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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

Graham C. Burdge[1]*, Sabine Tricon[2], Rebecca Morgan[3], Kirsty E. Kliem[3], Caroline Childs[1], Emma Jones[2], Jennifer J. Russell[1], Robert F. Grimble[1], Christine M. Williams[2], Parveen Yaqoob[2] and Philip C. Calder[1]

[1]*Institute of Human Nutrition, Biomedical Sciences Building, University of Southampton, Bassett Crescent East, Southampton, SO16 7PX, UK*
[2]*Hugh Sinclair Unit of Human Nutrition, University of Reading, Reading, UK*
[3]*Centre for Dairy Research, Department of Agriculture, University of Reading, Reading, UK*

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The present study investigated whether consuming dairy products naturally enriched in *cis*-9, *trans*-11 (*c*9,*t*11) 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 *c*9,*t*11 CLA/d; 0·31 g *trans*-vaccenic acid (*t*VA)/d) or CLA-enriched (1·43 g *c*9,*t*11 CLA/d; 4·71 g *t*VA/d) dairy products for 6 weeks. After 7 weeks washout, this was repeated with the alternate products. *c*9,*t*11 CLA concentration in plasma lipids was lower after consuming the control products, which may reflect the two-fold greater *c*9,*t*11 CLA content of the commercial products. Consuming the CLA-enriched dairy products increased the *c*9,*t*11 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 *t*VA 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 *c*9,*t*11 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 (*c*9,*t*11) 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 (*trans*-vaccenic acid; *t*VA) and by Δ9 desaturase, which converts *t*VA to *c*9,*t*11 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 *c*9,*t*11 CLA and *t*10,*c*12 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 *c*9,*t*11 and *t*10,*c*12 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 *c*9,*t*11 or *t*10,*c*12 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 *c*9,*t*11 or *t*10,*c*12 CLA per d in highly enriched preparations also produced only modest effects on HDL- and LDL-cholesterol concentrations (Tricon *et al.* 2004*b*) and immune function (Tricon *et al.* 2004*a*), although the results suggested divergent outcomes for *c*9,*t*11 CLA and *t*10,*c*12 CLA with a relatively beneficial effect of the *c*9,*t*11 CLA isomer (Tricon *et al.* 2004*b*). One possible alternative

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

* **Corresponding author:** Dr Graham C. Burdge, fax +44 2380 594379, email g.c.burdge@soton.ac.uk

explanation may be low bioavailability of CLA from synthetic TAG preparations. This is supported by the observation that when *c*9,*t*11 and *t*10,*c*12 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 $<0{\cdot}5$ % and in leucocytes $<0{\cdot}2$ % (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 *c*9,*t*11 CLA (0·4 % total fatty acids) has been reported in the liver of rats fed diets containing chemically synthesised *c*9,*t*11 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 *c*9,*t*11 CLA on the concentrations of *c*9,*t*11 CLA and *t*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, forty-nine early lactation Holstein–British Friesian cows were fed total mixed rations containing 0 (control) and 45 g/kg (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/m^2 and <32 kg/m^2; 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 *c*9,*t*11 CLA/d (control) or 1·43 g *c*9,*t*11 CLA/d (CLA-enriched) (Table 2). These products also provided 0·31 g or 4·71 g *t*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

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

(Mean values and standard deviations)

	Pre-study (habitual)			Control			CLA-enriched		
					Consumption			Consumption	
Dairy products group…	Mean	SD	Run-in provision	Provision	Mean	SD	Provision	Mean	SD
Milk (ml/d)	212	23·8	500	500	470·2**	14·4	500	491·4**	8·6
Butter (g/d)	14·1	1·9	12·5	12·5	15·7	0·9	12·5	15·7	1·3
Cheese (g/d)	28·3	6·5	28·0	28·0	32·6*	2·1	36·6	40·4*†	2·8

Mean values were significantly different from those during the pre-study phase: *$P<0·05$, **$P<0·0001$ (Student's paired *t* test).
Mean value was significantly different from that for the control phase: †$P<0·001$ (Student's paired *t* test).

