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Dairy product consumption is associated with a lowering of linoleic acid within serum TAG in adolescent females with overweight or obesity: a secondary analysis

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Abstract

Dairy fat is rich in SFA such as palmitic acid (16:0) but low in linoleic acid (18:2*n*-6). The natural carbon 13 enrichment (δ13C) of 16:0 is higher in dairy fat than in most of the food supply. In adults, serum levels of pentadecanoic acid (15:0) and heptadecanoic acid (17:0) are recognised as biomarkers of dairy intake. In adolescents, no study has evaluated serum fatty acid levels or δ13C in response to chronic dairy consumption. The objectives of this study were to evaluate whether increased dairy product consumption can modulate (1) serum fatty acid levels and (2) 16:0 δ13C in adolescents with overweight/obesity who followed a 12-week weight management programme. This secondary analysis of a randomised control trial included two groups of adolescent females: recommended dairy (RDa; *n* 23) and low dairy (LDa; *n* 23). The RDa group was given 4 servings/d of dairy products while the LDa group maintained dairy intakes at ≤ 2 servings/d. Blood was sampled before and after the intervention. Lipids were extracted and separated, and fatty acids were quantified by GC. Isotope ratio MS was used to assess 16:0 δ13C. There were no group differences on serum changes of 15:0 or 17:0. Within TAG, 18:2*n*-6 was lowered by 7·4 % only in the RDa group ($P = 0{\cdot}040$). The difference in delta 16:0 δ13C between the LDa and RDa groups did not reach statistical significance ($P = 0{\cdot}070$). Reductions in serum 18:2*n*-6 by dairy consumption could have positive health implications, but more studies are needed to confirm this assertion.

Key words: **Dairy: Fatty acid: SFA: Linoleic acid: Weight management**

Dairy products can be part of a healthy diet as they contain a significant number of micronutrients, are rich in high-quality protein and have been associated with the consumption of a nutrient-dense diet[1]. These characteristics make dairy products a good food choice, particularly for adolescents who need to consume higher amounts of nutrients per kg of body weight than adults because of requirements for growth and development[2]. However, regular dairy products are also rich in fat and most notably saturated fats[3,4]. For example, one serving of cheddar cheese contains approximately 35 % fat of which more than half is saturated fat. Because of this, observational studies have looked at whether long-term consumption of a diet rich in dairy products is associated with an increased risk of developing metabolic diseases such as CVD or type 2 diabetes[5–7]. Interestingly, most studies find no adverse effect of higher dairy intake and some suggest benefit, and this does not appear to be specific to low-fat dairy products[8].

Dairy is rich not only in palmitate (16:0) but also in SFA shorter than 16 carbons such as myristic acid (14:0)[3]. Dairy also contains significant amounts of odd chain SFA such as pentadecanoic acid (15:0) and heptadecanoic acid (17:0), in addition to dairy-specific *trans* fatty acids such as 16:1 *trans*-9 or 18:1 *trans*-11. When compared with most vegetable oils and margarines, dairy fat contains very low amounts of the *n*-6 PUFA linoleic acid (18:2*n*-6), the major dietary PUFA in the western-style eating pattern[9]. Some fatty acids have been proposed as markers of dairy intake such as 15:0 and 17:0[10–14]. Serum levels of these fatty acids have been associated with a lower risk of developing CVD[15] and type 2 diabetes[16] in adults. Little is known, however, whether these markers are valid in a population of adolescent females who are generally low dairy consumers and may benefit from increased dairy product consumption because of growth and development requirements. Moreover, markers of dairy intake have never been validated in younger persons with overweight or obesity.

Abbreviations: FAME, fatty acid methyl ester; LDa, low dairy; mUr, milliUrey; RCT, randomised control trial; RDa, recommended dairy; TC, total cholesterol; 14:0, myristic acid; 15:0, pentadecanoic acid; 17:0, heptadecanoic acid; 18:2*n*-6, linoleic acid; δ13C, carbon 13 enrichment.

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Our group has recently developed a method to trace fatty acid metabolism using natural variations in carbon 13 enrichment (δ13C) called compound-specific isotope ratio analysis[17–20]. This technique uses variations in the carbon isotopic pools of the food supply to trace fatty acid metabolism. Carbon from maize, a food often included as part of bovine feed, is more enriched with 13C than most of the food supply. A significant portion of dairy 16:0 comes from bovine lipogenesis and thus the δ13C of dairy 16:0 might be higher than serum 16:0 in humans. However, whether increased dairy consumption is associated with elevations in serum 16:0 δ13C in humans is unknown. The primary objective of this study was to evaluate whether increased dairy consumption can modulate fatty acid levels within serum lipid classes in adolescent females with overweight/obesity who followed a 12-week weight management, lifestyle modification (diet and exercise) programme as part of a randomised control trial (RCT). The secondary objective was to evaluate whether increased dairy consumption can elevate serum 16:0 δ13C. We hypothesised that increased dairy consumption will be associated with elevated serum levels of 15:0 and 17:0 in addition to increased δ13C of 16:0.

Methods

This study is a secondary analysis of the 'I.D.E.A.L. (Improving Diet, Exercise and Lifestyle) for Adolescents Study', a 12-week RCT with a parallel design carried out in adolescent females with overweight and obesity. The I.D.E.A.L study was a weight management intervention trial aimed at evaluating body composition changes and bone health in these adolescent females who underwent a 12-week lifestyle modification programme that included dietary counselling with dairy intake manipulations and an individualised exercise programme. The main study was registered at www.clinicaltrials.gov (NCT#02581813), and the major findings and related details such as RCT reporting and randomisation have been published previously[21–23]. Participants were recruited from the community surrounding Brock University (St. Catharines) over a 2-year period starting in May 2016. To be eligible, participants had to have overweight (BMI > 85th–97th percentile) or obesity (BMI > 97th percentile), be between 10- and 18-year-old, menarcheal, low dairy consumers (<2 servings/d; validated by a food record), sedentary (physical activity no more than 2 times/week) and have no allergy to dairy or lactose intolerance. All participants (and their parents/guardians) gave written informed assent and consent, respectively, to participate, and the Brock University Biosciences Research Ethics Board approved the study (BREB #14–284).

