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ORIGINAL COMMUNICATION

Lipid-lowering effects of a modified butter-fat: a controlled intervention trial in healthy men

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Objective: To investigate the lipid-lowering potential of a butter-fat modified through manipulations in bovine feeding to increase the unsaturated : saturated fatty acid ratio.
Design: Double-blind, randomised, cross-over intervention trial.
Setting: University of Auckland Human Nutrition Unit, New Zealand.
Subjects: Twenty healthy, male subjects.
Intervention: A residential trial in which all foods and beverages were provided during two intervention periods, comprising 3 weeks of high unsaturated 'modified' *vs* 3 weeks of saturated 'control' butter feeding separated by a 4 week washout. Diets were of typical composition of 39 percentage energy (en%) fat (20 en% butter-fat), 48 en% CHO, 13 en% protein.
Results: There was a significant decrease in both total ($P < 0.05$, -7.9%) and LDL-cholesterol ($P < 0.01$, -9.5%) during modified butter feeding. There was no significant effect of treatment on a range of other risk factors including HDL-cholesterol, triglyceride, apolipoprotein A or B, nonesterified fatty acids (NEFA), haemostatic clotting factor VII and fibrinogen or glucose ($P > 0.05$). Subjects were maintained in energy balance and there was no significant change in body weight during intervention. Butter-fat composition alone differed between treatments.
Conclusions: A significant improvement in cardiovascular risk can be achieved by moderate changes in dietary fatty acid profile, achieved through a common and well accepted food source, butter-fat.
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Introduction

High-fat diets, particularly those high in saturated fats, have long been shown to have adverse effects on cardiovascular disease (CVD) risk factors such as serum total and LDL-cholesterol (Grundy & Vega, 1988). For many years the recommendation to replace dietary saturated fats with carbohydrates has been an important public health message both for weight loss and improvements in cardiovascular health *per se* (NIH clinical guidelines the evidence report, June 1998). However this has been questioned and considerable controversy has arisen (Katan *et al*, 1997). Whilst rigorously controlled, residential trials of well-motivated compliant participants have clearly shown that a low-fat high-CHO diet can result in weight loss (Prewitt *et al*, 1991; Stubbs *et al*, 1995; Poppitt *et al*, 1998), in larger, longer-term community trials the results have been predominantly (Sheppard *et al*, 1991; Jeffrey *et al*, 1995; Willett, 1998) although not entirely (Saris *et al*, 2000; Poppitt *et al*, 2001) disappointing. Of equal concern are the purported adverse effects on circulating lipids. Whilst the replacement of saturated fat by CHO is well established in reducing circulating

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LDL-cholesterol, it may be accompanied by a concomitant reduction in HDL-cholesterol and/or increase in serum triacylglycerol (TG), both adverse factors for CVD risk (Katan *et al*, 1997; Katan, 1998).

An alternate approach to improving cardiovascular risk is to make alterations in the quality of the fat consumed. Many trials have shown that replacement of dietary saturated fatty acids with predominantly mono- (MUFA) and/or polyunsaturated (PUFA) fatty acids can improve serum lipid profile considerably (Grundy & Vega 1988, Berry *et al*, 1991; Hu *et al*, 1997), possibly by increasing the activity of LDL receptors in the liver. Most studies have investigated extreme manipulations of diet. Strategies in which saturated fatty acids are replaced by MUFAs or PUFAs within a normal diet would be of considerable importance to public health policy if it could be shown that significant reductions in risk could be achieved through simple physiological changes in commonly eaten foods. One of the most important food groups known to be naturally high in saturates, particularly myristic and palmitic acids, are the dairy fats. Dairy products comprise a considerable proportion of the diet in countries such as the United States, Europe and New Zealand and thus make an excellent tool through which reductions in adverse lipid and lipoprotein profiles may possibly be achieved. Three previous bovine feeding trials in Australia and Europe have investigated the effect of a reduced-saturates dairy diet on serum lipid profile and associated CVD risk factors, using a range of methods and with conflicting outcomes (Nestel *et al*, 1973; Noakes *et al*, 1996; Tholstrup *et al*, 1998). Only the Danish trial investigated the effect of modifying butter-fat alone, and this trial failed to show an improvement in risk profile (Tholstrup *et al*, 1998).

The aim of our current study was to determine whether a natural butter-fat, modified to replace a proportion of saturates with MUFAs and PUFAs through bovine feeding methods, could improve established CVD risk factors including circulating lipid profile and haemostatic clotting factors in healthy men following a strictly controlled dietary regime.

