Incorporation of cis-9, trans-11 conjugated linoleic acid and vaccenic acid (trans-11 18:1) into plasma and leucocyte lipids in healthy men consuming dairy products naturally enriched in these fatty acids

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The present study investigated whether consuming dairy products naturally enriched in cis-9, trans-11 (c9,t11) conjugated linoleic acid (CLA) by modification of cattle feed increases the concentration of this isomer in plasma and cellular lipids in healthy men. The study had a double-blind cross-over design. Subjects aged 34–60 years consumed dairy products available from food retailers for 1 week and then either control (0.17 g c9,t11 CLA/d; 0.31 g trans-vaccenic acid (tVA)/d) or CLA-enriched (1.43 g c9,t11 CLA/d; 4.71 g tVA/d) dairy products for 6 weeks. After 7 weeks washout, this was repeated with the alternate products. c9,t11 CLA content of plasma lipids was lower after consuming the control products, which may reflect the two-fold greater c9,t11 CLA content of the commercial products. Consuming the CLA-enriched dairy products increased the c9,t11 CLA concentration in plasma phosphatidylcholine (PC) (38%; P=0.035), triacylglycerol (TAG) (22%; P<0.0001) and cholesteryl esters (205%; P<0.0001), and in peripheral blood mononuclear cells (PBMC) (238%; P<0.0001), while tVA concentration was greater in plasma PC (65%; P=0.035), TAG (98%; P=0.001) and PBMC (84%; P=0.004). Overall, the present study shows that consumption of naturally enriched dairy products in amounts similar to habitual intakes of these foods increased the c9,t11 CLA content of plasma and cellular lipids.

Conjugated linoleic acid: Blood lipids: Human diet: Dairy products

Conjugated linoleic acid (CLA) describes a group of eighteen-carbon fatty acids, which differ in the geometry and position of the two conjugated double bonds. The principal sources of CLA in the human diet are dairy products and meat from ruminants (Lawson et al. 2001), with cis-9, trans-11 (c9,t11) CLA being by far the major isomer consumed (Lawson et al. 2001). This isomer is formed as a result of biohydrogenation reactions carried out by bacteria in the rumen, which produce the precursor trans-11 octadecenoic acid (tVA) and by Δ9 desaturase, which converts tVA to c9,t11 CLA, primarily in the mammary gland (Lawson et al. 2001).

Studies in animal models in which CLA intakes were increased show anti-tumorigenic activity (Ip et al. 1991; Belury, 2002), decreased atherogenesis (Lee et al. 1994; Nicolosi et al. 1997; Munday et al. 1999), decreased adiposity and increased lean body mass (Park et al. 1997; West et al. 1998; DeLany et al. 1999). Thus, greater CLA consumption may have beneficial effects on human health (Roche et al. 2001). However, the positive outcomes indicated by the animal studies have not been replicated in human studies (Calder, 2002; Kelley & Erickson, 2003; Watkins et al. 2004). This may reflect the type of the CLA preparations used in human studies. Some studies in human subjects have used synthetic mixtures of CLA isomers, mainly c9,t11 CLA and t10,c12 CLA, with smaller amounts of other isomers, in which the amount of each isomer consumed is low relative to the total CLA intake (Benito et al. 2001; Kelley et al. 2001; Von Loeffelholz et al. 2003). Other authors have reported the effects of 50:50 and 80:20 preparations of c9,t11 and t10,c12 CLA (Mougios et al. 2001; Masters et al. 2002; Noone et al. 2002; Albers et al. 2003) and others have used encapsulated triacylglycerols (TAG) highly enriched (≥80%, w/w) in c9,t11 or t10,c12 CLA (Burdge et al. 2004). While low intakes of individual isomers in CLA mixtures may account for the limited effectiveness in altering biological outcomes in human subjects, consumption of approximately 2.5 g c9,t11 or t10,c12 CLA per d in highly enriched preparations also produced only modest effects on HDL- and LDL-cholesterol concentrations (Tricon et al. 2004b) and immune function (Tricon et al. 2004a), although the results suggested divergent outcomes for c9,t11 CLA and t10,c12 CLA with a relatively beneficial effect of the c9,t11 CLA isomer (Tricon et al. 2004b). One possible alternative

Abbreviations: c9, t11, cis-9, trans-11; CE, cholesterol ester; CLA, conjugated linoleic acid; FAME, fatty acid methyl esters; PBMC, peripheral blood mononuclear cells; PC, plasma phosphatidylcholine; TAG, triacylglycerol; tVA, trans-vaccenic acid; UHT, ultra-high temperature processed.