Study design

From the main RCT, a total of forty-six participants were included in this analysis. Participants were randomised into two intervention groups: recommended dairy (RDa; *n* 23) or low dairy (LDa; *n* 23). Both groups received individualised dietary counselling from a registered dietitian at regular intervals (weeks 0, 2, 4, 8 and 12). The dietary counselling focused primarily on adopting general healthy eating patterns. For example, advice to both groups included minimising/avoiding processed foods, higher fat foods, sugar-sweetened beverages, pastries, and candy/confection and to replace these foods with vegetables, fruits, foods higher in fibre and whole grains, lean meats and meat alternatives. In terms of dairy-specific counselling, participants in the LDa group were advised to maintain their low habitual dairy intake (≤ 2 servings/d) and to consume non-dairy protein sources. They were also asked to avoid calcium-fortified beverages and juices (e.g., soya, almond, rice and orange/fruit juices). Participants in the RDa group were provided with and instructed to consume 4 servings/d of a mixture of dairy products including 1 % milk, 1 % chocolate milk, 0 or 2 % flavoured Greek yogurt (most often 2 %) or full-fat cheddar cheese. The supplied dairy products (from Danone Canada (Greek yogurt) and Parmalat Canada (cheese and milk)) were the same (aside from preferred yogurt flavour) for all participants in the RDa group and adherence with their consumption was very high (100 % adherent). Similarly, adherence to low dairy food consumption was high in the LDa group (100 % adherent). Specifically, dairy adherence was assessed by noting the average consumption of dairy product servings from the week 4, 8 and 12 food records. Participants were considered adherent if they consumed ≥ 3 (RDa) or ≤ 2 (LDa) servings/d of dairy products (as previously reported in[22]). Participants in both groups also received individualised exercise programmes over the 12-week period. The exercise intervention, which was primarily the same between the groups, consisted of three, guided (with a personal trainer), 60–90-minute mixed-exercise sessions/week. Briefly, each session included an aerobic exercise workout, combined with either a plyometric or resistance exercise workout. Aerobic exercise was completed on either a treadmill, stationary bike, rowing ergometer, or an elliptical machine. Resistance exercise utilised 4–5 different machines including seated row, chest press, leg curl and leg press to facilitate a whole-body workout. Plyometric exercise consisted of 4–5 jumping exercises including box jumps and jumping jacks (3 sets of 8–15 jumps per exercise) for a total of 96–225 jumps per workout. Further details about both the dietary counselling and exercise programme have been reported elsewhere[21,22].

Participant characteristics and biochemical analyses

Fasted, rested blood samples were collected by a trained phlebotomist before and after the 12-week dietary intervention. Similar collection patterns were employed for each participant pre- and post-intervention (i.e., blood was collected within the same menstrual phase (follicular) and at the end of the study, within days of official study completion). Specifically, they were collected from a vein in the antecubital fossa using standard venipuncture procedures between 08.00 and 10.00 following an overnight (10–12 h) fast into vacutainer tubes (with SST clot activator) and centrifuged at ≤ 1300 relative centrifugal field (g) for 15 min. Serum was then aliquoted into 0·5 ml cryovials and stored at –80°C until post-study analysis (i.e., following the completion of the data collection phase of the entire study). With regard to lipid analyses, the Cholestech LDX System (A&D Medical, Mississauga) was used to analyse total cholesterol (TC), TAG, HDL-C, LDL-C and glucose from frozen serum, post-study. Also, anthropometric and body composition measures, including height, weight, waist circumference, fat mass, % body fat and lean mass (*via* the BodyMetrix™ System, BX-2000,

IntelaMetrix, Inc.), were collected at the same timepoints (before and after the 12-week intervention). Specific details about these procedures have been reported previously[21–23].

Lipid extraction, separation by thin-layer chromatography and fatty acid quantification

Total lipids were extracted from 200 µl of serum using a modified version of the Folch et al. method[24]. Serum samples were homogenised by vortex in a solution of 2:1 chloroform: methanol. The mixtures were kept at 4°C overnight and brought to room temperature the next day. Potassium chloride (0·88 % (w/v)) was added to separate phases. The mixtures were vortexed, and phase separation was achieved by a 10 min centrifugation at 500 × g. The lipid-containing bottom chloroform phase was transferred into new test tubes and kept. Chloroform was added a second time to the methanol and potassium chloride mixture. Samples were vortexed and centrifuged a second time, and the bottom phase was collected again to ensure optimal extraction efficiency. The total lipid extracts were then dried down under a stream of nitrogen and reconstituted in 100 µl of chloroform. Lipid class separation was performed by thin-layer chromatography using TLC-G plates (Millipore Sigma) using heptane: diethyl ether: glacial acetic acid (60:40:2, by volume) as the migration solvent. Lipids were revealed by spraying a 0·1 % w/v solution of 8-anilino-1-naphthalene sulfonic acid in methanol and visualised under UV light. The phospholipid and TAG fractions were then scraped and transferred into new test tubes containing 27·6 µg of docosatrienoic acid ethyl ester (22:3*n*-3) as an internal standard for total fatty acid quantification. Fatty acids were transmethylated at 100°C for 1 hour with 14 % (v/v) boron trifluoride methanol. Fatty acid methyl esters (FAME) were quantified on a Varian 430 GC (Bruker) equipped with a SP-2560 biscyanopropyl siloxane capillary column (100 m length × 0·25 mm diameter × 0·2 µm film thickness; Supelco). Helium was the carrier gas, and 1 µl of samples were injected in splitless injection mode at 250°C. The GC oven temperature programme was: 60°C initially for 2 min followed by a 10·0°C/min ramp to 170°C with a 4 min hold, a 6·5°C/min ramp to 175°C, a 2·6°C/min ramp to 185°C, a 1·3°C/min ramp to 190°C and a 8·0°C/min ramp to 240°C with a 19 min hold for a total run time of 50·71 min. FAME were quantified by flame ionisation detection, and the peaks were identified through comparison with an external mixed FAME standard (GLC-569, Nu-Chek Prep Inc.).

