European Journal of Clinical Nutrition (2004) 58, 819–827
 0954-3007/04 $25.00
www.nature.com/ejcn

ORIGINAL COMMUNICATION

Effect of moderate changes in dietary fatty acid profile on postprandial lipaemia, haemostatic and related CVD risk factors in healthy men

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Objective: To investigate the effect of moderate changes in dietary fatty acid profile on postprandial risk factors for cardiovascular disease (CVD).
Design: Double-blind, randomised, crossover, intervention trial.
Setting: University of Auckland Human Nutrition Unit, New Zealand.
Subjects: A total of 18 lean healthy men.
Intervention: A dairy butter fat modified to reduce the saturated:unsaturated fatty acid ratio and a conventional high saturated butter fat were given on two separate occasions as a high-fat test meal (59 ± 4 g fat; 71 en% fat) at breakfast. A fat exclusion lunch, dinner and snacks were also given. Blood samples were collected at 0 (baseline), 1, 3, 6, 10 and 24 h.
Results: Maximum peak in total triacylglycerol (TAG) occurred 3 h postprandially and was highest on modified treatment (diet, $P<0.05$) due predominantly to increased TAG within the chylomicron-rich fraction. Transient peaks in total-, LDL- and HDL-cholesterol occurred postprandially, but did not differ between dietary treatments ($P>0.05$). There were no differential effects of diet on postprandial free fatty acids, apo A, apo B, glucose, insulin, amylin or haemostatic clotting factors ($P>0.05$).
Conclusions: In a group of healthy young men, replacement of 16% of total saturated fatty acids by mono- and polyunsaturated fats within a dairy lipid did not induce postprandial changes in CVD risk that may be considered beneficial for health.
Sponsorship: Fonterra, Wellington; New Zealand.
European Journal of Clinical Nutrition (2004) **58,** 819–827. doi:10.1038/sj.ejcn.1601882

Keywords: dietary fatty acids; postprandial lipaemia; serum triacylglycerol; serum cholesterol; haemostatic clotting factors; randomised controlled trial (RCT); lean men

Introduction

More than 20 years ago, Zilversmit (1979) suggested that postprandial lipoprotein metabolism may play a significant role in the process of atherogenesis through triacylglycerol-rich lipoprotein (TRL) metabolism. While fasting levels of TRL and cholesterol-rich lipoproteins can be significantly modulated through dietary change, and the strategy of altering dietary lipid quality has long been shown to be successful in reducing cardiovascular (CVD) risk (Grundy, 1986; Mensink & Katan, 1989; Mata *et al*, 1992; Katan, 1997; Willett, 1998; Poppitt *et al*, 2002), the relationship between diet, transient postprandial changes in lipids and CVD risk is not as well established (Patsch *et al*, 2000; Parks, 2001). There is, however, a growing body of evidence that changes in postprandial triacylglycerol (TAG) and high-density lipoprotein cholesterol (HDL-C) are associated with changes in CVD risk (Havel, 1994, 1997a,b; Bergeron & Havel, 1997;

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Received 14 April 2003; revised 30 August 2003; accepted 4 September 2003

Karpe, 1999; Berglund, 2002) and that modulation of postprandial lipid profile is an important target for dietary intervention.

Both direct and indirect mechanisms for the relationship between postprandial TAG and CVD risk have been proposed. Poor chylomicron clearance may lead to deposition of lipid on the arterial wall (Hazzard & Bierman, 1976; Cortner *et al*, 1987; Groot *et al*, 1991) or there may be reciprocal crossing of cholesteryl esters (CE) and TAG between lipoproteins, which can lead to the 'atherogenic lipoprotein phenotype' (Miesenbock & Patsch, 1992). For example, CE transferred to TRL may result in CE-enriched particles that accumulate as part of atheromatous plaques, and TAG transferred to low-density lipoprotein cholesterol (LDL-C) and HDL-C may more readily undergo lipase hydrolysis, which leads to reduction in lipoprotein size and formation of a preponderance of small HDL and small, dense LDL that are easily oxidised (de Graaf *et al*, 1993), toxic to endothelial cells (Sattar *et al*, 1998) and atherogenic (Patsch *et al*, 1992). Decreased postprandial HDL-C is also associated with an increase in CVD risk (Gordon *et al*, 1977; Patsch *et al*, 1992), although decrease in HDL-C may be a direct consequence of an increase in TRLs rather than indication of an independent marker of risk (Patsch *et al*, 1984; Miesenbock & Patsch, 1992; Muesing *et al*, 1995; Thomsen *et al*, 1999).

By eating three meals a day, we are repeatedly exposed to elevated levels of circulating lipoproteins and while there are strong relationships between the total fat content of the meal and the lipaemic response (Tall *et al*, 1982; Cohen *et al*, 1988; Rifai *et al*, 1990; deBruin *et al*, 1991; Dubois *et al*, 1994), there is little consensus as to the differential effects of fat quality such as fatty acid composition on postprandial response (Zampelas *et al*, 1994; Muesing *et al*, 1995; Higashi *et al*, 1997; Roche & Gibney, 1997, 2000; Roche *et al*, 1998; Tholstrup *et al*, 1998; Thomsen *et al*, 1999; Mekki *et al*, 2002). While there is also growing evidence that haemostatic clotting factors, such as total Factor VII coagulant activity (FVIIc), may be affected postprandially by increasing fat load in the diet (Miller *et al*, 1991; Larsen *et al*, 2000), there also remains little consensus as to the differential effects of individual fatty acids (Miller, 1998; Sanders *et al*, 2001).