Methods

Subjects

Twenty healthy, male volunteers were recruited into the study following a wide advertising campaign for interested participants. All were of normal body weight (body mass index (BMI) = 18–25 kg/m^2) and, following a screening panel for clinical biochemistry, were shown to be normal for lipid profile, liver function, thyroid function (as assessed by T4, TSH), plasma glucose and insulin levels, and blood pressure. None had a known history of CVD or diabetes, nor were currently or previously treated for hypertension or any metabolic disorder. All volunteer subjects provided written informed consent. Ethics approval for this study was obtained from the University of Auckland and the Auckland North Health Authority Ethics Committees.

Protocol

This study was a double-blind, randomised, cross-over dietary intervention in which compliance was ensured by provision of all foods and beverages. Subjects were randomly assigned to enter either the treatment or placebo arm of the trial. All were required to be residential at the University of Auckland Human Nutrition and Metabolic Unit throughout both dietary intervention periods. Each of the two intervention periods was 21 days in length and each separated by a minimum washout period of 4 weeks during which time all volunteers returned home and resumed their normal diet. Blood and urine samples were routinely collected throughout the intervention. Fasted blood samples were collected by venipuncture on the morning of days 0 and 1 (pre-intervention baseline), 7, 14, 21 and 22. Twenty-four-hour urine samples to assess dietary compliance by nitrogen balance were collected on days 10 and 20 on both arms of the intervention. Body weight was measured daily whilst subjects were fasted and after voiding of the bladder. Blood samples were analysed for total cholesterol and fractions, TG, apolipoprotein A, apolipoprotein B, nonesterified fatty acids (NEFA), glucose, insulin and haemostatic clotting factors fibrinogen and factor VII.

Butter-fat composition

The composition of the two butter-fats used in this trial is shown in Table 1. In the modified butter a proportion of the saturated fats were replaced by MUFAs and PUFAs. The two butters were fed to subjects such that the total intake of butter fat was identical on each arm. The modified low saturate butter-fat was manufactured for this trial using bovine feeding methods. Lactating dairy cows were fed a diet enriched with unsaturated fatty acids, protected from saturation in the rumen by an encapsulating coat, to primarily promote the MUFA and PUFA and reduce the saturated fat content of the milk from which the butter fat was derived. As these cows were also feeding on pasture, the colour of the butter produced was comparable to the control

Table 1 Composition of the control and modified butter-fat

Composition (%)	*Control butter*	*Modified butter*	*Delta*
Total fat content (percentage w/w)	85.2	81.7	−3.5
Moisture (percentage w/w)	12.4	15.4	+3.0
Total saturated fat (percentage 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 (percentage 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 (percentage 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	191	−31

butter. During production, the control butter was reworked to reduce the hardness and hence reduce any perceived difference in the texture of the butters. The quality of the modified butter was excellent, with a clean taste and good keeping properties. The only dairy fat product given to subjects in this intervention was the control and modified dairy butter. No cheese, yoghurt, spreads or other dairy derived lipid products of any kind were included in the diet. The butter-fat was incorporated into meals and snacks throughout the day, as butter on toast for breakfast, pre-prepared sandwiches for lunch, into sauces and desserts for the evening meal and cakes and biscuits between meals.

Diet

The background diet was designed to be identical on both arms of the intervention to ensure that the only variable in the diet was the fatty acid profile driven by the composition of the control and modified butter–fats. The total dietary intake, including the butter-fat supplement, for all subjects is shown in Table 2. The diet was controlled for total fat and cholesterol, total CHO and fibre, total protein and protein fractions, and micronutrients including Na, K and Ca. To ensure both treatments were identical all food ingredients were weighed to the nearest gram during diet preparation. The energy and macronutrient content of the diet was initially calculated using the dietary program 'Diet 1' (Crop and Food Research, Palmerston North, New Zealand) and then verified by direct chemical analyses of duplicate diet samples. The duplicate diet methodology was such that on 12 occasions during the intervention a duplicate 4 day diet from a single subject was collected, homgenised and an aliquot frozen for later chemical analysis. This enabled the absolute composition of the diet to be verified and also demonstrated that there were no significant trends caused by seasonal variability in food products included in the diet. Butter-fat provided half of the total fat in the diet ($=20\%$ of total energy), and hence was scaled to total energy intake and body weight for each individual.

Subjects were fed to energy balance, based on a multiple of predicted basal metabolic rate (BMR, Schofield *et al*, 1985) and diets were altered on a daily basis to maintain a constant body weight during each intervention period. A combination of change in body weight, reported activity and hunger levels was used to assess total daily energy requirements. A 4 day dietary rotation was used during the study such that every fifth day the entire diet repeated. Subjects were provided with breakfast, lunch, dinner and between-meal snacks. Breakfast and dinner were eaten under supervision at the Metabolic Unit, whilst lunch and snacks were packed and volunteers were able to take them to college or their place of work as required. Decaffeinated, sugar-free beverages and decaffeinated tea and coffee were freely available. Subjects were required to eat only and all of the foods provided. Alcohol was prohibited throughout the intervention. The subjects were self-selected and highly motivated. Independent dietary compliance was assessed from 24 h urinary nitrogen balance data, where urinary losses of nitrogen were directly compared with dietary protein intake (where g protein $=6.25\times$gN).