Carbon 13 isotopic analysis, reporting and normalisation

For this exploratory analysis, carbon 13 isotopic evaluation was carried out from the TAG-derived FAME from the serum of a randomly selected subset of participants (*n* 11–12/group/timepoint). We looked at the isotopic enrichment of 16:0, which is the main saturated fatty acid in dairy fat, and used 18:2*n*-6 as a control since dairy fat is very low in 18:2*n*-6 and thus dairy consumption should not affect serum 18:2*n*-6 δ13C. FAME δ13C was analysed by GC-isotope ratio mass spectrometry using a Delta V isotope ratio mass spectrometry system (Thermo Fisher Scientific) as described previously[18,19]. For each dairy product, we measured δ13C of 16:0 and 18:2*n*-6 in duplicate. Serum δ13C of 16:0 was quantified by multipoint linear regression, normalised and converted to the international carbon isotope reference scale, Vienna Peedee Belemnite, as described previously[17–20]. FAME reference standards with calibrated δ13C values (USGS70, USGS71 and USGS72; Reston Stable Isotope Laboratory, USA Geological Survey) were used to produce the standard curve and the linear regression equation. The δ13C reporting unit is milliUrey (mUr). A 1 mUr change equals to a 0·1 % (or 1‰) change in ^{13}C:^{12}C compared with a reference material. Therefore, a positive delta δ13C value is indicative of an increase in δ13C.

Statistical analyses

Because this study is a secondary analysis, the sample size was originally calculated with 95 % power to detect significant differences in changes in lean mass between the intervention groups[22]. For participant characteristics, serum biochemistry and fatty acid profiles within serum TAG or phospholipids, statistical analyses were carried out using repeated measures ANOVA in SPSS (version 20, IBM Corp.). Group (RDa and LDa) and time (baseline and post) were the fixed factors in the model. When the group × time interaction was statistically significant, it was further explored using paired *t* tests to compare baseline and post-intervention results within each group separately. Independent samples *t* tests were also performed to compare the results between groups at each timepoint separately. Bivariate correlations were also performed on post intervention values to assess the relationships between 16:0 and 18:2*n*-6 content within serum TAG and serum HDL-C, LDL-C and TC/HDL-C. For 16:0 and 18:2*n*-6, δ13C comparisons were made between the groups, and all results are presented as delta δ13C. Delta δ13C is defined as fatty acid δ13C post intervention – fatty acid δ13C at baseline. Because data were not normally distributed in one of the groups for this analysis, the difference between the groups was assessed by a non-parametric Mann–Whitney *U* test. Data are presented as mean ± standard errors of the mean (SEM), and statistical significance was set at $P < 0·05$.

Results

Dairy product fatty acid profiles

The fatty acid profiles of the different dairy products that were consumed are presented in Table 1. The 0 % Greek yogurt is not presented as its fat content was < 0·1 % of the food mass (data not shown). For all other dairy products, 16:0 was the most abundant fatty acid representing between 37·50 ± 0·10 % and 41·58 ± 0·34 % of the total fatty acids depending on the dairy product (Table 1). The most abundant odd chain fatty acid was 15:0, representing between 1·18 ± 0·01 % and 1·49 ± 0·01 % of the total fatty acids depending on the dairy product whereas total 18:1 *trans* was the most abundant *trans* fatty acid group (Table 1) with the vast majority being 18:1 *trans*-11 (data not shown). Accurate quantification of 18:1 *trans*-11 was not possible due to significant overlap with other 18:1 *trans* fatty acids.

Participant characteristics and serum biochemistry

The mean age of the participants at baseline was similar between the LDa and RDa groups (online Supplementary Table 1). There

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Table 1. Fatty acid relative percent in dairy food (mean values and standard errors of the mean)

	White milk 1 %		Chocolate milk 1 %		Full-fat cheddar cheese		2 % Greek yogurt	
Fatty acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
14:0	11·01	0·52	11·02	0·07	14·63	0·10	12·83	0·43
15:0	1·29	0·04	1·18	0·01	1·49	0·01	1·36	0·01
16:0	41·33	0·78	37·50	0·10	41·58	0·34	39·07	0·53
17:0	0·81	0·01	0·73	0·01	0·82	0·01	0·85	0·01
18:0	12·92	0·16	14·37	0·09	10·28	0·12	12·58	0·26
20:0	0·20	0·00	0·28	0·01	0·14	0·01	0·19	0·01
Total SFA	67·56	1·48	65·08	0·13	68·95	0·54	66·88	0·52
14:1*n*-5	0·70	0·06	0·94	0·01	1·24	0·03	1·01	0·06
16:1*n*-7	2·03	0·11	2·05	0·01	2·27	0·04	2·19	0·03
16:1 *trans*-9	0·52	0·09	0·37	0·01	0·35	0·03	0·41	0·03
17:1*n*-7	0·21	0·02	0·21	0·01	0·22	0·01	0·24	0·01
18:1*n*-7	0·68	0·01	0·65	0·01	0·60	0·01	0·69	0·03
18:1*n*-9	22·68	1·34	24·99	0·08	21·30	0·18	23·70	0·27
18:1 *trans* (all isomers)	2·44	0·37	2·19	0·03	1·86	0·27	1·69	0·34
20:1*n*-9	0·12	0·01	0·12	0·01	0·10	0·01	0·12	0·01
Total MUFA	29·38	1·19	31·53	0·07	27·94	0·47	30·05	0·46
18:2*n*-6	2·23	0·24	2·55	0·07	2·19	0·07	2·23	0·06
18:2*n*-6 *cis*-9, *trans*-11	0·26	0·01	0·22	0·01	0·24	0·01	0·16	0·05
20:3*n*-6	0·12	0·01	0·11	0·01	0·09	0·01	0·11	0·01
Total *n*-6 PUFA	2·61	0·25	2·88	0·08	2·52	0·07	2·50	0·08
18:3*n*-3	0·45	0·05	0·51	0·01	0·59	0·01	0·56	0·01
Total *n*-3 PUFA	0·45	0·05	0·51	0·01	0·59	0·01	0·56	0·01

n 4 per dairy product.

were group × time interactions for dairy servings per day. Post-intervention and compared with baseline, dairy servings per day decreased by 59 % in the LDa group and increased by 181 % in the RDa group ($P = 0·003$ and $P < 0·001$, respectively). Post-intervention, dairy servings per day was 882 % higher in the RDa group compared with the LDa group ($P < 0·001$; online Supplementary Table 1). There were no significant differences between the dairy groups at any timepoint and no effect of the intervention on serum glucose, HDL-C, LDL-C, TC and TC/HDL-C (online Supplementary Table 1).