A trial recently published from our laboratory has shown that small changes in the fatty acid profile can help to lower fasting total and LDL-C even in apparently healthy men (Poppitt *et al*, 2002) and thereby potentially improve CVD risk. In that trial, a modified butter fat, in which 16% of saturates were replaced with mono (MUFA) and polyunsaturates (PUFA), was fed over a 3-week period as part of a healthy, moderate-fat diet. The aim of the current study was to determine whether such moderate changes in the fatty acid profile may also have protective effects on postprandial outcomes related to CVD risk, including TRL and cholesterol-rich lipoproteins, haemostatic and other associated metabolic parameters.

Methods

Subjects

A total of 18 lean (BMI $22.9 \pm 2.0\,kg/m^2$), healthy, male volunteers aged 19–33 y were recruited into this intervention following screening to confirm normal clinical biochemistry as assessed by lipid profile, liver function, thyroid function (T4, TSH), plasma glucose and blood pressure (Table 1). None had a current or previous history of treatment for significant disease, nor were they taking medications for lipid, blood pressure or metabolic disorders. All subjects completed both arms of the intervention. There were no subjects who withdrew or who were excluded for noncompliance. All volunteer subjects provided written informed consent. Ethics approval for this study was obtained from the Auckland Ethics Committees, Auckland, New Zealand.

Protocol

This study was a double-blind, randomised crossover trial in which participants were required to complete two treatments each of 24 h duration, during which a control or a modified dairy lipid was given as a high-fat bolus within a breakfast meal. The breakfast meal comprised a sweet blueberry muffin, a milk and sugar-free decaffinated hot beverage and/or a glass of cold water. The fatty acid profiles of the two butter fats are shown in Table 2. Participants were randomised to treatment using stratification methods, such that nine of the subjects were given modified and nine were given control butter fat as their first treatment. They were then all crossed over on to the remaining treatment regime. Treatments were separated by a minimum 3-day washout period. Subjects were confined within the human nutrition facility at the University of Auckland throughout each 24-h treatment. They arrived fasted at the nutrition unit at 0730 on day 1, an indwelling venous cannula was inserted and a baseline blood sample was collected. At 0800, the butter fat bolus was served. Further blood samples were then collected at 1, 3, 6, 10 and 24 h after the breakfast meal. A fat exclusion lunch containing 3.1 g dietary fat and comprising vegetarian pasta, bread roll and orange juice was served immediately

Table 1 Subject characteristics at screening of the 18 male subjects who completed both arms of the intervention

Characteristic	*Mean*	*s.d.*
Age (y)	23	4.2
Body weight (kg)	72.8	6.7
Body mass index (kg/m^2)	22.9	2.0
Systolic blood pressure (mmHg)	123	10.5
Diastolic blood pressure (mmHg)	78	9.0
Waist (cm)	79.5	6.1
Fasting plasma glucose (mmol/l)	4.7	0.3
Total cholesterol (mmol/l)	4.3	0.8
LDL-C (mmol/l)	2.5	0.7
HDL-C (mmol/l)	1.4	0.4
Triacylglycerol (mmol/l)	0.8	0.3

Table 2 Composition of the control and modified butter fat, indicating the major constituents of the fatty acid profile

	Control butter	*Modified butter*	δ
Total fat content (% w/w)	85.2	81.7	−3.5
Moisture (% w/w)	12.4	15.4	3.0
Total saturated fat (% fat)	70.5	54.4	−16.1
Lauric C12:0	3.8	2.7	−1.1
Myristic C14:0	12.0	8.3	−3.7
Palmitic C16:0	31.5	18.8	−12.7
Stearic C18:0	10.1	13.4	3.3
Total PUFA (% fat)	3.0	10.5	7.5
Linoleic C18:2	1.2	7.2	6.0
α-Linolenic C18:3	0.8	2.3	1.5
Total MUFA (% fat)	22.1	32.0	9.9
$C18:1_{total}$	18.6	30.0	11.4
$C18:1_{trans}$	4.3	4.7	0.4
Cholesterol mg/100 g butter	222.0	191.0	−31.0

Table 3 Average composition of the fat-loaded breakfast muffin test meal

	Control butter fat muffin		*Modified butter fat muffin*	
Nutrient composition	*Mean*	*s.d.*	*Mean*	*s.d.*
Energy (kJ)	3130	193	3140	193
Protein (% en)	4.9	0.5	4.9	0.5
Carbohydrate (% en)	23.2	1.7	23.1	1.7
Fat (% en)	70.8	4.9	70.9	4.9
Fat (g)	59.1	3.7	59.5	3.9

following the 6 h blood sample. A fat exclusion snack containing 0.7 g dietary fat and comprising fruit cake and apple juice was served mid-afternoon, 8 h postbreakfast. A fat exclusion dinner containing 1.3 g dietary fat and comprising vegetarian risotto, raspberry desert and a soft beverage was served following the 10 h blood sample. These meals and snacks were identical on both treatment arms and contained an average of 5.1 g of dietary fat on each occasion. Subjects slept at the unit that evening and a final blood sample was collected fasted at 24 h.