Table 2 Composition of the diet including the butter supplements as measured by direct chemical analysis; mean ± s.d.[a]

	Control butter	*Modified butter*	*Delta*
Energy intake, EI (range, MJ/day)	10.5–15.5	10.5–16.0	
EI (mean, MJ/day)[b]	13.1 ± 0	13.2 ± 0.2	+0.1
CHO, en%[b]	47 ± 0.6	48 ± 0.6	+1
Protein, en%[b]	13 ± 0.7	13 ± 0.7	0
Fat, en%	40 ± 0.8	39 ± 0.8	−1
Cholesterol (mg/day)[c]	298 ± 2	280 ± 2	−18
Total SFA (calculated, en%)	20 ± 0.3	15 ± 0.3	−5
SFA profile (mg/g)			
C10:0	3.1	2.6	−0.5
C12:0	12.9	14.4	+1.5
C14:0	16.1	8.8	−7.3
C16:0	37.4	26.6	−10.8
C18:0	12.7	16.8	+4.1
Total MUFA (calculated, en%)	6 ± 0.2	8 ± 0.1	+2
MUFA profile (mg/g)			
C16:1	3.2	2.3	−0.9
C18:1	31.6	44.0	+12.4
Total PUFA (calculated, en%)	14 ± 0.1	16 ± 0.2	+2
PUFA profile (mg/g)			
C18:2	34.1	44.8	+10.7
C18:3	2.7	3.6	+0.9

[a]Results are the mean values for six duplicate portions analysed from both control and modified diets. The major effects were a reduction in C14:0, C16:0 and an increase in C18:0, C18:1, C18:2 in the Modified diet.
[b]No significant difference between treatments ($P>0.05$). Minor fractions of fatty acid profiles not shown. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.
[c]Cholesterol intake shown for a typical 13.0 MJ diet.

Statistical analyses

t-Test analyses were used to identify any differences in dietary energy or macronutrient composition between the modified and control diets as eaten by the subjects (background diet + butter-fat supplement). All anthropometric and metabolic variables including body weight, total-, LDL- and HDL-cholesterol, TG, apolipoprotein A and B, fibrinogen and factor VII were analysed for between-diet effects with time and subject interactions, using the mixed model procedure of split-plot-in-time repeated measure ANOVA. These data was also analysed for longitudinal changes between baseline and the end of the intervention on each treatment separately from repeat measures ANOVA, assessing the change in slope over the entire 21 days of the intervention. All baseline metabolic data were calculated as the mean (± s.e.m.) of the two pre-intervention blood samples collected on days 0 and 1. The repeat measure on each individual was performed to increase the accuracy of baseline. 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 bovine feeding regimen that was followed in this trial was able to achieve a ~16% decrease of saturated fatty acids within the butter fat, replaced by ~9 and ~8% increases in mono- and polyunsaturates, respectively. The major reductions were in palmitic (C16 : 0, − 12.7%) and myristic (C12 : 0, − 3.7%) acids. Octadecanoic acid (C18 : 1) increased by 11.4%, linoleic (C18 : 2) by 6.0% and α-linolenic by 1.5%. The macronutrient composition of the total diet consumed, including the butter supplement, was on average 39% of total energy derived from fat, 48% from carbohydrate and 13 from protein (Table 2). There was no significant difference between total energy or total macronutrient composition between treatments ($P > 0.05$). As intended in the study design, the considerable differences in composition between the two butter-fats resulted in a significant difference in fatty acid profile between the two treatment diets. There was also a difference in dietary cholesterol between treatments, reflecting the lower content in the modified butter-fat.

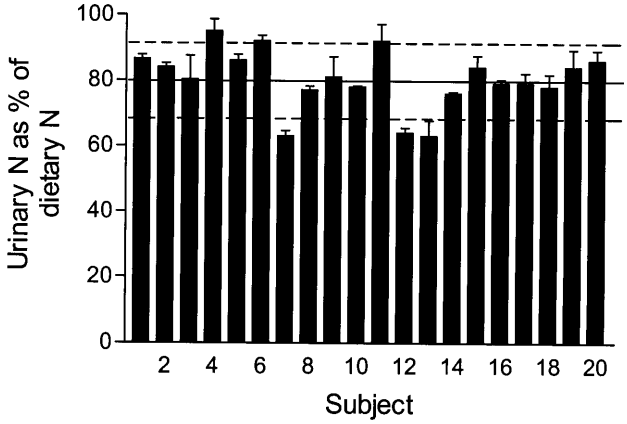

Figure 1 Twenty-four-hour urinary nitrogen excretion calculated as a percentage of 24 h dietary nitrogen intake during 4 days' measurement for each subject. Range 70–90% indicated by the dotted lines, mean ± s.e.m.