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explanation may be low bioavailability of CLA from synthetic TAG preparations. This is supported by the observation that when c9,t11 and t10,c12 CLA were consumed at 2.5 g/d for 8 weeks the increment in the concentrations of these isomers in plasma phosphatidylcholine (PC), cholesteryl esters (CE) and NEFA, although significant, was <0.5% and in leucocytes <0.2% (Burdge et al. 2004). Similar changes in the concentrations of these isomers in plasma or cellular lipids have been reported in other studies in human subjects where CLA intakes were increased (Benito et al. 2001; Masters et al. 2002; Noone et al. 2002; Petridou et al. 2003). Although direct comparisons between studies in human subjects and rodents are difficult, a similar magnitude of incorporation of c9,t11 CLA (0–4% total fatty acids) has been reported in the liver of rats fed diets containing chemically synthesised c9,t11 CLA (Sebedio et al. 2001; Alasnier et al. 2002). This suggests that a difference between species in the activity of CLA may account for differences in biological outcomes.

Even if CLA has a lower biological activity in man, its effectiveness may be enhanced by optimising the amount of CLA available to the body. It is possible that consumption of foods enriched in CLA by modifying fatty acid metabolism in ruminants may increase the bioavailability of CLA from human diets, which will be important if human consumers are to gain health benefits from increased intakes of specific CLA isomers. Chardigny et al. (2003) showed that TAG structure is an important determinant of CLA bioavailability in rats. Incorporation of CLA into TAG synthesised naturally may enhance bioavailability by presenting the fatty acid to the body in a structure more readily hydrolysed by lipase enzymes. In the present study, we describe the effect of consuming dairy products naturally enriched in c9,t11 CLA on the concentrations of c9,t11 CLA and IV/VA in plasma and cellular lipids using a double-blind, cross-over design. We report the incorporation of these fatty acids in plasma PC, CE, TAG and NEFA fractions and into peripheral blood mononuclear cells (PBMC).

**Experimental procedures**

**Materials**

Solvents were purchased from Fisher Scientific UK Ltd (Loughborough, Leicestershire, UK), and all other reagents were from Sigma (Poole, Dorset, UK). Fatty acid standards were from Sigma and Nu-Chek-Prep (Elysian, MN, USA).

**Production of dairy products**

A detailed description of the dietary interventions in the cattle to produce the control and CLA-enriched dairy products, the preparation of ultra-high temperature processed (UHT) milk, cheese and butter and the organoleptic properties of these foods will be published elsewhere (Jones et al. 2005). In brief, four-nine early lactation Holstein–British Friesian cows were fed total mixed rations containing 0% (control) and 45% (on DM basis) of a mixture (1:2, w/w) of fish oil and sunflower-seed oil during two consecutive 7 d periods to produce a control and CLA-enhanced milk, respectively. Milk produced from these cows was used to produce UHT milk, cheese and butter according to standard practices. Triangle tests conducted on UHT milk (1 week after processing), butter (2 weeks after manufacture), and cheese (1 week after maturation) by a panel (n 20) of consumer volunteers showed that there were no significant differences in the flavour or visual properties of the products.