Diet

The butter bolus given to each subject was calculated individually and scaled to body size. Each individual was given 5.3 g butter fat per megajoule intake, such that on a 10 MJ diet the butter fat bolus was 53 g. Table 3 shows the composition of the blueberry muffin including the bolus of butter fat. Subjects were kept in energy balance throughout the day, calculated as 1.4 times predicted basal metabolic rate (BMR), equivalent to a sedentary day. The average daily energy requirement for the subjects was estimated to be 11.2 ± 0.2 MJ/day, and was matched by intake. The average butter fat intake was 59 g, equivalent to approximately 73 g of dairy butter. The total average energy content of the test breakfast was 3.5 MJ (high fat), lunch was 3.5 MJ (high CHO), afternoon snack was 1.5 MJ (high CHO) and dinner was 2.4 MJ (high CHO). The energy and macronutrient content of the diet was calculated using the dietary program Foodworks™ (Crop & Food Research, Palmerston North, New Zealand). Subjects were required to consume only the foods and beverages provided throughout the day. They had access to no other food and were kept within the facility throughout each arm of the trial.

Butter fat composition

The modified butter fat was manufactured for this trial using bovine feeding methods in which lactating dairy cows were fed a diet enriched with unsaturated fatty acids, in turn protected from saturation in the rumen by an encapsulating protein coat. Further details of the normal and modified products have been provided in a previous publication (Poppitt *et al*, 2002). This method resulted in a proportion of the saturated fatty acids in the control butter being replaced by unsaturated fatty acids (see Table 2).

Analytical methods

Blood samples from the 0, 1, 3, 6, 10 and 24 h venous collections were analysed for circulating total cholesterol (TC) , LDL-C, HDL-C, total TAG, TAG-poor (VLDL) chylomicron, TAG-rich chylomicron, free fatty acids (FFA), apo A, apo B, glucose, insulin, amylin, and the haemostatic clotting factors fibrinogen and FVIIc activity. Serum TC, LDL-C, HDL-C, total TAG, FFA, apo A, apo B and glucose concentrations were measured in triplicate using a COBAS Mira™ auto-analyser (Hoffman-La Roche Ltd, Basel, Switzerland). A three-step enzymatic colour method utilising cholesterol esterase, cholesterol oxidase and peroxidase was used to analyse serum TC in triplicate. Fasting and nonfasting LDL-C was measured directly using an autoLDL-C™ Cholesterol Reagent Set (Pointe Scientific, Inc.™). Step 1 used a detergent to solubilise the chylomicron, HDL-C and VLDL-C fractions, with subsequent removal of these fractions by cholesterol esterase and cholesterol oxidase. Step 2 utilised another detergent to solubilise the remaining LDL-C, and a chromogenic coupler produced colour formation that is proportional to the amount of LDL-C present in the sample. A two-step HDL-C precipitating reagent set (Pointe Scientific, Inc.™) followed by enzymatic cholesterol analysis was used for analyses of HDL-C. A triglyceride-GPO Reagent Set™ multistep, enzymatic, colour reaction method (Pointe Scientific, Inc.™) was used to analyse total TAG in triplicate. TAG fractions were prepared following the method of Thomsen *et al* (1999). A measure of 4.2 ml EDTA plasma was overlaid with 1 ml of HEPES-buffered saline (HBS) 10 mM, pH 7.4 and centrifuged at $26\,000 \times g$ at 4°C for 30 min (Sorval Discovery 100S, Asheville USA, SW65 head). The lower, chylomicron-poor fraction was aspirated by placing an HPLC probe into the bottom of the tube and pumping away the fraction

(peristaltic pump, Pharmacia P-1, NJ, USA) at a rate of 1 ml/min. The chylomicron-rich layer was washed off the tube walls into the body of the tube using 1 ml of HBS. This was then transferred to a 1.5 ml Eppendorf tube (Hamburg, Germany) and ultracentifuged at $12\,000 \times g$ for 20 min at 4°C. The bottom layer was again aspirated. All chylomicrons were washed off the aspiration needle and tube wall using 1 ml of HBS and mixed well. Both fractions were then frozen at −80°C for later analysis. The recovery of chylomicron-rich and chylomicron-poor TAG was calculated as a percentage of the recovery of total TAG to assess the accuracy of the ultracentrifugation method. An enzymatic colorimetric method (Roche™) based on the method of Shimizu *et al* (1980) was used to analyse FFA. Apolipoprotein A-1 Reagent Set™ and apolipoprotein B Reagent Set™ immunoturbidimetric analysis methods (Pointe Scientific, Inc.™) were used to analyse apo A and apo B. The serum glucose concentration was measured enzymatically using the Glucose Hexokinase Reagent Set™ method (Pointe Scientific, Inc.™). The serum insulin concentration was directly measured in duplicate using a double antibody radioimmunoassay (RIA) kit method (Peninsula Laboratories, Inc.™, Belmont, USA). The serum amylin concentration was directly measured in duplicate using a solvent extraction kit method (Peninsula Laboratories, Inc.™, Belmont, USA) and subsequent RIA measurement of the peptide concentration. Haemostatic clotting FVIIc and fibrinogen concentrations were analysed as single measures using a Behring Coagulation System™ batch analyser (PA, USA).