Within serum phospholipids, 15:0, 17:0 and dairy trans fatty acid changes were not different between the dairy groups. There were no significant group × time interactions or group effects for any of the fatty acids within the serum phospholipid fraction (Table 2). There were, however, significant time effects for 17:0, 20:0, total saturated fatty acids, 17:1*n*-7 and 20:4*n*-6 (Table 3). Post intervention, the relative percents of 17:0, 17:1*n*-7 and 20:4*n*-6 were on average 4·2, 14·2 and 4·4 % higher than baseline, respectively, and this was independent of group (Table 2). Post intervention, the relative percents of 20:0 and total saturated fatty acids were on average 5·7 and 1·0 % lower than baseline, respectively, and this was independent of group (Table 2).

Within serum TAG, 18:2n-6 was lowered only in the RDa group. There were no group x time interactions or group effects for 15:0, 17:0, or any of the dairy-specific *trans* fatty acids within the serum TAG fraction (Table 3). There was, however, a significant group × time interaction for 24:0 and trends for 16:0 and total SFA, but the post hoc analysis for 24:0 revealed no statistically significant differences between the groups at either timepoint or over time within each group (Table 3).

Interestingly, there were group × time interactions for relative percents of 18:2*n*-6 and 20:2*n*-6 within serum TAG (Table 3). Post-intervention, 18:2*n*-6 was 7·4 % lower compared with baseline in the RDa group only ($P = 0·040$). On the other hand, 20:2*n*-6 was 11·6 % higher post-intervention compared with baseline in the LDa group only ($P = 0·004$). There was also a time effect for 22:5*n*-6 for which its relative percent was 12·0 % higher post intervention compared with baseline and this was independent of group (Table 3).

Within serum TAG, 16:0 correlated with serum cholesterol in the LDa group only. In the LDa group, relative percent of 16:0 within serum TAG was inversely correlated with serum HDL-C (Fig. 1(a)). An absolute increase of 10 % in 16:0 was associated with 30 % lower HDL-C levels. In the LDa group, relative percent of 16:0 within serum TAG was correlated with serum TC/HDL-C (Fig. 1(e)). An absolute increase of 10 % in 16:0 was associated with 40 % higher TC/HDL-C levels. In the RDa group, there were no statistically significant correlations between 16:0 within serum TAG and any of the cholesterol biomarkers (Fig. 1(b), (d), (f)).

Within serum TAG, 18:2n-6 content correlated with serum TC/HDL-C in the RDa group. In the RDa group, relative percent of 18:2*n*-6 within serum TAG was positively correlated with serum TC/HDL-C (Fig. 2(f)). An absolute decrease of 10 % in 18:2*n*-6 was associated with 39 % lower TC/HDL-C levels. In the LDa group, there were no statistically significant correlations between 18:2*n*-6 within serum TAG and any of the cholesterol biomarkers (Fig. 2(a), (c), (e)).

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Table 2. Fatty acid relative percent in serum phospholipids (mean values and standard errors of the mean)

	LDa				RDa						
	Baseline		Post		Baseline		Post				
Fatty acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P_{group}	P_{time}	$P_{interaction}$
14:0	0·34	0·02	0·31	0·01	0·33	0·02	0·35	0·02	0·484	0·589	0·087
15:0	0·19	0·01	0·19	0·01	0·20	0·01	0·21	0·01	0·164	0·171	0·295
16:0	26·77	0·31	26·79	0·28	27·24	0·33	27·18	0·28	0·255	0·917	0·874
17:0	0·40	0·02	0·42	0·02	0·41	0·01	0·42	0·01	0·686	0·049	0·991
18:0	15·06	0·31	14·72	0·27	14·74	0·27	14·51	0·25	0·461	0·079	0·714
20:0	0·29	0·02	0·29	0·02	0·30	0·03	0·27	0·02	0·902	0·043	0·249
22:0	0·71	0·06	0·70	0·07	0·70	0·08	0·65	0·07	0·735	0·182	0·369
23:0	0·15	0·02	0·13	0·02	0·13	0·01	0·13	0·01	0·652	0·347	0·399
24:0	0·58	0·06	0·58	0·06	0·56	0·06	0·50	0·06	0·534	0·081	0·095
Total SFA	44·50	0·26	44·12	0·24	44·60	0·29	44·23	0·33	0·783	0·020	0·964
16:1*n*-7	0·56	0·04	0·54	0·04	0·57	0·03	0·56	0·03	0·760	0·506	0·646
16:1 *trans*-9	0·17	0·02	0·17	0·01	0·18	0·01	0·19	0·01	0·378	0·927	0·498
17:1*n*-7	0·13	0·01	0·14	0·02	0·12	0·01	0·14	0·02	0·894	0·022	0·517
18:1*n*-7	1·34	0·03	1·37	0·05	1·38	0·04	1·38	0·04	0·691	0·509	0·623
18:1*n*-9	9·22	0·21	9·11	0·21	9·14	0·25	9·47	0·28	0·609	0·532	0·229
18:1 *trans* (all isomers)	0·43	0·03	0·50	0·04	0·45	0·02	0·46	0·02	0·640	0·125	0·251
20:1*n*-9	0·16	0·01	0·16	0·01	0·16	0·01	0·16	0·01	0·986	0·713	0·952
24:1*n*-9	1·09	0·11	1·09	0·11	1·11	0·10	0·96	0·10	0·698	0·055	0·053
Total MUFA	13·10	0·19	13·09	0·24	13·10	0·28	13·32	0·28	0·700	0·568	0·509
18:2*n*-6	23·70	0·48	24·02	0·48	24·08	0·52	23·40	0·57	0·846	0·628	0·185
18:2 *cis*-9, *trans*-11	0·04	0·01	0·04	0·01	0·04	0·01	0·04	0·01	0·362	0·062	0·456
18:2 *trans*-9, 12	0·03	0·02	0·01	0·01	0·02	0·01	0·01	0·01	0·460	0·285	0·360
18:3*n*-6	0·09	0·01	0·07	0·01	0·09	0·01	0·08	0·01	0·888	0·092	0·528
20:2*n*-6	0·34	0·01	0·33	0·01	0·33	0·01	0·34	0·01	0·953	0·986	0·351
20:3*n*-6	3·37	0·17	3·26	0·21	2·78	0·25	3·09	0·18	0·156	0·384	0·079
20:4*n*-6	9·61	0·40	9·98	0·36	9·84	0·35	10·32	0·32	0·545	0·043	0·801
22:2*n*-6	0·12	0·01	0·11	0·00	0·11	0·01	0·11	0·01	0·307	0·392	0·266
22:4*n*-6	0·36	0·01	0·38	0·02	0·36	0·01	0·38	0·02	0·810	0·072	0·974
22:5*n*-6	0·36	0·02	0·37	0·01	0·36	0·02	0·36	0·02	0·764	0·603	0·705
Total *n*-6 PUFA	38·03	0·22	38·59	0·27	38·00	0·44	38·14	0·42	0·585	0·173	0·417
18:3*n*-3	0·29	0·02	0·26	0·01	0·27	0·02	0·27	0·02	0·800	0·240	0·277
20:5*n*-3	0·64	0·05	0·59	0·04	0·67	0·05	0·69	0·04	0·278	0·487	0·251
22:5*n*-3	0·79	0·03	0·81	0·03	0·88	0·04	0·87	0·03	0·096	0·847	0·537
22:6*n*-3	2·64	0·14	2·56	0·10	2·48	0·10	2·48	0·12	0·407	0·550	0·524
Total *n*-3 PUFA	4·37	0·16	4·21	0·11	4·30	0·14	4·31	0·13	0·933	0·362	0·330
Total (μg/ml)	990·63	31·22	979·55	34·90	1003·16	26·58	983·87	27·96	0·829	0·416	0·825

