes risk [9].

As with other dietary biomarkers, the potential to investigate C17:0 and C15:0 blood levels in large prospective cohort studies is likely to increase with the expansion in use of more cost-effective methods for collecting biological samples, such as dried blood spots (DBS) [10, 11]. However, the usefulness of C17:0 and C15:0 as measures of exposure is contingent on the within-individual reproducibility over time in concentration of these biomarkers, i.e. on evidence that a single baseline measure –as is common in cohort studies– provides an accurate account of individuals' typical or habitual dietary exposure [12]. The relevance of C17:0 and C15:0 as biomarkers of high-fat dairy intake also requires evidence of the sensitivity of these fatty acids to changes in diets. Therefore, the objective of this study was to explore the reproducibility over time of repeated measurements done on the same

study participants, and the sensitivity to reported change in dairy intake, of blood C17:0 and C15:0 levels. The hypothesis was that C17:0 and C15:0 fatty acids would demonstrate good reproducibility over time in individuals with stable dairy intakes, and sensitivity to change with change in dairy intake habits in a similar population of adults.

2 Materials and methods

2.1 Overview

Data originate from the web-based Food4Me personalized nutrition proof-of-principle study (Clinicaltrials.gov RCT number NCT01530139) [13]. Briefly, the study compared the effects of different levels of personalised nutrition advice on health-related outcomes with the same outcomes in a group receiving only generic nutrition advice, as described in detail in Celis-Morales et al. [14]. Recruitment was done across seven European countries (Germany, Greece, Ireland, the Netherlands, Poland, Spain and the United Kingdom). Participants in each country were randomized into the Generic-advice[1] group or any of three personalised-advice[2] Intervention groups. Baseline measurements (t0) of dietary intakes and biomarkers were followed up at three (t3) and six months (t6) for all participants. Variability over time of C15:0 and C17:0 levels was studied using the Generic-advice arm of the Food4Me study (individuals with greater stability in dietary intakes over time), while sensitivity to change in dairy intake of C15:0 and C17:0 levels was studied using pooled data from participants randomised to the Intervention groups (individuals with changing diets over time). Results for C17:0 and C15:0 levels in whole blood were compared with values obtained for the sum of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), a well-established

[1] Dietary advice for this group was based on (European) population guidelines. Information was also given on body weight and physical activity based on (European) population guidelines.

[2] One group was given dietary advice based on individual dietary intake data alone, another group was given advice based on individual dietary intake an phenotypic data, and a third group was given information on individual dietary intake, phenotypic data and genotypic data.

biomarker of oily fish intake [15], as a measure to benchmark the reproducibility and sensitivity values of C15:0 and C17:0.

2.2 Study sample

DBS samples, dietary intakes and anthropometric data were collected from an adult sample (≥18 years) over six months from August 2012 to February 2014 as part of the Food4Me study.

2.3 Assessment of blood fatty acids

Two DBS cards (Vitas Ltd, Oslo) were used to collect approximately 5 drops of blood (150µl) per card on each occasion [14]. The blood spots were left to dry at room temperature for at least 2 hours but not more than 4 hours, and then sent for processing and analysis (Vitas Ltd, Norway). Once removed from the DBS sample, the dried human whole blood was directly methylated with sodium methylate. After incubation at 50^{o}C, the formed fatty acid methyl esters (FAMES) were extracted into hexane and analysed on an Agilent Technologies gas-liquid chromatograph with a flame-ionization detector (GC-FID). The fatty acid methyl esters were separated on a Supelco 30µm x 250µm x 0.2µm column and reported as g/100 gram FAME (weight percentage).

2.4 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 frequently consumed in all the countries represented in the study and developed specifically for the Food4Me intervention. Participants replied how often in the past month they had eaten each of the food items using nine response categories ranging from “never (<1 per month)” to “6+ per day”, after which they indicated their usual serving size among seven possibilities from a range of portion size colour photographs. The list of dairy food products covered in the FFQ included cream

(double and single), high-fat cheese (e.g. stilton, cheddar and brie), medium-fat cheese (e.g. goats' cheese, camembert, feta and emmental), low-fat cheese (e.g. fresh mozzarella, cream cheese and katiki), very low-fat cheese (e.g. cottage cheese and quark), butter, milk (full-fat, low-fat and zero-fat), fruit mousse and yoghurt (fruit, full-fat, Greek and low-fat). The FFQ also covered other food categories that potentially contribute to C15:0 and C17:0 levels, such as ruminant meats (e.g. beef and beef products) and fish and fish products (e.g. oily fish, smoked salmon and sushi).