Statistical analyses

Body weight and metabolic outcomes were analysed using linear mixed model ANOVA (SAS: PROC MIXED, SAS version 8.0, SAS Institute Inc., Cary, NC, USA, 2001). Split-plot-in-time repeated measure ANOVA tested within- and between-diet interactions over time periods of 6 and 24 h. Treatment groups, ID, study day and run order effects were included in the procedure. Total TAG, TAG-rich and TAG-poor chylomicron were also analysed for within- and between-diet effects with time, subject and run order interactions at 3 h (peak height), and as the area under the curve (AUC) between 0 and 6 h. In all measures, when there was no differential effect of fat quality, the two treatments were combined and the quantitative effects of the dietary lipid were analysed over time. All biochemical assays were analysed in triplicate and presented as a mean ± s.e.m. Statistical significance was based on 95% limits ($P<0.05$).

Results

The feeding regimen carried out in the lactating dairy cows reduced saturated fatty acids by 16% and increased MUFA and PUFA by 10 and 7.5%, respectively, in the modified butter fat product (see Table 2). The major reductions in saturates were in palmitic (C16:0, −12.7%) and myristic (C14:0, −3.7%) acids. These were replaced by oleic (C18:1, +11.4%), linoleic (C18:2, +6.0%) and linolenic acids (C18:3, +1.5%). There was no significant increase in the content of *trans* fats, which was between 4 and 5% of total fat in both products. The cholesterol component of the butter decreased by 31 mg/100 g butter fat in the modified product, which represented an average decrease of 18 mg cholesterol in the butter bolus as fed to the subjects at breakfast.

When analysed independent of dietary treatment, total TAG increased postprandially in response to the high-fat test breakfasts ($time_{0-6\,h}$, $P<0.001$; Figure 1). There were no significant between-treatment effects (diet × time, $P>0.05$) when analysed over 6, 24 h or as the AUC over 6 h ($AUC_{0-6\,h}$), but the maximal 3 h peak height was significantly greater on modified butter fat treatment than on control treatment (diet, $P<0.05$). When analysed independent of dietary

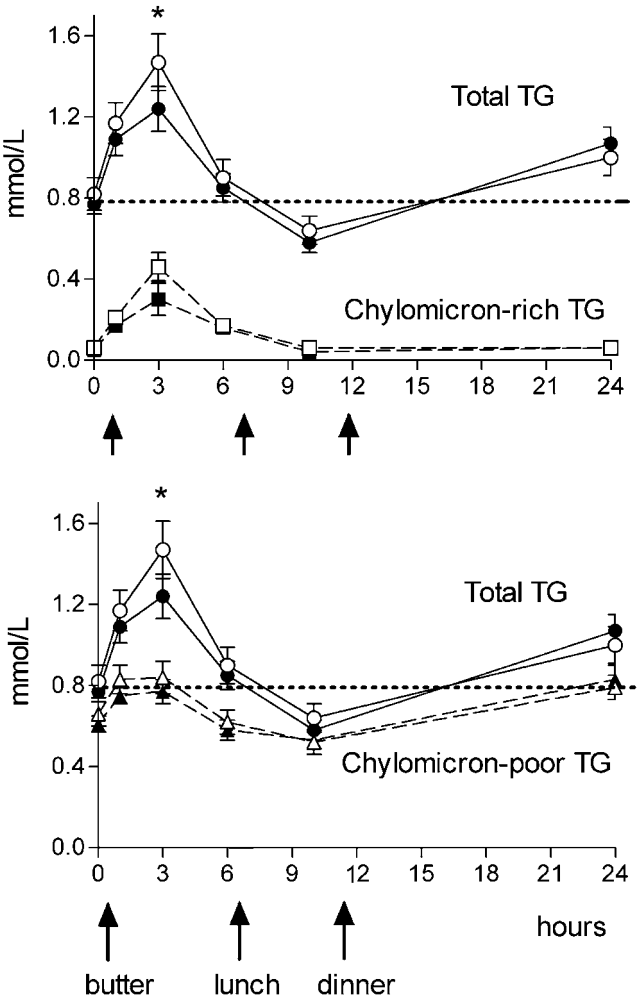

Figure 1 Postprandial changes in total, chylomicron-rich and chylomicron-poor TAG fractions following the consumption of a high-fat breakfast containing a control (●) or modified (○) dairy-derived lipid, in 18 lean healthy men. Statistical significance shown for between-treatment effects (ANOVA), *$P<0.05$. Mean ± s.e.m.