n 23 per group, LDa, low dairy; RDa, recommended dairy.

Within serum TAG, there were no differences in 16:0 carbon 13 enrichment between the groups. The 16:0 δ13C of the fat-containing dairy products was on average 3·5 ± 0·5 mUr higher than the participants' baseline serum TAG 16:0 δ13C. This is an estimate calculated using the mean 16:0 δ13C of the four fat-containing dairy foods compared with the mean 16:0 δ13C value within TAG of all participants. Within serum TAG, 16:0 delta δ13C was –0·36 ± 0·39 mUr in the LDa group *v.* 0·57 ± 0·44 mUr in the RDa group, but this difference did not reach statistical significance ($P = 0·070$, Fig. 3). Serum 18:2*n*-6 delta δ13C was not different between the groups (Fig. 3).

Discussion

The results presented in this study suggest that 15:0, 17:0 and dairy *trans* fatty acids within serum phospholipids or TAG are not elevated following a dairy-rich diet in adolescent females with overweight/obesity who underwent a 12-week lifestyle modification programme for weight management. TAG content of 18:2*n*-6, however, was lowered significantly by the increased consumption of dairy foods which is in agreement with Abdullah et al.[14]. Interestingly, both 16:0 and 18:2*n*-6 correlated differently with serum cholesterol depending on the group suggesting that changes in TAG fatty acid profile associated with dairy intake might positively influence lipoprotein metabolism. To our knowledge, this is the first clinical intervention study to investigate the relationship over time between dairy intake and the serum fatty acid response in a population of adolescent girls with overweight/obesity.

The human body cannot synthesise odd chain fatty acids in substantial amounts, and therefore, their presence in tissues is believed to originate mostly from the diet. These fatty acids are almost exclusively found in ruminant animals (i.e. cows) with dairy products being the main dietary source[11,25]. Observational studies have linked adipocyte, plasma and erythrocyte levels of 15:0, 17:0 and dairy *trans* fatty acids with dairy food intake[10,11]. A randomised cross-over study also confirmed 15:0 and 17:0 as biomarkers of dairy intake in a large population of healthy Canadians[14]. In a RCT of 180 healthy volunteers, it

Table 3. Fatty acid relative percent in serum TAG (mean values and standard errors of the mean)