Validation of the FFQ was done against the weighed food diaries and it was compared to the printed EPIC-Norfolk FFQ (CAMB/PQ/6/1205) [16-18]. Correlation of energy intake between the Food4Me FFQ and the EPIC-Norfolk FFQ was 0.68 (52% of cases in exact agreement), with the lowest correlation between the two instruments for polyunsaturated fatty acids (PUFA % total energy) at 0.43 (37% of cases in exact agreement). Correlations for dairy foods ranged from 0.55 (milk, 49% of exact agreement) to 0.7 (yoghurts, 58% of exact agreement) [17]. Correlations for red meat and meat products was greater than 0.67 (> 50% of exact agreement), and for fish and fish products > 0.70 (> 50% of exact agreement) [17]. Validity of the FFQ against a 4-day weighed food record (n=49) showed exact cross-classification values from 41% (cheese) to 51% (milk) [18]. The final Food4Me FFQ showed good test-retest reproducibility (n=100, 2 occasions, 4 weeks apart) with Spearman correlation values between measurements ranging from 0.66 (cheese, 52% exact agreement) to 0.79 (yoghurt, 58% of exact agreement) [18]. The categorization of dairy products into high-fat and low-fat was based on the fat content (g) per 100g of product with high fat defined as > 20g of fat /100g.

Under-reporting of energy intake was identified using the lowest estimated energy requirement of 1.1 times the predicted basal metabolic rate [19]. Over-reporting of total energy intake was defined as values exceeding 18.84 MJ/day [13].

2.5 Statistical analyses

Differences in demographic characteristics and dietary intakes were explored using a repeated measures Skilling-Mack test (Friedman's ANOVA with missing values) for continuous non-normal variables, and logistic regression for binary variables. Comparison of blood fatty acid levels across time periods was done using a repeated measures ANOVA using Box's conservative correction factor *p*-values.

Reproducibility analyses

Reproducibility over time of fatty acid levels was explored using three methods. Intra-class correlation coefficients (ICCs) were calculated on the log-transformed fatty acid levels using the variance components from a random effects model (not controlling for time trend). The ICC in a one-way random-effects model measures the ratio of between-person variation over the total variation in the data. Higher ICC values indicate that more of the variance in the data comes from differences across individuals than from differences over time within individuals, therefore providing a degree of the reproducibility of replicate measures from the same subject [20: p.563]. In addition to ICCs, the within-subject coefficient of variation (WCVs) was calculated on the logarithm of the fatty acid levels using a one-way random effects model with MLE estimation [21]. The WCV provides a more generalizable estimation of reproducibility because, unlike the ICC, its value is not specific to a population or sample. Smaller values of the WCV indicate better reproducibility, i.e. lower within-person change across time. Finally, Spearman correlations of fatty acid measurements across the three time points of the study were also explored. Bootstraps with 1000 replications were used to calculate confidence intervals for all the measures of reproducibility.

Sensitivity to dietary change analyses

The change in fatty acid levels from changes in dairy intake (for C15:0 and C17:0) and fish intake (for EPA and DHA) were explored using first-difference linear regression [22] with clustered standard errors over individual participants (the model equations can be found in the online supplementary material). For the purpose of analyses, dairy intake was classed into daily portions of total dairy, high-fat dairy and low-fat dairy. High-fat dairy included double cream, high-fat cheese, medium-fat cheese and butter. Low-fat dairy included 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. In addition, individual products were grouped by product category into daily portions of milk, cream, yoghurt, cheese and butter. Intake of oily fish included consumption of cooked, raw and canned oily fish (the sample mean portions of the different product categories can be found in the online supplementary material). Models were run separately for all of these categories, with basic adjustment for total energy intake (MJ/day), age, smoking status and BMI. In addition, for the dairy intake models, results were adjusted for intake of ruminant meat and oily fish portions. Other covariates that did not change over the study period (e.g. sex and country of residence) were not included in the analyses [22].