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 aminopropylsilica 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 Griinari *et al.* (1998) as described elsewhere (Shingfield *et al.* 2003). FAME were resolved on a 100 m × 0·25 mm × 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 × 0·25 mm × 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 *c*9,*t*11 CLA or *t*VA 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 *t*VA 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 *c*9,*t*11 and *t*VA 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 *c*9,*t*11 CLA during the run-in period was about twice that when subjects consumed the control products, but approximately one-quarter of the amount provided in the CLA-enriched dairy products (Table 2). *c*9,*t*11 CLA provision in the CLA-enriched products was therefore about 8-fold greater compared with controls. Total *t*VA 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 2. *Cis*-9, *trans*-11 (*c*9,*t*11) conjugated linoleic acid (CLA) and *trans*-vaccenic acid (*t*VA) content of dairy products and provision of these fatty acids during the periods of consuming the run-in, control and CLA-enriched dairy products

	*c*9,*t*11 CLA			*t*VA		
	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/d)						
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

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 *c*9,*t*11 CLA and *t*VA.

*c*9,*t*11 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 *c*9,*t*11 CLA present at the start of the intervention period (ANOVA; $P<0{\cdot}0001$). The proportion of *c*9,*t*11 CLA in plasma TAG was significantly greater ($P<0{\cdot}0001$) than in PC, NEFA and CE, which did not differ significantly, while PBMC contained the least amount ($P<0{\cdot}0001$ compared with plasma lipids) of this isomer (Table 2). The distribution of *c*9,*t*11 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 *c*9,*t*11 CLA between the start of the control intervention and the start of the intervention with the CLA-enriched dairy products (Table 3).

There was no significant difference between dietary regimens in the concentration of *c*9,*t*11 CLA within each lipid pool at the start of the intervention period (Table 3). Between the start and end of the intervention period when subjects consumed the control products there was a decrease in *c*9,*t*11 CLA concentration in plasma PC (38 %), TAG (22 %), NEFA (37 %) and CE (25 %), although there was no change in the *c*9,*t*11 CLA content of PBMC (Table 3). Consumption of the CLA-enriched dairy products resulted in an increase in the fractional concentration of *c*9,*t*11 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 *c*9,*t*11 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 *c*9,*t*11 CLA in plasma PC and in plasma TAG (r 0·62; $P=0{\cdot}0002$) and PBMC (r 0·36; $P=0{\cdot}047$). The proportion of *c*9,*t*11 CLA in plasma TAG was positively related to the concentration of this isomer in the CE fraction (r 0·7; $P<0{\cdot}0001$).

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

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

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)					CLA-enriched (*n* 31)						
	Start		End			Start		End			Comparison between food products	
	Mean	SD	Mean	SD	Start *v.* end: *P* *	Mean	SD	Mean	SD	Start *v.* end: *P* *	Start *v.* start: *P*	End *v.* end: *P*
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	<0·0001	NS	<0·0001
NEFA	0·27	0·21	0·17	0·12	0·018	0·24	0·25	0·35	0·25	NS	NS	<0·001
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

PC, phosphatidylcholine; TAG; triacylglycerol; CE, cholesteryl ester.
* Statistical comparisons between samples collected at the start and end of the study were by Student's paired *t* test.

Table 4. Fractional *trans*-vaccenic acid concentration (g/100 g) in plasma lipids and peripheral blood mononuclear cells (PBMC) (Mean values and standard deviations)

	Control (*n* 31)					CLA-enriched (*n* 31)						
	Start		End			Start		End			Comparison between food products	
	Mean	SD	Mean	SD	Start *v.* end: *P**	Mean	SD	Mean	SD	Start *v.* end: *P**	Start *v.* start: *P*	End *v.* end: *P*
PC	0·28	0·12	0·18	0·08	0·001	0·34	0·12	0·56	0·51	0·035	NS	<0·0001
TAG	0·36	0·24	0·26	0·16	NS	0·46	0·28	0·91	0·60	0·001	NS	<0·001
NEFA	0·35	0·38	0·16	0·16	NS	0·15	0·49	0·24	0·27	NS	NS	NS
CE	ND		ND			ND		ND				
PBMC	0·49	0·35	0·58	0·37	NS	0·45	0·32	0·83	0·64	0·004	NS	<0·05