	LDa				RDa						
	Baseline		Post		Baseline		Post				
Fatty acid	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	P_{group}	P_{time}	$P_{interaction}$
14:0	1·81	0·15	1·62	0·09	1·71	0·15	1·85	0·19	0·722	0·828	0·175
15:0	0·27	0·01	0·26	0·01	0·27	0·01	0·29	0·02	0·281	0·559	0·391
16:0	24·31	0·66	23·35	0·76	24·29	0·65	25·12	0·81	0·331	0·888	0·078
17:0	0·32	0·01	0·35	0·02	0·34	0·01	0·36	0·02	0·456	0·065	0·893
18:0	3·21	0·16	3·22	0·14	3·05	0·11	3·41	0·16	0·946	0·149	0·167
20:0	0·06	0·01	0·06	0·01	0·06	0·01	0·08	0·01	0·391	0·188	0·267
22:0	0·03	0·01	0·03	0·01	0·03	0·01	0·03	0·01	0·789	0·706	0·790
23:0	0·07	0·01	0·06	0·01	0·06	0·01	0·06	0·01	0·337	0·234	0·403
24:0	0·06	0·01	0·05	0·01	0·05	0·01	0·06	0·01	0·728	0·461	0·033
Total SFA	30·14	0·85	29·00	0·90	29·87	0·76	31·26	1·04	0·367	0·845	0·056
16:1*n*-7	3·48	0·19	3·25	0·20	3·52	0·22	3·34	0·22	0·810	0·133	0·884
16:1 *trans*-9	0·12	0·01	0·13	0·01	0·12	0·01	0·13	0·01	0·849	0·460	0·909
17:1*n*-7	0·21	0·01	0·22	0·01	0·24	0·01	0·23	0·02	0·164	0·747	0·221
18:1*n*-7	2·35	0·06	2·45	0·07	2·47	0·08	2·39	0·09	0·706	0·925	0·188
18:1*n*-9	37·82	0·81	38·41	0·76	38·25	0·63	38·63	0·79	0·708	0·447	0·861
18:1 *trans* (all isomers)	0·37	0·03	0·43	0·05	0·42	0·04	0·43	0·05	0·457	0·391	0·530
20:1*n*-9	0·32	0·01	0·34	0·02	0·33	0·01	0·36	0·02	0·378	0·101	0·795
24:1*n*-9	0·05	0·01	0·05	0·01	0·05	0·01	0·04	0·01	0·499	0·308	0·693
Total MUFA	44·71	0·77	45·27	0·80	45·41	0·64	45·54	0·76	0·557	0·604	0·747
18:2*n*-6	19·59	0·71	20·22	0·77	19·39	0·66	17·96*	0·77	0·184	0·415	0·037
18:2 cis-9, *trans*-11	0·09	0·01	0·09	0·01	0·10	0·01	0·11	0·01	0·105	0·874	0·494
18:2 *trans*-9, 12	0·10	0·01	0·11	0·01	0·11	0·01	0·12	0·01	0·237	0·533	0·601
18:3*n*-6	0·39	0·05	0·35	0·04	0·35	0·03	0·33	0·03	0·464	0·156	0·629
20:2*n*-6	0·25	0·01	0·28**	0·01	0·26	0·01	0·25	0·01	0·392	0·144	0·009
20:3*n*-6	0·34	0·02	0·34	0·02	0·32	0·01	0·31	0·01	0·269	0·950	0·659
20:4*n*-6	1·41	0·16	1·29	0·11	1·23	0·07	1·28	0·11	0·555	0·608	0·146
22:2*n*-6	0·19	0·03	0·16	0·01	0·15	0·02	0·15	0·02	0·339	0·346	0·252
22:4*n*-6	0·22	0·02	0·21	0·01	0·22	0·02	0·22	0·01	0·867	0·538	0·948
22:5*n*-6	0·10	0·01	0·11	0·01	0·09	0·01	0·10	0·01	0·440	0·030	0·356
Total *n*-6 PUFA	22·68	0·85	23·18	0·82	22·23	0·70	20·83	0·83	0·176	0·381	0·068
18:3*n*-3	1·60	0·10	1·67	0·13	1·58	0·10	1·48	0·11	0·402	0·870	0·366
20:5*n*-3	0·18	0·02	0·16	0·02	0·18	0·02	0·18	0·02	0·761	0·319	0·304
22:5*n*-3	0·33	0·02	0·34	0·02	0·37	0·02	0·36	0·02	0·315	0·891	0·488
22:6*n*-3	0·37	0·03	0·38	0·02	0·36	0·03	0·36	0·03	0·701	0·850	0·607
Total *n*-3 PUFA	2·48	0·10	2·55	0·13	2·49	0·12	2·37	0·13	0·559	0·822	0·372
Total (μg/ml)	682·60	67·47	670·85	68·02	804·11	96·21	814·03	87·93	0·220	0·983	0·797

n 23 per group, LDa, low dairy; RDa, recommended dairy; *or **different from baseline.

was demonstrated that increasing dairy consumption by three servings for 1 month was associated with 16·5 and 12·5 % increases in 15:0 and 17:0 plasma levels, respectively[12]. Within plasma, phospholipid levels of 15:0 have been inversely associated with CVD and type 2 diabetes[15,16].

We did not find any significant difference between the groups with regard to 15:0, 17:0, 16:1 *trans*-9 and 18:1 *trans*. It is important to note that in most of the studies showing 15:0 or 17:0 as biomarkers of dairy intake, the effect size was between 10 and 20 % and the statistical power was high[10,14]. It is therefore likely that the present study was not sufficiently powered to detect small differences in 15:0 or 17:0 between the groups consuming different levels of dairy foods. Indeed, our study represents an exploratory secondary analysis from a larger RCT[22].

The absence of an elevation for 15:0 and 17:0 within serum phospholipids may also relate to the inherent fatty acid composition and content of the dairy foods consumed. In a study from New Zealand[12], the reported fatty acid profile from whole milk was strikingly different from what we report here and what has been reported in other studies conducted in Europe and North America[3,4]. For example, in the milk from the New Zealand study, 16:0 was 22 % lower than the sum of 14:0, 15:0 and 17:0, whereas in the dairy foods from the present study, 16:0 was over 200 % higher than the sum of 14:0, 15:0 and 17:0. Interestingly, the three reported markers of dairy intake were 14:0, 15:0 and 17:0 in that study[12], whereas none of these fatty acids were elevated by dairy consumption in the present study. Variability in dairy-derived fatty acid composition of the milk used to make dairy foods might therefore be important to consider when evaluating these fatty acid markers of dairy intake.

To our knowledge, no other study has evaluated the fatty acid response to dairy consumption within the serum TAG fraction. In serum TAG, we report no difference between the LDa and RDa groups on 15:0, 17:0, 16:1 *trans*-9 or 18:1 *trans*. Interestingly, and in accordance with a previous study[14], dairy consumption was associated with a significant reduction in 18:2*n*-6 within serum TAG and this was not caused by a difference in total PUFA intake between the dairy groups in our study (data not shown). There is a debate pertaining to the role of 18:2*n*-6 for the prevention of CVD and the maintenance of healthy blood lipids. On one hand,