3 Results

3.1 Sample characteristics

After excluding observations with over- and under-reporting of energy intake (n=820) and extreme values of the fatty acid variables (n=11)[3], the total number of observations over the three periods was 3148; 760 from the Control group and 2388 from the Intervention group. The total number of participants at was 334 in the Generic-advice group and 1054 in the Intervention arm of the Food4Me study. Table 1 presents the descriptive statistics for the

[3] Cases which had unrealistically large or small values identified through box-plots of the data.

Generic-advice and Intervention groups. Over the three periods, the average (±sd) age of participants was 40 years (±13) across both groups, with an average BMI of 27 kg/m^2 (±4.2). Over time BMI and waist circumference decreased slightly for both groups, but the Intervention group had a larger reduction in total energy intake from fat, from an average of 10.7% to 9.8% (Generic-advice group: 10.4% to 10.0%). Both groups were similar in the percentage energy intake from dairy (Generic-advice group: 13% ±6.6%), and the Generic-advice group had a slightly higher contribution of fat from dairy (17.3% ±9%) compared to the Intervention group (16.1% ±8.2%).

3.2 Dietary intakes

Across the three periods, median (± interquartile range) intake of dairy products in the Generic-advice group was 314 (±277) g/day and 292. (±266) g/day in the Intervention group (Table 2). In both the Intervention and Generic-advice groups, intake of low-fat dairy was much higher than intake of high-fat dairy. Low-fat dairy intake was stable over time for both the Intervention and the Generic-advice group (p>0.05). Both the Intervention and Generic-advise groups showed a reduction in high-fat dairy intake (p<0.001 and p=0.01, respectively), although median intake levels were more stable in the Generic-group. Across product categories and groups, milk exhibited the highest median intake at around 170 g/day, followed by yoghurt (around 53 g/day) and cheese (around 24 g/day). There was no change over time in daily intake in any of the individual product categories for the Generic-advice group, while in the Intervention group there was a decrease in cream, cheese, and butter, with the largest relative change for butter.

Median intake of oily fish at baseline (t0) was about 16 g/day in the Generic-advice and Intervention groups, increasing at t6 to 21.1 (±27.6) g/day in the Generic-advice group and 23.5 (±29.9) g/day in the Intervention group.

3.3 Reproducibility over time of biomarkers of dairy intake

Across the three time points, levels of C15:0 and C17:0 in the Generic-advice group were 0.21% (sd=±0.06) and 0.32% (±0.06) respectively. Similar values were observed in the Intervention group (Table 3). Over time mean C17:0 levels showed an increase in t3 and a subsequent decrease in t6 (p<0.001). In contrast, average C15:0 levels remained stable in the Generic-advice group, but increased slightly in the Intervention group (p=0.014). In an attempt to benchmark the C15:0 and C17:0 results the levels of the well-established marker of oily fish were examined (EPA+DHA). EPA+DHA levels were 3.8% in t0 for the Generic-advice and Intervention groups, significantly increasing (p<0.05) to 3.9% and 4.1% in t3 for the Generic-advice group and the Intervention group, respectively.

Measures of agreement between the baseline and repeat blood fatty acid levels are shown in Table 4. Blood levels of C15:0 showed high correlation over time (ICC of 0.62, 95% confidence interval (CI): 0.57, 0.68), but the ICC for C17:0 was much lower, at 0.32 (95% CI: 0.28, 0.46). The ICC for C15:0 was comparable to the values obtained for EPA+DHA (0.67, 95% CI: 0.62, 0.71). The WCV for C15:0 was 16.6% (95% CI: 14.9%, 18.3%), and WCV for C17:0 was 14.6% (95% CI: 13.3%, 16.0%). In comparison, EPA+DHA had a WCV of 17.4% (95% CI: 16.1%, 18.8%). Finally, the highest Spearman rank correlation coefficients were observed between the baseline (t0) and t3 measurements. As with the ICCs, the highest values were observed for the dietary biomarker C15:0 (t0-t3: 0.69, 95% CI: 0.61, 0.78). C17:0 exhibited correlation coefficients approximately half the values of C15:0. EPA+DHA had the highest correlation coefficients of the three biomarkers (t0-t3: 0.72, 95% CI: 0.69, 0.82) (Table 4).