treatment, chylomicron-rich TAG (upper panel) also increased postprandially ($time_{0–6\,h}$, $P<0.001$). The maximal 3 h peak height mimicked the pattern of total TAG, but the difference was not significant (diet, $P>0.05$). There were no significant between-treatment effects of diet (diet × time, $P>0.05$) when analysed over 6, 24 h or as $AUC_{0–6\,h}$. Chylomicron-poor TAG (lower panel) comprised the major portion of total TAG at baseline on both treatment days. When analysed independent of dietary treatment, there was an increase postprandially ($time_{0–6\,h}$, $P<0.01$), but no differential effect of treatment at the 3 h peak (diet, $P>0.05$). There were no significant between-treatment effects of diet (diet × time, $P>0.05$) when analysed over 6, 24 h or as $AUC_{0–6\,h}$. During the analytical process, the average recovery of chylomicron-rich and chylomicron-poor TAG fractions across both treatments and all time periods was 89 ± 12 (s.d.)%. A total of 197 individual blood samples were analysed from 216 time points on the two treatment arms (19 missing data points). Recovery of <80% was obtained in 16 samples and >110% in one sample only, indicating that the method used was reasonably informative.

Changes in the circulating levels of cholesterol-rich lipoproteins following the two separate test meals are shown in Figure 2. There was little differential response of cholesterol

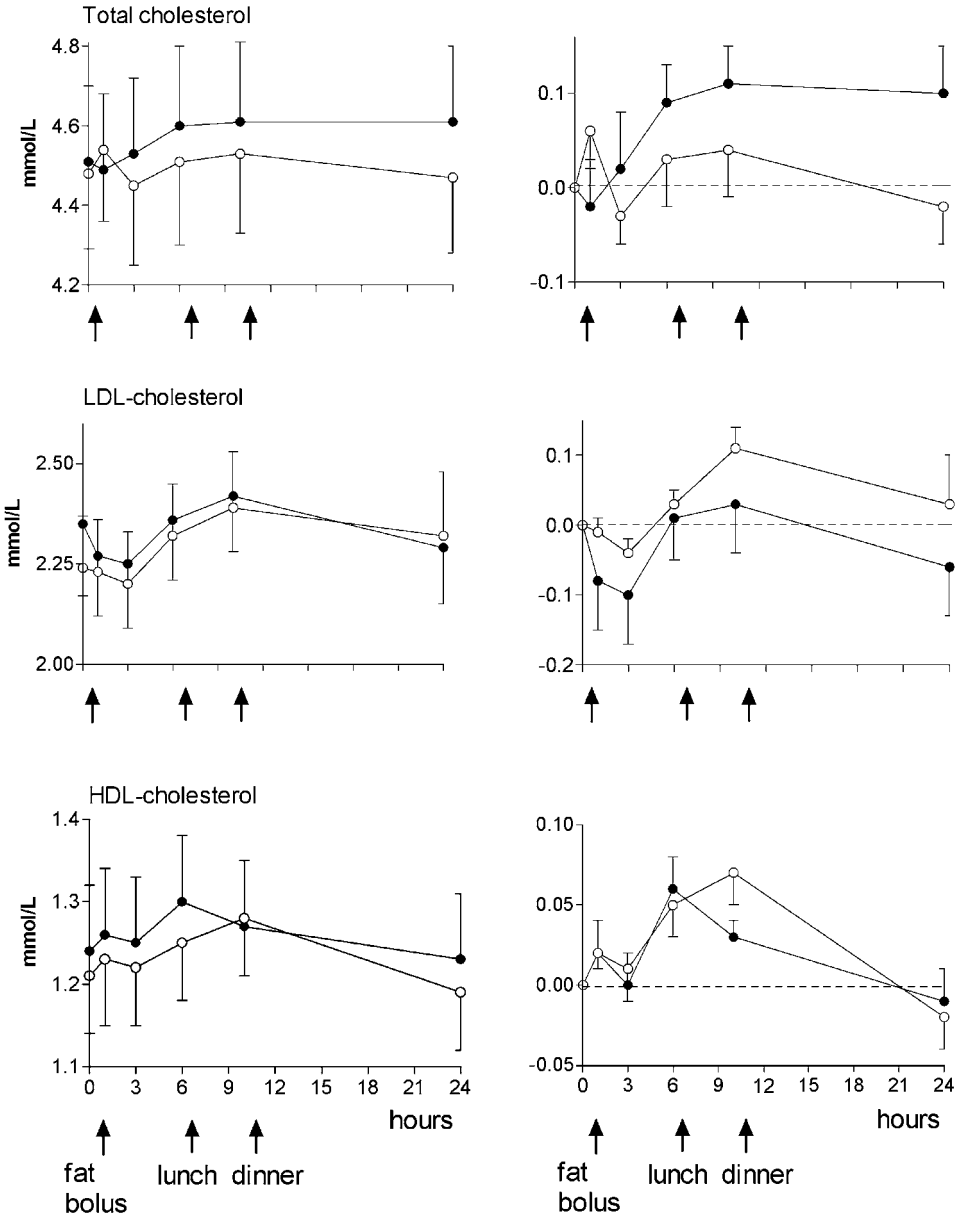

Figure 2 Postprandial changes in total, LDL and HDL cholesterol following the consumption of a high-fat breakfast containing a control (●) or modified (○) dairy-derived lipid. Changes from baseline shown in the RH panels. Mean ± s.e.m.