**Subjects and study design**

The study was carried out at two centres (about 60 miles apart) in the south of the UK and had a double-blind, cross-over design. Inclusion criteria were: male sex; BMI > 18 kg/m² and < 32 kg/m²; absence of diagnosed CVD, diabetes, liver or endocrine dysfunction, chronic inflammatory disease; no medication use; omnivorous eating habits; smoking ten or less cigarettes per d; alcohol consumption twenty-one units or less per week; no supplement (such as vitamins, fish oils or evening primrose oil) use. Volunteers fitting these criteria were screened for fasting plasma concentrations of cholesterol (3.0–8.0 mmol/l), TAG (0.5–3.0 mmol/l), and glucose (3.9–6.8 mmol/l). Ultimately thirty-two healthy men aged 34–60 years were recruited into the study. One subject withdrew after the first phase of the intervention for reasons not directly related to the study. Thirty-one subjects completed the study (eleven in Southampton, UK and twenty in Reading, UK). Identical protocols were used at both sites for administering the intervention trial and for the collection and storage of samples. The study was approved by the University of Reading Ethics and Research Committee and the South and West Hampshire Local Research Ethics Committee. Subjects gave written consent.

Subjects were assigned to consume either the control or CLA-enriched dairy products in random order, and asked to substitute their habitual dairy products for the experimental ones. The study took the following format. Subjects consumed commercially prepared milk, cheese and butter during a 7 d run-in period before consuming the experimental dairy products. The daily provision and consumption of individual dairy products is summarised in Table 1. The subjects then consumed the experimental products in amounts designed to provide 0.17 g c9,t11 CLA/d (control) or 1.43 g c9,t11 CLA/d (CLA-enriched) (Table 2). These products also provided 0.31 g or 4.71 g IV/VA/d, respectively (Table 2). Daily provision of cheese was greater during the period when subjects consumed the CLA-enriched products in order to provide the same total intake of fat. This was because the fat content of the CLA-enriched milk was lower than that of the control milk. Subjects consumed either the control or CLA-enriched products for 6 weeks and then returned to consuming their habitual dairy products for a 7-week washout period. Subjects then consumed the commercial products for a second run-in period of 7 d before consuming alternate experimental products for a further 6 weeks.

After an overnight fast, blood samples (40 ml) were collected at baseline, after the run-in period, and after the 6 weeks intervention period. Lithium heparin was used as an anticoagulant. Blood was separated into plasma and cellular fractions by centrifugation using Histopaque mononuclear cell separation media (Sigma) (Kew et al. 2003). Plasma was collected and frozen in tubes containing butylated hydroxytoluene and stored at −20°C. PBMC were aspirated from the interface, washed with PBS and frozen at −20°C.

**Analysis of food intakes**

Subjects completed food diaries for 3 d, including two weekdays and one weekend day, during the recruitment and screening period before commencing the study as a marker of habitual intakes, and during each of the periods of consumption of the
experimental products. Nutrient intakes were determined by using Foodbase software (Institute of Brain Chemistry, London, UK). All dairy products used in the intervention were analysed for their fatty acid compositions by GC and this information was added to the Foodbase database. Diet diaries were analysed for nutrient composition using Foodbase, with amounts of foods consumed being quantified using household measures, standard food portion sizes and weights of foods provided in manufacturers’ information.