3.4 Change in dietary intake and change in fatty acid levels

Results indicate that an additional portion of high-fat dairy increased the DBS level of C15:0 by 0.32% (95% CI: 0.05%, 0.6%), whereas an increase of one portion per day of

cheese and of butter increased C15:0 DBS levels by 1.8% (95% CI: 0.5%, 3.0%) and by 3.3% (95% CI: 1.3%, 5.3%), respectively (Table 5). The effect of an increase of butter intake on C15:0 levels was similar to the change observed on EPA + DHA, with an increase of 2.6% (95% CI: 1.3%, 3.9%, $p<0.001$) for an increase of one portion per day of oily fish. Greater intakes of high-fat dairy also raised the levels of C17:0 by 0.3% (95% CI: 0.098%, 0.45%); in addition, levels of this fatty acid rose by 9.4% (95% CI: 3.4%, 15.4%) in response to an additional daily portion of dairy cream. There were no significant effects detected in the levels of C15:0 and of C17:0 for changes in total low-fat dairy and lower-fat dairy products, such as total milk and yoghurt[4].

4 Discussion

Using data from a Pan-European dietary intervention study, this study provides evidence on the reproducibility over time for repeated measures of blood C15:0 levels in DBS. Furthermore, the results demonstrate the association between changes in daily intakes of specific high-fat dairy products and blood levels of the odd-chain fatty acids C15:0 and C17:0.

For C15:0, the ICC value and Spearman correlation were satisfactory and comparable to the values found for the well-established biomarker EPA+DHA. In contrast, C17:0 levels showed low correlations over time (ICC<0.35). The ICC results for C17:0 may have been weakened by the temporary increase in the levels of this fatty acid between baseline (t0) and t3, although sensitivity analyses excluding t3 observations did not show any improvement in the ICC for this fatty acid. Further evidence of reproducibility for C15:0 was found from the WCV analyses, which also presented good evidence of reproducibility for C17:0. The apparent contradiction in the evidence of reproducibility for C17:0 from the ICC and the

[4] On average, the proportion in the sample of semi-skimmed and skimmed milk over total milk consumption was 79.7% (95% CI: 78.5%, 80.9%), and the proportion of plain or flavoured low-fat yoghurts over total yoghurt consumption was 82.4% (95% CI: 81.3%, 83.4%).

WCV values could result from endogenous metabolic synthesis pathways for odd-chain saturated fatty acids such as C15:0 and C17:0. The existence of such pathways has been postulated on the basis of the higher levels of C17:0 observed in different lipid fractions in humans compared to what would be expected from the ratio of these fatty acids in milk fat [4, 23-25]. More recently strong evidence has emerged that supports the endogenous biosynthesis of C17:0 [26]. This biosynthesis of C17:0 could explain higher mean levels of heptadecanoic acid in the current data and the lower ICCs.

Associations between changes in dairy intake and C15:0 and C17:0 DBS levels were not significant for low-fat dairy products. This supports our previous work [3], and is probably explained by the fact that low-fat dairy products have low levels of milk fats including the odd-chained fatty acids C15:0 and C17:0 [2]. Higher intakes of high-fat dairy, total dairy, cheese and butter were associated with an increase in C15:0 DBS levels, with the largest effects for butter followed by cheese. The strong link between change in butter intake and C15:0 levels observed here reinforces previous evidence [3] and underscores the potential usefulness of this fatty acid as a biomarker of butter intake for prospective studies of cardiovascular health [27]. C17:0 levels changed in response to higher consumption of high-fat dairy and cream. In particular, cream consumption had a strong effect on C17:0 levels (≈ 10% increase). Cream is not a fermented dairy product and this may have played a role in the magnitude of this effect since bacteria in fermented products may affect lipid metabolism and interfere with plasma fatty acid levels [28], although this explanation remains to be explored for C17:0 and C15:0.