fractions to the two fat challenges and no evidence of significant between-treatment effects in either TC, LDL-C or HDL-C when analysed over the immediate postprandial 6 h (diet × time, $P>0.05$) or the entire 24-h period (diet × time, $P>0.05$). When analysed independent of treatment over the 6-h postprandial period, there was a significant increase in TC and HDL-C and a significant decrease in LDL-C ($time_{0–6\,h}$, $P<0.001$), a consequence of lipaemia *per se*.

There were no significant between-treatment effects on circulating FFA, apo A, apo B, glucose or insulin when analysed over either 6 or 24 h (Figure 3; diet × time, $P>0.05$). Serum amylin tended to be higher during modified treatment when analysed over 24-h (diet × time, $P=0.052$). Temporal changes in FFA were driven by meal pattern, a rapidly decrease post-fat bolus followed by a significant rise prior to the lunch meal ($time_{0–6\,h}$, $P<0.001$). When analysed independent of treatment, there was little effect of the fat challenge on apo A ($time_{24\,h}$, $P>0.05$), while apo B decreased at 6 and 10 h and increased during the overnight fast ($time_{24\,h}$, $P<0.05$). Glucose and insulin responded in an entirely predictable manner, increasing rapidly postprandially. Amylin, cosecreted with insulin, also altered in response to meal pattern increasing at 1 and 10 h ($time_{24\,h}$, $P<0.05$).

There were no differential effects of dietary treatment on the activity of FVIIc or fibrinogen when analysed over the immediate 6 h postprandial period or over the 24 h test (diet × time, $P>0.05$; Figure 4). When analysed independent of treatment, the high-fat bolus produced a transient drop in FVIIc over 6 h ($time_{0–6\,h}$, $P<0.01$), but no effect on plasma fibrinogen ($time_{0–6\,h}$, $P>0.05$).

Discussion

While there is no doubt that there is a strong positive relationship between the total fat content of a meal and the magnitude of postprandial lipaemia (Tall *et al*, 1982; Cohen *et al*, 1988; Rifai *et al*, 1990; deBruin *et al*, 1991; Dubois *et al*, 1994), there remains little firm consensus as to the differential effect of fatty acid classes or individual fatty acids on postprandial markers of CVD risk. Trials have shown both unsaturated (Harris *et al*, 1988, 1990; Weintraub *et al*, 1988; Zampelas *et al*, 1994; Tholstrup *et al*, 1998; Thomsen *et al*, 1999) and saturated fats (Edelin *et al*, 1968; Schlierf *et al*, 1977; Avarim *et al*, 1986; Muesing *et al*, 1995; Higashi *et al*, 1997; Mekki *et al*, 2002) to preferentially decrease or have no differential effect (Roche & Gibney, 1997; Roche *et al*, 1998) on postprandial TAG. When unsaturated fats are considered in more detail, there is emerging evidence that MUFA may have no differential effect relative to saturates (Roche & Gibney, 1997; Roche *et al*, 1998), while PUFA may reduce postprandial TAG relative to SFA (Harris *et al*, 1988, 1990; Weintraub *et al*, 1988; Zampelas *et al*, 1994; Roche & Gibney, 2000).

When cholesterol-rich lipoproteins are measured, saturates may decrease (Thomsen *et al*, 1999) or have no differential effect (Avarim *et al*, 1986; Muesing *et al*, 1995; Higashi *et al*, 1997) on postprandial HDL-C, despite changes in triglyceridaemia. Postprandial changes in TC and LDL-C remain equally poorly defined (Avarim *et al*, 1986; Daumerie *et al*, 1992; Muesing *et al*, 1995; Tholstrup *et al*, 1998). Postprandial TRL and HDL-C are of particular importance since there are established relationships between these circulating lipoproteins and consequent risk of CVD (Patsch *et al*,