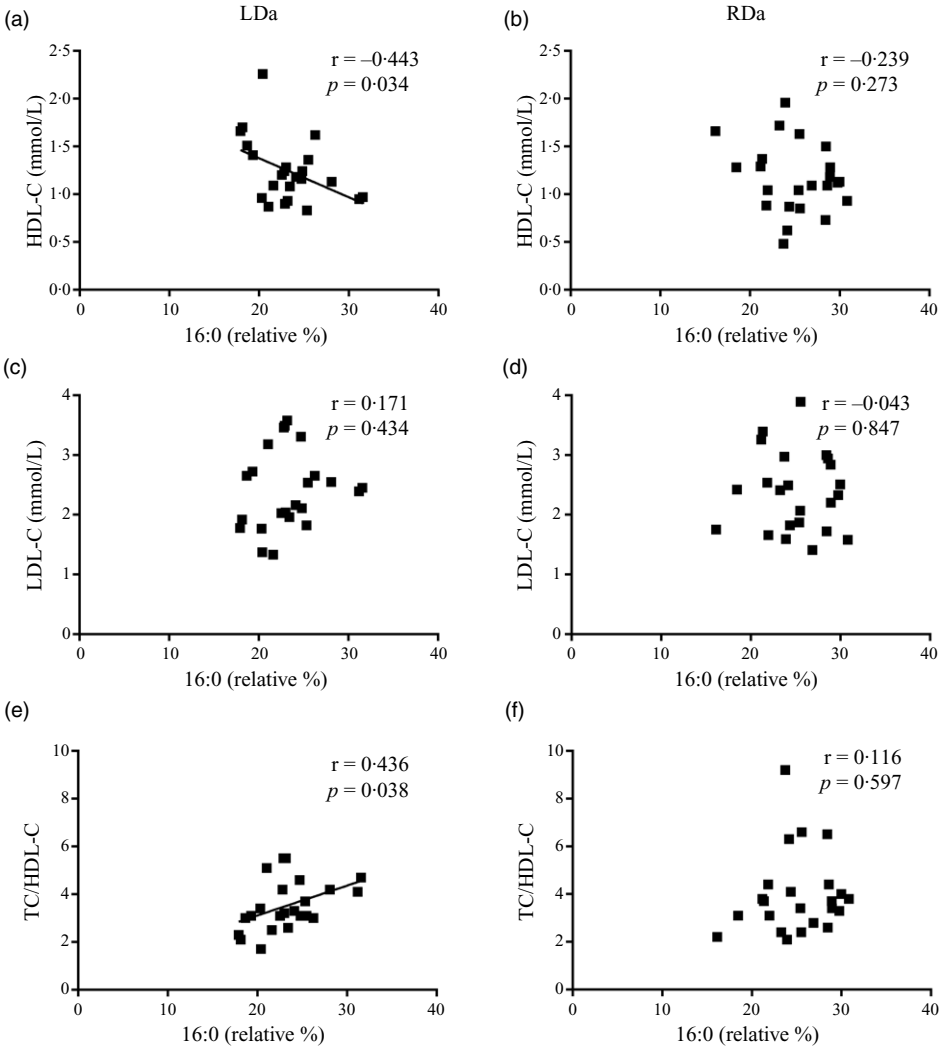

Fig. 1. Bivariate correlations between the relative percent of 16:0 within serum TAG and HDL-C (a) and (b), LDL-C (c) and (d), or TC/HDL-C (e) and (f) in participants consuming a diet low in dairy (LDa, *n* 23; left panel) or with recommended dairy (RDa; *n* 23; right panel) over 12 weeks. TC, total cholesterol.

serum levels of 18:2*n*-6 are inversely correlated with the risk of developing CVD[26,27]. On the other hand, dietary intervention studies have yielded conflicting results, suggesting a clear reduction in serum LDL-C with increased intake of 18:2*n*-6[28] but no CVD prevention[29]. Moreover, although this idea is controversial, researchers have suggested that North-Americans consume too much *n*-6 PUFA[30], most notably through vegetable oils and margarines, and that this could impair *n*-3 PUFA metabolism[31], leading to increased inflammation and CVD. It has also been proposed that increased dairy product intake could increase the capacity to synthesise *n*-3 PUFA by lowering 18:2*n*-6 serum levels[32], which could provide health benefits. However, in the present study, we did not see any statistically significant differences in serum *n*-3 PUFA levels between the LDa and RDa groups. To lower dietary intake of *n*-6 PUFA, foods rich in *n*-6 PUFA need to be replaced by other foods low in *n*-6 PUFA. Dairy, with a very low 18:2*n*-6 content, could be a good food choice to lower 18:2*n*-6 as evidenced by the results presented herein. Importantly, any potential health consequences associated with the lowering of dietary *n*-6 PUFA depend on the composition and source of the replacement food. This is also the case with saturated fats and CVD risk. Replacing dietary saturated fats with unsaturated fats lowers CVD risk, but the opposite effect is found if refined carbohydrates are used as the replacement[33].

None of the classic fatty acid markers of dairy intake were validated in the present study and thus we next evaluated whether variations in fatty acid δ13C could be more sensitive to trace dairy consumption in studies with smaller sample sizes. We showed that 16:0 from the dairy products used in the study had higher δ13C than baseline serum TAG 16:0 in our study sample. However, delta δ13C for 16:0 was not statistically different between the RDa and LDa groups. It is important to note that although we report the two-tailed *P* value as our main statistic, for this comparison our hypothesis was directional, and the 1-tailed *P* value was 0·035. This result was obtained despite the low sample size and the fact that most dairy products used in this study contained lower amounts of fat. More studies are needed to evaluate the potential of the