An important strength of this study is the use of three blood DBS measurements over six months in a large sample of participants in groups of both relatively stable dairy intakes and changing dairy intakes in order to study the reproducibility and sensitivity of blood C15:0 and C17:0 levels. The dietary intake data for the study came from purposely-designed and

validated FFQ questionnaires [17, 18], nonetheless, consideration when evaluating the results should be given to the possible attenuation in the strength of the observed diet-biomarker relationships from using FFQ to capture intakes [4].

In conclusion, blood C15:0 levels showed strong evidence of reproducibility over time and sensitivity to change in intake of high-fat dairy products, comparable to the values found for the well-established biomarker EPA+DHA for oily fish intake, which are important conditions for the usefulness of biomarkers in the study of dietary intakes and nutrient-disease relationships. Results for C17:0 question its role as a dietary biomarker and supports recent research demonstrating substantial biosynthesis of C17:0 [26, 29]. This taken together with our previous results supports C15:0 only as a biomarker of dairy intake [3].

Table 1. Demographic and anthropometric characteristics of sample participants

	Generic-advice group				Intervention group			
	t0	**t3**	**t6**		**t0**	**t3**	**t6**	
	N=289	N=243	N=228	**p-value**	N=889	N=777	N=722	**p-value**
Men (%)	38	41	42	0.995	40	42	41	0.946
Age (years)	39.4 (13.2)	40.3 (13.1)	40.9 (13.1)	N/A	40.6 (12.9)	40.9 (13.1)	41.5 (12.9)	N/A
Smoker (%)	15	14	15	0.978	11	10	10	0.792
BMI (kg/m2)	25.1 (4.6)	24.4 (3.9)	24.4 (3.9)	0.048	25.3 (4.9)	24.9 (4.5)	24.7 (4.1)	p<0.001
Waist circumference (cm)	84.6 (13.4)	83.4 (12.5)	83.6 (12.5)	0.042	84.9 (13.3)	84.1 (12.8)	83.8 (12.2)	p<0.001
Total energy intake (MJ/day)	10.4 (3.1)	10.2 (2.9)	10 (3.1)	0.008	10.7 (3)	9.7 (2.7)	9.8 (2.8)	p<0.001
Energy intake from dairy (%)	13.1 (7)	13.1 (6.7)	13 (6.2)	0.178	12.3 (6.3)	12.5 (6.2)	12.1 (6.1)	0.233
Total energy from fat (%)	35.6 (5.8)	35.5 (5.8)	35.7 (6.5)	0.829	35.9 (5.8)	35 (5.4)	34.8 (5.6)	0.003
Total fat intake from dairy (%)	17.3 (9.6)	17.5 (9)	16.9 (8.4)	0.250	16.2 (8.3)	16.3 (8.3)	15.8 (8.1)	0.293
Energy intake from meat and fish (%)	13.4 (7)	13.4 (8)	14.5 (8.1)	0.022	13.9 (7.2)	14.1 (6.8)	14.1 (7)	0.007
Total fat intake from meat and fish (%)	17.9 (9.3)	17.8 (9.8)	18.7 (9.1)	0.006	18.6 (9.3)	18.8 (8.8)	19 (8.9)	0.003

Standard deviation in parenthesis. t0 is baseline, t3 and t6 are three months and six months form baseline, respectively. *p*-value from a repeated measures Skilling-Mack test (continuous variables) and logistic regression (binary variables).

Table 2. Mean and median daily (g/day) dairy and oily fish intake across study periods