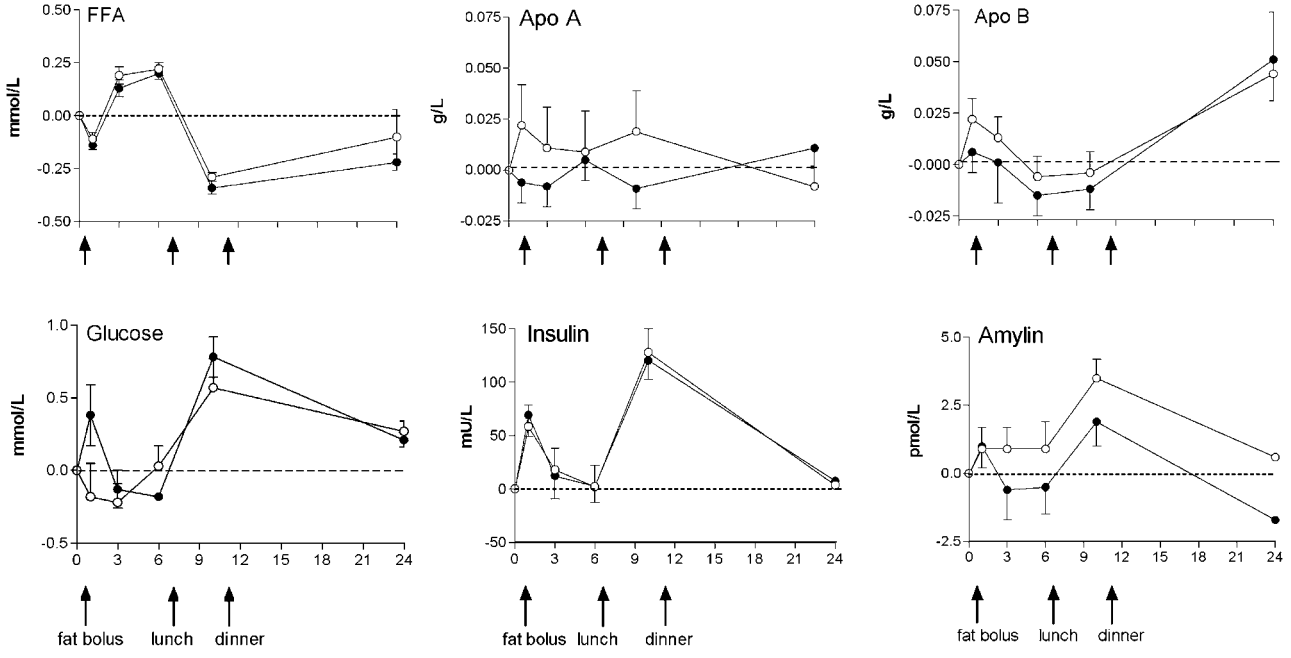

Figure 3 Postprandial changes in FFA, apo A and apo B (top panel), glucose, insulin and amylin (bottom panel) following the consumption of a high-fat breakfast containing a control (●) or modified (○) dairy-derived lipid. Mean ± s.e.m.

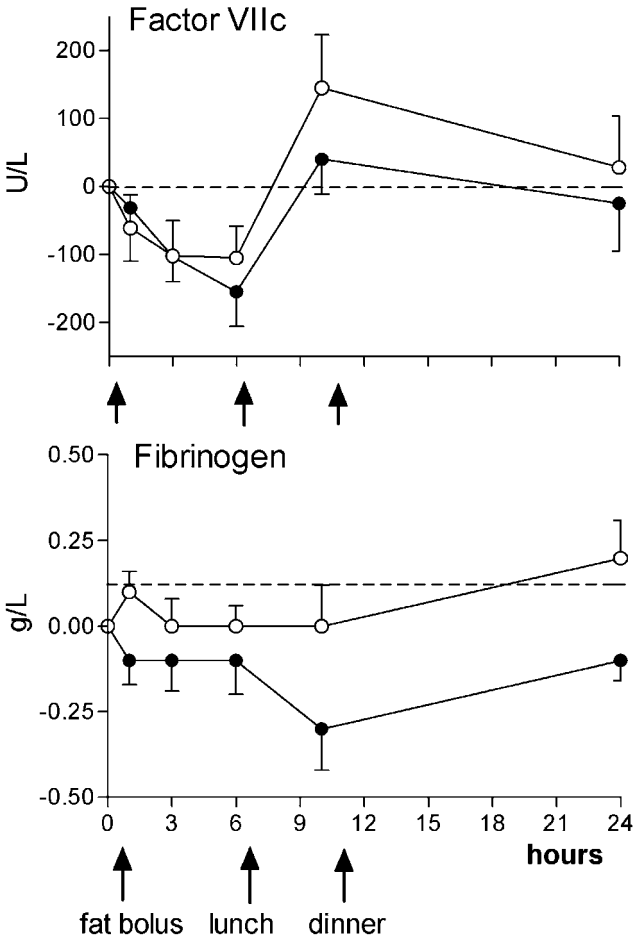

Figure 4 Postprandial changes in haemostasis following the consumption of a high-fat breakfast containing a control (●) or modified (○) dairy-derived lipid. Mean ± s.e.m.

1992; Havel, 1994, 1997a,b; Bergeron and Havel, 1997; Karpe, 1999) although it has been suggested that the negative relationship with HDL-C may be a direct result of lipaemia rather than as an independent risk factor *per se* (Miesenbock & Patsch, 1992; Muesing *et al*, 1995).