Analysis of plasma and peripheral blood mononuclear cell fatty acid compositions

The methods for the analysis of plasma and PBMC fatty acid compositions were as described elsewhere (Burdge et al. 2004). Briefly, total lipids were extracted from plasma or PBMC using chloroform–methanol (2:1, v/v) (Folch et al. 1957). For plasma, the total lipid fraction was separated into PC, CE, TAG and NEFA by solid-phase extraction using BondElut aminopropylsila cartridges (Varian, Walton-on-Thames, Surrey, UK) (Burdge et al. 2000). The total lipid extracts from PBMC were not fractionated further. Fatty acid methyl esters (FAME) were prepared from CE and PC by the methods of Christie (1982) and Grinari et al. (1998) as described elsewhere (Shingfield et al. 2003). FAME were resolved on a 100 m x 0.25 mm x 0.2 µm CPSIL-88 fused silica capillary column (Chrompack, Middelburg, The Netherlands) using a 3400 CX gas chromatograph (Varian Instruments, Walnut Creek, CA, USA) equipped with flame ionisation detection as described elsewhere (Burdge et al. 2004). FAME were prepared from the plasma TAG and NEFA and from PBMC as described elsewhere (Folch et al. 1957; Burdge et al. 2004), and resolved on a 6890 gas chromatograph equipped with flame ionisation detection (Hewlett Packard, Wokingham, Berkshire, UK) using a 100 m x 0.25 mm x 0.2 µm CPSIL-88 fused silica capillary column (Chrompack) (Burdge et al. 2004). The differences in analytical techniques reflect established methodologies in the two participating laboratories, which produced comparable resolution of FAME and relative proportions of individual fatty acids. FAME were identified routinely by comparison of retention times with authentic standards and fractional concentrations calculated from baseline-corrected peak areas. The number of fatty acids detected in each lipid fraction was PC 15, CE 14, TAG 17, NEFA 12 and PBMC 24. The lower limit of detection was 0.01 g/100 g (Burdge et al. 2004). There was no difference in amount of cis9,t11 CLA or tVA recovered or an increase in degradation products when standards were prepared using these methods (data not shown).

Statistical analysis

There were no period or period–treatment interactions between the arms of the cross-over study. CLA and tVA concentrations did not differ significantly from a normal distribution and so were analysed using parametric statistical tests. Comparisons between dietary interventions were by Student’s unpaired t test, while comparisons between the start and end of the intervention period within a lipid class were by Student’s paired t test. Comparisons between lipid classes within the same intervention were by one-way ANOVA with Bonferroni’s post hoc correction. The relationship between cis9,t11 and tVA contents in plasma and cellular lipids was determined by linear regression analysis.

Results

Food intakes and consumption of cis-9, trans-11 conjugated linoleic acid and trans-vaccenic acid

Consumption of milk was significantly greater when subjects consumed the control (122 %) and CLA-enriched (132 %) products compared with habitual intakes (Table 1). Consumption of cheese was greater when subjects consumed the CLA-enriched products compared with their habitual intakes (42 %) or when they consumed the control dairy products (24 %). There was no difference in butter intake between habitual intakes and periods when subjects consumed the control or CLA-enriched products (Table 1). Since subjects consumed all of the dairy products provided (with the exception of a few subjects who did not reach their target milk intake of 500 ml), total CLA intake was at least the amount provided in these foods. Daily provision of cis9,t11 CLA during the run-in period was about twice when subjects consumed the control products, but approximately one-quarter of the amount provided in the CLA-enriched dairy products (Table 2), and cis9,t11 CLA provision in the CLA-enriched products was therefore about 8-fold greater compared with controls. Total tVA provision was similar during the run-in period and when subjects consumed the control products, but was 17-fold greater during the period when the CLA-enriched products were consumed (Table 2).

Table 1. Provision of dairy foods and intakes of dairy products before the run-in period of the study and during the period of consumption of the control or conjugated linoleic acid (CLA)-enriched foods

<table>
<thead>
<tr>
<th>Dairy products group (habitual)</th>
<th>Control</th>
<th>CLA-enriched</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>Milk (ml/d)</td>
<td>500</td>
<td>14.4</td>
</tr>
<tr>
<td>Butter (g/d)</td>
<td>12.5</td>
<td>0.9</td>
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<tr>
<td>Cheese (g/d)</td>
<td>28.0</td>
<td>32.6*</td>
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Mean values were significantly different from those during the pre-study phase: *P<0.05, **P<0.001 (Student’s paired t test). Mean value was significantly different from that for the control phase: †P<0.001 (Student’s paired t test).
Concentration of cis-9, trans-11 conjugated linoleic acid in plasma and peripheral blood mononuclear cell lipids

There were no significant differences in the concentrations of the fatty acids routinely detected in each lipid fraction apart from c9,t11 CLA and rVA.