	Generic-advice group				Intervention group			
	t0 N=289	**t3** N=243	**t6** N=228	***p*-value**	**t0** N=889	**t3** N=777	**t6** N=722	***p*-value**
Total dairy								
Mean (sd)	371.9 (295.3)	362.4 (243.4)	354.4 (229.8)	0.033	357.2 (260.8)	335 (232.1)	336.1 (232.7)	0.007
Median (iqr)	306.7 (290.5)	327.6 (263.5)	301.8 (265.6)		301.6 (272.5)	288.6 (256.8)	288.9 (269)	
High-fat dairy								
Mean (sd)	26.1 (26.2)	25.5 (26.7)	23.5 (22.4)	0.010	27.3 (27.5)	18.6 (18.4)	18.4 (18.9)	p<0.001
Median (iqr)	17.1 (31.7)	20.3 (24.9)	18.6 (27.2)		19.3 (30.5)	13 (21)	12.8 (20.2)	
Low-fat dairy								
Mean (sd)	317.8 (269.1)	308.9 (232.1)	308.8 (219.6)	0.568	307.9 (252.5)	287.6 (219.9)	297.6 (221.2)	0.189
Median (iqr)	264.8 (288.3)	269.9 (266.9)	258.3 (259.9)		249 (263.3)	250 (246.4)	252 (261.5)	
Milk								
Mean (sd)	217.9 (232.7)	210.9 (201.7)	210.7 (191.9)	0.535	212.3 (226.3)	186.3 (202)	193.3 (197.4)	p<0.001
Median (iqr)	174.4 (270)	189 (262.5)	189 (262.5)		177.7 (262.5)	148.5 (216.3)	161.1 (239)	
Cream								
Mean (sd)	2.3 (4)	2.3 (3.8)	2.2 (3.7)	0.719	2.7 (6.5)	2.1 (3.8)	2 (4)	p<0.001
Median (iqr)	0 (3.2)	0 (3.9)	0 (3.2)		1.1 (3.8)	0 (2.9)	0 (3)	
Yoghurt								
Mean (sd)	84.8 (96.9)	82.9 (91.4)	79.5 (90.9)	0.351	78.9 (101)	82.6 (93.1)	85.6 (99.8)	0.849
Median (iqr)	53.6 (107.1)	53.6 (111.6)	53.6 (110.5)		53.6 (89.3)	53.6 (98.2)	53.6 (104.9)	
Cheese								
Mean (sd)	34.8 (32.7)	33.1 (35.2)	35.1 (35.9)	0.228	35 (35.3)	31.5 (30.5)	31.4 (32.2)	p<0.001
Median (iqr)	25.7 (42.4)	22.9 (31.8)	25.4 (37.4)		24.1 (34.4)	22.3 (31.6)	21.9 (30.9)	
Butter								
Mean (sd)	4.6 (8.4)	5.3 (10.8)	4.7 (10.9)	0.186	6.2 (12)	3.8 (7.6)	3.7 (8.5)	p<0.001
Median (iqr)	0.6 (6.4)	1.1 (6.4)	0.6 (6.4)		1.1 (6.4)	0.6 (3.9)	0.9 (3.9)	
Oily fish								
Mean (sd)	23.9 (26.2)	30.1 (29.5)	27.6 (26)	p<0.001	24.4 (27.4)	30.2 (28.4)	31.2 (38.1)	p<0.001
Median (iqr)	16.6 (24.5)	21.9 (27.1)	21.1 (27.6)		16.1 (25.4)	22.4 (30.9)	23.5 (29.9)	

sd: standard deviation, iqr: interquartile range. t0 is baseline, t3 and t6 are three months and six months form baseline, respectively. *p*-value from a repeated measures Skilling-Mack test. High-fat dairy category includes double cream, high-fat cheese, medium-fat cheese and butter. Low-fat dairy category 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 3. Mean and standard deviations across study periods of blood levels of fatty acids pentadecanoic (C15:0), heptadecanoic (C17:0), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)

	Generic-advice group					Intervention group				
	t0	**t3**	**t6**	**Total**	***p*-value**	**t0**	**t3**	**t6**	**Total**	***p*-value**
	(289)	(243)	(228)	(760)		(889)	(777)	(722)	(2388)	
Pentadecanoic (C15:0)										
Mean	0.20	0.21	0.21	0.21	0.145	0.20	0.21	0.21	0.21	0.014
sd	0.06	0.05	0.06	0.06		0.06	0.05	0.06	0.06	
Between sd				0.05					0.05	
Within sd				0.03					0.03	
Heptadecanoic (C17:0)										
Mean	0.32	0.34	0.31	0.32	p<0.001	0.32	0.34	0.31	0.32	p<0.001
sd	0.05	0.07	0.05	0.06		0.06	0.07	0.07	0.06	
Between sd				0.05					0.05	
Within sd				0.04					0.04	
EPA+DHA										
Mean	3.75	4.01	3.93	3.89	0.033	3.77	4.04	4.07	3.95	p<0.001
sd	1.17	1.19	1.17	1.18		1.25	1.30	1.29	1.28	
Between sd				1.09					1.17	
Within sd				0.49					0.56	

sd: standard deviation. Between sd: between-subject standard deviation, Within sd: within-subject standard deviation. t0 is baseline, t3 and t6 are three months and six months form baseline, respectively. Number of observations in parentheses. *p*-value from a repeated measures ANOVA with Box's conservative correction factor.