In this trial, we showed that the modified lipid, with a high content of unsaturated fatty acids, preferentially increased the total circulating TAG in the 3 h immediately following ingestion of the bolus. There was, however, no evidence of differential effects of fat quality on cholesterol-rich lipoprotein fractions, nor on other associated markers of CVD risk. The response to the quantity of fat eaten was relatively predictable in all measured outcomes. Both high-fat breakfasts caused total TAG to increase to a peak at around 3 h postprandially. This was due predominantly to an increase in chylomicron-rich TAG, although there was also a small increase in chylomicron-poor TAG, which may suggest that some of this was of intestinal rather than hepatic origin. The decline 10 h after the fat challenge, which drove TAG below baseline fasting levels, has previously been reported and may be due to meal-induced changes in the activity of the lipoprotein lipase system (Groot & Scheek, 1984). HDL-C increased several hours after the serum TAG peak, at 6 h post-meal. This delayed rise with respect to TAG has also previously been shown in a number of trials following high-fat feeding, accompanied by a decrease in HDL density (Havel *et al*, 1973; Groot & Scheek, 1984; Muesing *et al*, 1995) and an increase in HDL constituents predominantly through increases in the HDL_3 fraction (Tall *et al*, 1982; Groot & Scheek, 1984).

There are a number of factors that may be responsible for the variability in response between the published trials, including the subject groups studied, the effects of prior diet (Harris *et al*, 1988), the nature of the lipid emulsion or mixed meal (Mekki *et al*, 2002) and the detailed fatty acid profile of the test bolus. Butter fat is one of the most complex dietary lipids both in terms of fatty acid (predominantly palmitic, myristic, stearic, oleic acids) and TAG components and its physico-chemistry. In this trial, we modified the dairy fat mainly through replacement of palmitic and myristic acids with oleic, linoleic and a small fraction of α-linolenic acids. Despite the large fat bolus of almost 60 g given to the men in this trial, the absolute change in dietary fatty acids was relatively small. Total saturates were decreased by approximately 10 g and were replaced by an average of 6 and 4 g MUFA and PUFA, respectively. This moderate change, which we have previously shown to reduce circulating levels of fasting TC and LDL-C significantly in long-term trials (Poppitt *et al*, 2002), had a modest effect on TAG alone. A number (Edelin *et al*, 1968; Schlierf *et al*, 1977; Muesing *et al*, 1995; Higashi *et al*, 1997; Tholstrup *et al*, 1998; Mekki *et al*, 2002), although certainly not all (Harris *et al*, 1988, 1990; Weintraub *et al*, 1988; Zampelas *et al*, 1994; Roche *et al*, 1998; Tholstrup *et al*, 1998; Thomsen *et al*, 1999) previous trials have shown that unsaturated fats tend to produce a greater TAG response than saturates. This has been attributed to a range of factors including: (i) the physical form of the lipid droplet within the gastric emulsion, whereby saturated fats are less easily lipolysed in the gut; (ii) differential rates of absorption of the lipolysis products; and (iii) differential rates of catabolism of chylomicron remnants (Apgar *et al*, 1987; Schrijver *et al*, 1991; Muesing *et al*, 1995; Phan *et al*, 1999).

Less work has been carried out in the area of postprandial haemostatic response to high-fat feeding. There is evidence to suggest that the total dietary fat may increase postprandial FVIIc transiently and hence increase CVD risk (Miller *et al*, 1991; Salomaa *et al*, 1993; Oakley *et al*, 1998; Hunter *et al*, 2001), but conflicting evidence of differential effects of fatty acids. Five trials have failed to show a differential effect of dietary fat on FVIIc when comparing SFA, MUFA and PUFA (Larsen *et al*, 1997; Hunter *et al*, 2001), SFA and MUFA (Oakley *et al*, 1998), SFA and PUFA (Miller *et al*, 1991), and MUFA and PUFA (Larsen *et al*, 1999), while other trials showed that stearic acid may increase and PUFA may decrease postprandial FVIIc (Miller, 1989, 1998; Sanders

et al, 2001). Our trial showed no evidence of either an increase in FVIIc following the large fat bolus, instead there was a transient decrease following the bolus of fat, or a differential effect of fatty acids. There is no previous evidence of either total fat or fatty acid composition having an effect on circulating levels of fibrinogen (Freese & Mutanen, 1995) and our trial also failed to show any significant changes.

In conclusion, in response to an acute bolus of lipid given at breakfast, there were rapid and transient increases in glucose, insulin and amylin and decreases in FFA during the first hour. There was a gradual increase in TRL, TC and HDL-C and a decrease in LDL-C over 6 h and little change in the markers of haemostatic function, FVIIc and fibrinogen. Previous trials have shown TAG, HDL-C and FVIIc to be important postprandial markers of CVD risk, which have the potential to be modified through dietary change. Changes in the composition of the fatty acids within the lipid load significantly affected TAG-rich lipoproteins only, and there were no differential effects of the two test lipids on other postprandial markers of CVD risk that were measured.

Acknowledgements

We thank Glyn Muir who ran the metabolic kitchen during this trial. We are also grateful to Jenneke van Drunen, Esther de Swart and Joanna Lawrence for their considerable assistance during studentships. Cynthia Tse provided laboratory and administrative support throughout. We also thank the subjects who participated in this trial.

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