c9,t11 CLA was detected in all of the plasma and PBMC samples collected at the start and end of the intervention period. There were significant differences between plasma lipid classes and PBMC in the proportion of c9,t11 CLA present at the start of the intervention period (ANOVA; P<0.0001). The proportion of c9,t11 CLA in plasma TAG was significantly greater (P<0.0001) than in PC, NEFA and CE, which did not differ significantly, while PBMC contained the least amount (P<0.0001 compared with plasma lipids) of this isomer (Table 2). The distribution of c9,t11 CLA between these lipid pools did not change when the subjects consumed the control or CLA-enriched dairy products, although the absolute concentrations varied according to the dietary intervention. There were no significant differences in the concentration of c9,t11 CLA between the start of the control intervention and the start of the intervention or after consuming the control products. However, after consumption of the CLA-enriched dairy products there was a positive relationship between the concentration of c9,t11 CLA in plasma PC (42 %), TAG (161 %), CE (205 %) and PBMC (238 %), while there was no significant change in the concentration of this isomer in plasma NEFA (Table 3). These effects were reflected in significant differences at the end of the intervention period between consuming the control or CLA-enriched dairy products (Table 3).

There was no significant relationship between the proportion of c9,t11 CLA in any of the lipid pools measured at the start of the intervention or after consuming the control products. However, after consumption of the CLA-enriched dairy products there was a positive relationship between the concentration of c9,t11 CLA in plasma PC and in plasma TAG (r 0.62; P=0.0002) and PBMC (r 0.36; P=0.047). The proportion of c9,t11 CLA in plasma TAG was positively related to the concentration of this isomer in the CE fraction (r 0.7; P<0.0001).

Concentration of trans-vaccenic acid in plasma and peripheral blood mononuclear cell lipids

rVA was detected in plasma PC, TAG and NEFA fractions and PBMC at baseline and after the intervention periods. However, rVA was not detected in the CE fraction (Table 4). There was no significant difference in the fractional concentration of rVA between plasma PC, TAG and NEFA, and PBMC at the start of the intervention period. There were no significant differences in

| Table 2. Cis-9, trans-11 (c9,t11) conjugated linoleic acid (CLA) and trans-vaccenic acid (rVA) content of dairy products and provision of these fatty acids during the periods of consuming the run-in, control and CLA-enriched dairy products. |
|---|---|---|---|---|---|---|---|---|
| c9,t11 CLA |  |  |  |  |  |  |  |  |
| Run-in | Control | CLA-enriched | Run-in | Control | CLA-enriched |
| Content in dairy products |  |  |  |  |  |  |  |  |
| Milk (g/100 ml) | 0.02 | 0.02 | 0.14 | 0.03 | 0.03 | 0.45 |
| Butter (g/100 g) | 1.07 | 0.34 | 2.92 | 0.49 | 0.67 | 9.88 |
| Cheese (g/100 g) | 0.39 | 0.12 | 0.98 | 0.23 | 0.26 | 3.33 |
| Intakes (g/9) |  |  |  |  |  |  |  |  |
| Milk | 0.1 | 0.1 | 0.70 | 0.15 | 0.16 | 2.25 |
| Butter | 0.13 | 0.04 | 0.37 | 0.06 | 0.08 | 1.24 |
| Cheese | 0.11 | 0.03 | 0.36 | 0.06 | 0.07 | 1.22 |
| Total | 0.34 | 0.17 | 1.43 | 0.27 | 0.31 | 4.71 |

| Table 3. Fractional cis-9, trans-11 conjugated linoleic acid (CLA) concentration (g/100 g) in plasma lipids and peripheral blood mononuclear cells (PBMC) (Mean values and standard deviations) |
|---|---|---|---|---|---|---|---|---|---|---|
| Control (n 31) |  |  |  |  |  |  |  |  |  |  |
| Start | End | Start | End | Start v. end: P* |
| Mean | SD | Mean | SD | Mean | SD |
| PC | 0.26 | 0.09 | 0.16 | 0.06 | <0.0001 | 0.24 | 0.07 | 0.34 | 0.12 | 0.035 | NS | <0.0001 |
| TAG | 0.51 | 0.18 | 0.40 | 0.16 | 0.017 | 0.46 | 0.19 | 1.20 | 0.66 | 0.001 | NS | <0.0001 |
| NEFA | 0.27 | 0.21 | 0.17 | 0.12 | 0.018 | 0.24 | 0.25 | 0.35 | 0.25 | NS | NS | <0.0001 |
| CE | 0.24 | 0.11 | 0.18 | 0.08 | 0.016 | 0.21 | 0.07 | 0.64 | 0.23 | <0.0001 | NS | <0.0001 |
| PBMC | 0.07 | 0.05 | 0.08 | 0.05 | NS | 0.08 | 0.06 | 0.27 | 0.19 | <0.0001 | NS | <0.0001 |