Table 4. Estimates for intra-class correlation coefficients (ICC), within-subject coefficient of variation (WCV) and Spearman correlations for measurement of blood fatty acid levels over time

Fatty acid	N (n)	ICC	95% CI[§] (Lower limit)	95% CI (Upper limit)
C15:0	760 (334)	0.62	0.57	0.68
C17:0	760 (334)	0.32	0.28	0.46
EPA+DHA	760 (334)	0.67	0.62	0.71
Fatty acid	**N**	**WCV**	**95% CI (Lower limit)**	**95% CI (Upper limit)**
C15:0	760 (334)	16.6	14.9	18.3
C17:0	760 (334)	14.6	13.3	16.0
EPA+DHA	760 (334)	17.4	16.1	18.8
Fatty acid	**(n)**	**Spearman correlation***	**95% CI (Lower limit)**	**95% CI (Upper limit)**
C15:0				
t0 – t3	205	0.69	0.61	0.78
t3 – t6	196	0.67	0.58	0.76
t0 – t6	194	0.66	0.57	0.76
C17:0				
t0 – t3	205	0.47	0.36	0.59
t3 – t6	196	0.38	0.26	0.51
t0 – t6	194	0.36	0.22	0.49
EPA+DHA				
t0 – t3	205	0.75	0.69	0.82
t3 – t6	196	0.68	0.60	0.76
t0 – t6	194	0.68	0.59	0.77

N (n): total number of observations (number of participants). ICC, Spearman correlations and coefficient of variation calculated for fatty acid levels in dry blood spot samples. ICC calculated on the log-transformed fatty acid levels using the variance components from a random effects model. WCV calculated on the logarithmic scale using a one-way random effects model with MLE estimation procedure. *Unadjusted Spearman correlations. [§]Confidence intervals from bootstrap with 1000 replications. Generic-advice group only.

Table 5. Association between change in dairy (portion/day) and % change in blood pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) levels*

	C15:0				C17:0			
	b	se	p	(95% CI)	b	se	p	(95% CI)
Total dairy	**1.02**	**0.5**	**0.023**	**(0.14,1.91)**	0.09	0.4	0.829	(-0.75,0.93)
High-fat dairy	**0.32**	**0.1**	**0.019**	**(0.05,0.58)**	**0.27**	**0.1**	**0.002**	**(0.1,0.45)**
Low-fat dairy	0.19	0.3	0.483	(-0.35,0.73)	0.21	0.3	0.415	(-0.29,0.71)
Milk	0.39	0.8	0.649	(-1.28,2.05)	-0.26	0.8	0.736	(-1.74,1.23)
Cream	4.74	2.8	0.091	(-0.77,10.25)	**9.42**	**3.0**	**0.002**	**(3.43,15.4)**
Yoghurt	0.46	1.0	0.635	(-1.44,2.36)	-0.79	0.9	0.399	(-2.64,1.05)
Cheese	**1.77**	**0.6**	**0.006**	**(0.52,3.02)**	0.69	0.6	0.208	(-0.39,1.78)
Butter	**3.34**	**1.0**	**0.001**	**(1.34,5.35)**	1.3	1.2	0.271	(-1.01,3.61)

*All regressions adjusted for total energy intake, age, BMI, and smoking status; as well as intake of alcohol, meat, oily fish, savoury pastries and sweet pastries. se: standard error, p: p-value. High-fat dairy category includes double cream, high-fat cheese, medium-fat cheese and butter. Low-fat dairy category 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. Intervention group only.

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7 Conflicts of interest

CAD is a cofounder, stock-owner, board member and consultant for Vitas AS. TEG is a cofounder, CEO and stock-owner of Vitas AS.