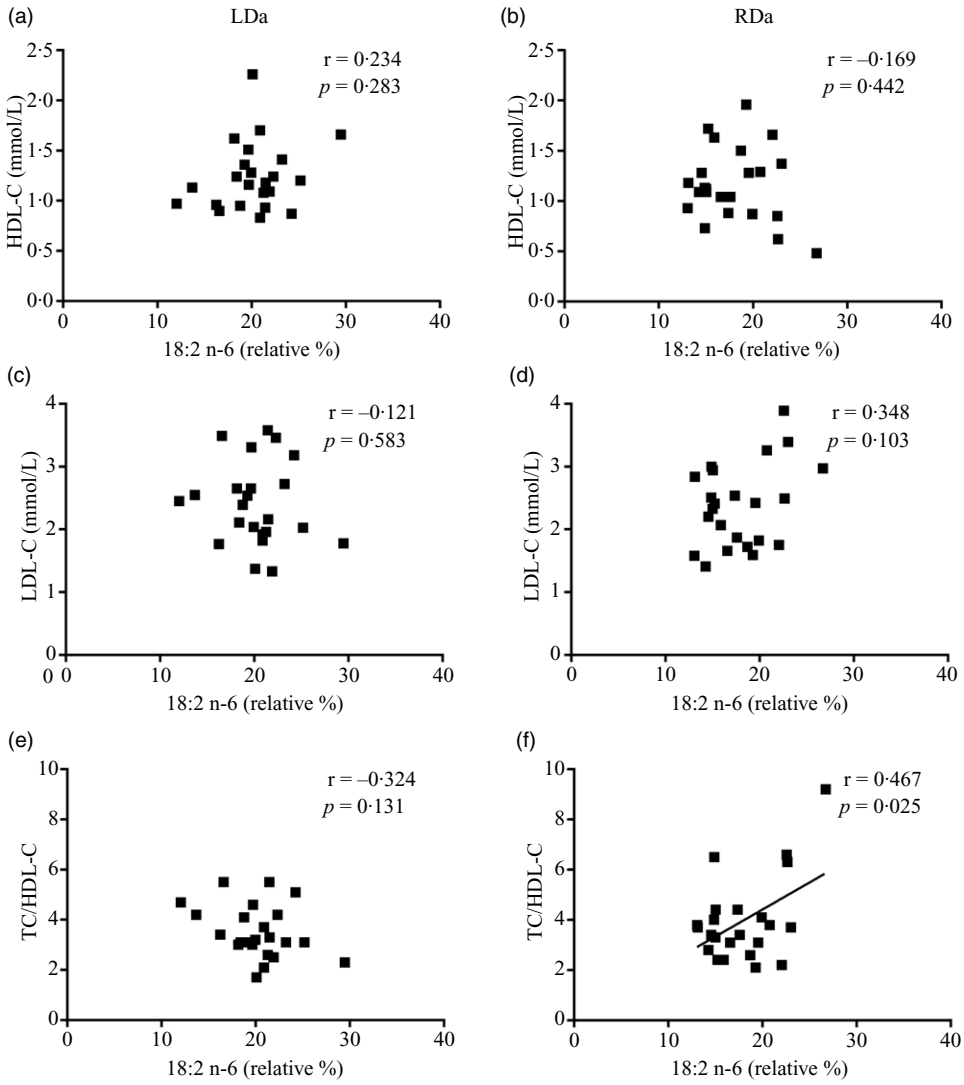

Fig. 2. Bivariate correlations between the relative percent of 18:2*n*-6 within serum TAG and HDL-C (a) and (b), LDL-C (c) and (d), or TC/HDL-C (e) and (f) in participants consuming a diet low in dairy (LDa, *n* 23; left panel) or with recommended dairy (RDa; *n* 23; right panel) over 12 weeks. TC, total cholesterol.

compound-specific isotope ratio analysis technique to trace dairy consumption, ideally by using designs that focus on dietary fat manipulations with dairy.

With regard to SFA within serum TAG, there were trends for group × time interactions for 16:0 and total SFA. However, the increases in the RDa group were not statistically significant. This result would warrant further investigation in a study with more power because dietary SFA might be associated with an increased risk of CVD although the literature on this subject is heterogenous and inconclusive[34–36]. Moreover, elevated serum SFA levels are not consistently associated with an elevated risk of CVD[37], and this could depend on the food source of SFA[38,39]. It was also recently reported in a cross-sectional study that youth with obesity have higher levels of 16:0 and lower levels of 18:2*n*-6 within serum TAG[40]. In a cohort of adults participants at risk for type 2 diabetes, concentrations of 16:0 within serum TAG have been shown to be inversely correlated with levels of HDL-C[41]. In the present study, we demonstrate a significant inverse correlation between 16:0 and HDL-C post-intervention and no significant correlation between 18:2*n*-6 and HDL-C in the LDa group at the end of the intervention and this is in agreement with the adult cohort study[41]. On the other hand, we did not observe a significant correlation between 16:0 and any of the cholesterol markers, but we did observe a significant correlation between 18:2 *n*-6 and TC/HDL in the RDa group. We therefore posit that the dietary source of SFA influences the serum TAG population which, in turn, could influence lipoprotein metabolism.

This study had several strengths, the main one being that this is the first dairy-focussed, multi-factorial, lifestyle modification RCT in adolescent girls with overweight/obesity; a population that is understudied. Another strength was the study design in that the main difference between the two intervention groups was the different intakes of dairy foods. This study also had several limitations. First, because the study involved multiple interventions as part of a total lifestyle programme (dairy nutrition, nutrition counselling, exercise, etc.), it is harder to isolate the effect of dairy alone. However, the study was designed such that the only

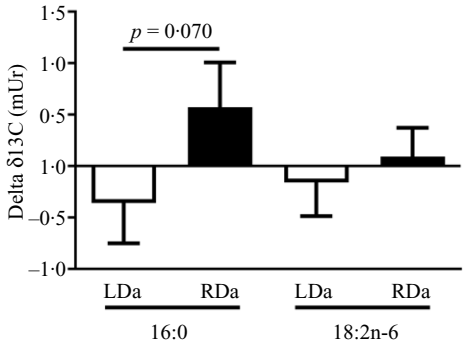

Fig. 3. Delta δ13C of 16:0 and 18:2*n*-6 within serum TAG in participants consuming a diet low in dairy (LDa, *n* 11) or with recommended dairy (RDa; *n* 12). Delta δ13C is defined as δ13C of the fatty acid post intervention – δ13C of the fatty acid at baseline. δ13C, carbon 13 enrichment.

difference between the groups was the dairy intake. Also, in some cases, one dairy product (0 % Greek yogurt) did not contain any fat, and most other dairy products except for cheddar cheese were partially skimmed/low fat. Lastly, dairy fat intake was not precisely measured in this study, and adherence to dairy product consumption was self-reported in food records. It is possible that the lower levels of absolute dairy fat ingested (despite the participants purportedly consuming 3–4 servings of dairy foods/d) could explain why we did not detect any statistical differences between the groups for the previously reported biomarkers of dairy intake. Future studies should ensure an adequate level of ingested dairy fat before making these comparisons. Moreover, ascertaining an objective marker of dairy fat intake, as we explored herein, would help to alleviate the universal issue of relying on self-reported intake data from food records.

In conclusion, this study shows that a 12-week diet and exercise intervention enriched with a variety of dairy products is associated with a 7·4 % lowering of 18:2*n*-6 within serum TAG in adolescent females with overweight/obesity. This modification in the TAG fatty acid profile could positively influence lipoprotein metabolism, which in turn could influence CVD risk. Additionally, our study provides promising results for the use of compound-specific isotope ratio analysis for assessing increased dairy intake in smaller clinical populations. Future research is necessary to further evaluate these relationships.

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All authors have read and approved the final manuscript. Authors' contribution: A. R. J. designed research, M. C. conducted research, R. C-W. and M. C. analysed data, R. C-W. wrote paper with scientific input from A. R. J. and R. P. B. R. C-W. had primary responsibility for final content.

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Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S0007114521001677

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