* Statistical comparisons between samples collected at the start and end of the study were by Student’s paired t test.
the concentration of \( \text{tVA} \) between the start of the control intervention and the start of the intervention with the CLA-enriched dairy products (Table 4). \( \text{tVA} \) concentration in plasma PC was lower (36%) at the end of the intervention period compared with the start after consumption of the control products, but there was no significant change in the \( \text{tVA} \) content of TAG, NEFA or PBMC (Table 4).

Consumption of the CLA-enriched products, which contained more than 10-fold higher \( \text{tVA} \) than the control products, was associated with an increase in the proportion of \( \text{tVA} \) in plasma PC (65%), TAG (98%) and PBMC (84%), but there was no significant difference in the \( \text{tVA} \) content of the NEFA fraction (Table 4). These effects were reflected in significant differences at the end of the intervention period between consuming the control or CLA-enriched dairy products (Table 4).

There was no significant relationship between the proportion of \( \text{tVA} \) in any of the lipid pools measured at the start of the intervention or after consuming the control foods. However, after consuming the CLA-enriched dairy products the \( \text{tVA} \) content of plasma PC was positively related to the concentration of this isomer in plasma \( \text{tVA} \) (Table 4). The concentration of \( \text{tVA} \) in plasma TAG was also positively related to the concentration of this isomer in the NEFA fraction (Table 4).

Discussion

The present study shows that consuming dairy products naturally enriched in CLA, especially \( \text{c9,t11 CLA} \), increases the concentration of the \( \text{c9,t11 CLA} \) isomer in plasma and cellular lipids. This was accompanied by an increase in the concentration of \( \text{tVA} \) plasma and cellular lipids.

One aim of the present study was to determine whether CLA status could be altered by consuming dairy products in amounts that would be achievable in the general population. The habitual consumption of milk was about half that required by the study intervention, while the intakes of cheese and butter were comparable. Milk provided approximately 50% of the \( \text{c9,t11 CLA} \) consumed per d during the intervention period, with an equal contribution being made by the butter and cheese combined. Thus it is possible that consuming CLA-enriched products in amounts typical of the UK population may provide a means for raising \( \text{c9,t11 CLA} \) intakes. For example, compared with commercially prepared products used during the run-in period, CLA-enriched cheese and butter alone would double the daily intake of this isomer.

\( \text{c9,t11 CLA} \) was present in all four plasma lipid classes and in PBMC total lipids at baseline, which would reflect intakes in the background diet. The concentration of this isomer was lower in plasma lipids after consumption of the control products compared with baseline, although there was no difference in the concentration of \( \text{c9,t11 CLA} \) in PBMC. One possible explanation is that the intake of \( \text{c9,t11 CLA} \) during the two run-in periods was greater than when subjects consumed the control products. This is consistent with previous findings that the concentration of \( \text{c9,t11 CLA} \) in plasma lipids is highly dependent upon dietary intakes (Noone et al. 2002; Burdge et al. 2004).