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.

the concentration of *t*VA between the start of the control intervention and the start of the intervention with the CLA-enriched dairy products (Table 4). *t*VA 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 *t*VA content of TAG, NEFA or PBMC (Table 4). Consumption of the CLA-enriched products, which contained more then 10-fold higher *t*VA than the control products, was associated with an increase in the proportion of *t*VA in plasma PC (65 %), TAG (98 %) and PBMC (84 %), but there was no significant difference in the *t*VA 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 *t*VA 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 *t*VA content of plasma PC was positively related to the concentration of this isomer in plasma TAG (r 0·42; $P=0·0195$) and in PBMC (r 0·54; $P=0·0019$). The proportion of *t*VA in plasma TAG was also positively related to the concentration of this isomer in the NEFA fraction (r 0·42; $P=0·019$).

Discussion

The present study shows that consuming dairy products naturally enriched in CLA, especially *c*9,*t*11 CLA, increases the concentration of the *c*9,*t*11 CLA isomer in plasma and cellular lipids. This was accompanied by an increase in the concentration of *t*VA 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 *c*9,*t*11 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 *c*9,*t*11 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.

*c*9,*t*11 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 *c*9,*t*11 CLA in PBMC. One possible explanation is that the intake of *c*9,*t*11 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 *c*9,*t*11 CLA in plasma lipids is highly dependent upon dietary intakes (Noone *et al.* 2002; Burdge *et al.* 2004).

Consumption of 1·4 g *c*9,*t*11 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 *c*9,*t*11 CLA concentration in the NEFA fraction. *c*9,*t*11 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), *c*9,*t*11CLA is preferentially incorporated into this lipid pool. One previous report described the use of dairy products fortified with *c*9,*t*11 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 *c*9,*t*11 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 *c*9,*t*11 CLA/d (Burdge *et al.* 2004). The concentration of *c*9,*t*11 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 *c*9,*t*11 CLA naturally incorporated into a food matrix does not appear to increase its bioavailability in man above synthetic sources of *c*9,*t*11 CLA.

Intakes of the *c*9,*t*11 CLA precursor *t*VA were similar during the run-in period and when subjects consumed the control dairy foods. However, the daily intake of *t*VA was 17-fold greater when subjects consumed the CLA-enriched dairy products. The greater *t*VA content of the CLA-enriched products reflects increased ruminal biohydrogenation required to increase synthesis of *c*9,*t*11 CLA. There was no indication of differential partitioning of *t*VA between plasma PC, TAG and NEFA fractions. There was a significant decrease in *t*VA concentration in plasma lipids, although this was only significant for PC, when

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 *t*VA concentration in plasma PC and TAG, and in PBMC, and a non-significant trend towards a higher *t*VA concentration in the NEFA fraction. Thus, intakes of dairy products naturally enriched in *c*9,*t*11 CLA that are sufficient to increase the concentration of this isomer in plasma and cellular lipid also increase the levels of *t*VA. 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 *t*VA. Man is able to convert *t*VA to *c*9,*t*11 CLA (Turpeinen *et al.* 2002). Thus, enrichment of dairy products with *t*VA may represent an additional means of increasing availability of *c*9,*t*11 CLA within the body.

Overall, the present study indicates that it is feasible to increase *c*9,*t*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 *t*VA in milk and this fatty acid is also readily incorporated into plasma and cellular lipid pools. Thus strategies to enrich milk with *c*9,*t*11 CLA without the accompanying increase in *t*VA 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 *c*9,*t*11 CLA in blood lipids and PBMC in attempting to increase *c*9,*t*11 CLA intake by providing modified dairy products against highly enriched encapsulated preparations.

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