Consumption of 1.4 g \( \text{c9,t11 CLA} \) per d in the CLA-enriched products resulted in significant increases in the concentration of this isomer in plasma PC, CE, TAG and in PBMC total lipids, and a trend towards an increase in \( \text{c9,t11 CLA} \) concentration in the NEFA fraction. \( \text{c9,t11 CLA} \) was present in highest concentrations in the TAG fraction at baseline and after consuming the control or CLA-enriched foods, which suggests that, as in the rat (Banni et al. 2001), \( \text{c9,t11 CLA} \) is preferentially incorporated into this lipid pool. One previous report described the use of dairy products fortified with \( \text{c9,t11 CLA} \) from a synthetic source, although the concentration of this isomer in plasma or tissue lipid pools was not reported (Malpuech-Brugere et al. 2004). The present study shows for the first time that substituting dairy products naturally enriched in \( \text{c9,t11 CLA} \) significantly increases the concentration of this isomer in plasma and cellular lipids. The concentrations achieved in plasma PC and CE were comparable with those obtained when subjects consumed encapsulated preparations containing 1.2 g \( \text{c9,t11 CLA/d} \) (Burdge et al. 2004). The concentration of \( \text{c9,t11 CLA} \) in PBMC total lipids was also similar to that reported previously using synthetic CLA preparations (Kelley et al. 2001; Burdge et al. 2004). Overall, these data suggest that provision of \( \text{c9,t11 CLA} \) naturally incorporated into a food matrix does not appear to increase its bioavailability in man above synthetic sources of \( \text{c9,t11 CLA} \).

Intakes of \( \text{c9,t11 CLA} \) precursor \( \text{tVA} \) were similar during the run-in period and when subjects consumed the control dairy foods. However, the daily intake of \( \text{tVA} \) was 17-fold greater when subjects consumed the CLA-enriched dairy products. The greater \( \text{tVA} \) content of the CLA-enriched products reflects increased ruminal biohydrogenation required to increase synthesis of \( \text{c9,t11 CLA} \). There was no indication of differential partitioning of \( \text{tVA} \) between plasma PC, TAG and NEFA fractions. There was a significant decrease in \( \text{tVA} \) concentration in plasma lipids, although this was only significant for PC, when

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<th>Table 4. Fractional trans-vaccenic acid concentration (g/100 g) in plasma lipids and peripheral blood mononuclear cells (PBMC)</th>
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<tr>
<td><strong>Control (n 31)</strong></td>
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<td>PBMC</td>
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CLA, conjugated linoleic acid; PC, phosphatidylcholine; TAG, triacylglycerol; CE, cholesteryl ester; ND, not detected.

*Statistical comparisons between samples collected at the start and end of the study were by Student’s paired t-test.
subjects consumed the control products despite similar intakes to the run-in periods. The explanation for this decrease is not clear.

Consumption of the CLA-enriched products resulted in increased rVA concentration in plasma PC and TAG, and in PBMC, and a non-significant trend towards a higher rVA concentration in the NEFA fraction. Thus, intakes of dairy products naturally enriched in cis9,11 CLA that are sufficient to increase the concentration of this isomer in plasma and cellular lipid also increase the levels of rVA. This may be a possible cause for concern because increased intakes of the trans fatty acid elaidic acid (trans-9 18:1) are associated with greater risk of CVD (Zock & Katan, 1992). However, there is insufficient evidence to indicate whether similar effects are produced by increased consumption of rVA. Man is able to convert rVA to cis9,11 CLA (Turpeinen et al., 2002). Thus, enrichment of dairy products with rVA may represent an additional means of increasing availability of cis9,11 CLA within the body.

Overall, the present study indicates that it is feasible to increase cis9,11 CLA intakes in human consumers by the intake of dairy products naturally enriched in this isomer at intakes of these foods that do not greatly exceed those of the UK population. However, one of the drawbacks of natural enrichment of dairy products with CLA by the dairy feeding regimen used here is that there is also an increase in the level of rVA in milk and this fatty acid is also readily incorporated into plasma and cellular lipid pools. Thus strategies to enrich milk with cis9,11 CLA without the accompanying increase in rVA may be important for the future development of CLA-enriched dairy products. Together these data suggest that there is no obvious advantage in terms of the concentration of cis9,11 CLA in blood lipids and PBMC in attempting to increase cis9,11 CLA intake by providing modified dairy products against highly enriched encapsulated preparations.

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