Table 3 Body weight and metabolic risk factors on the two control and modified butter treatments

	Control butter		*Modified butter*	
Variable	*Pre*	*Post*	*Pre*	*Post*
Body weight (kg)	68.7 ± 6.1	68.4 ± 6.0	69.4 ± 6.2	69.3 ± 5.9
Total cholesterol (mmol/l)	4.54 ± 0.5	4.31 ± 0.6	4.58 ± 0.7	4.22 ± 0.7*
LDL-cholesterol (mmol/l)	2.92 ± 0.5	2.85 ± 0.6	2.98 ± 0.6	2.70 ± 0.5**
HDL-cholesterol (mmol/l)	1.24 ± 0.3	1.16 ± 0.3	1.22 ± 0.3	1.19 ± 0.3
Triglyceride (mmol/l)	0.84 ± 0.4	0.69 ± 0.3	0.85 ± 0.3	0.74 ± 0.2
Apolipoprotein A (g/l)	1.67 ± 0.2	1.62 ± 0.2	1.66 ± 0.2	1.61 ± 0.2
Apolipoprotein B (g/l)	0.81 ± 0.1	0.75 ± 0.1	0.82 ± 0.2	0.74 ± 0.1
Fibrinogen (g/l)	2.78 ± 0.4	3.02 ± 0.8	2.95 ± 0.9	2.74 ± 0.7
Factor VII (U/l)	937 ± 218	915 ± 265	873 ± 252	853 ± 291

Mean ± s.d. No significant difference preintervention between control and modified butter treatments for any measured variables; pre-intervention calculated as the average of d0 + d1. Post-intervention, day 22. Significant effect of treatment, ANOVA, *$P < 0.05$; **$P < 0.01$.

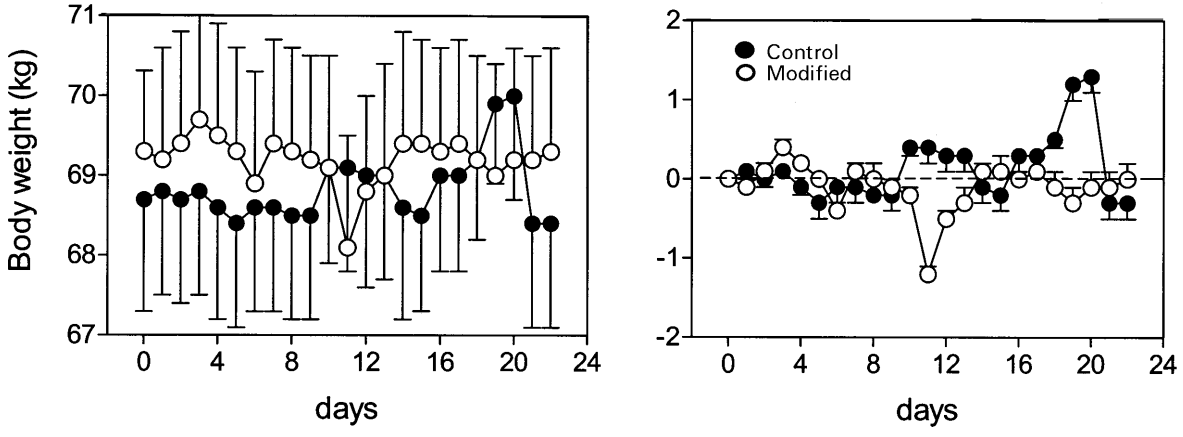

Figure 2 No significant change in body weight during 3 weeks on control and modified butter-fat treatments, mean ± s.e.m.

Subject motivation and compliance was maximised in this residential study by provision of all foods and beverages throughout both intervention periods. Figure 1 shows an estimate of compliance for each subject as assessed by 24 h N balance on four occasions during the trial. Body weight and metabolic outcomes pre- and post-intervention are shown in Table 3. There was no significant difference at baseline between the control and modified butters for any of the parameters measured ($P > 0.05$). Figure 2 shows that there was no significant difference in the average body weight of the subjects during the 3 weeks of modified or control butter feeding, nor was there a significant increase or decrease during either intervention period which would have influenced lipid profile ($P > 0.05$). Body weight was successfully

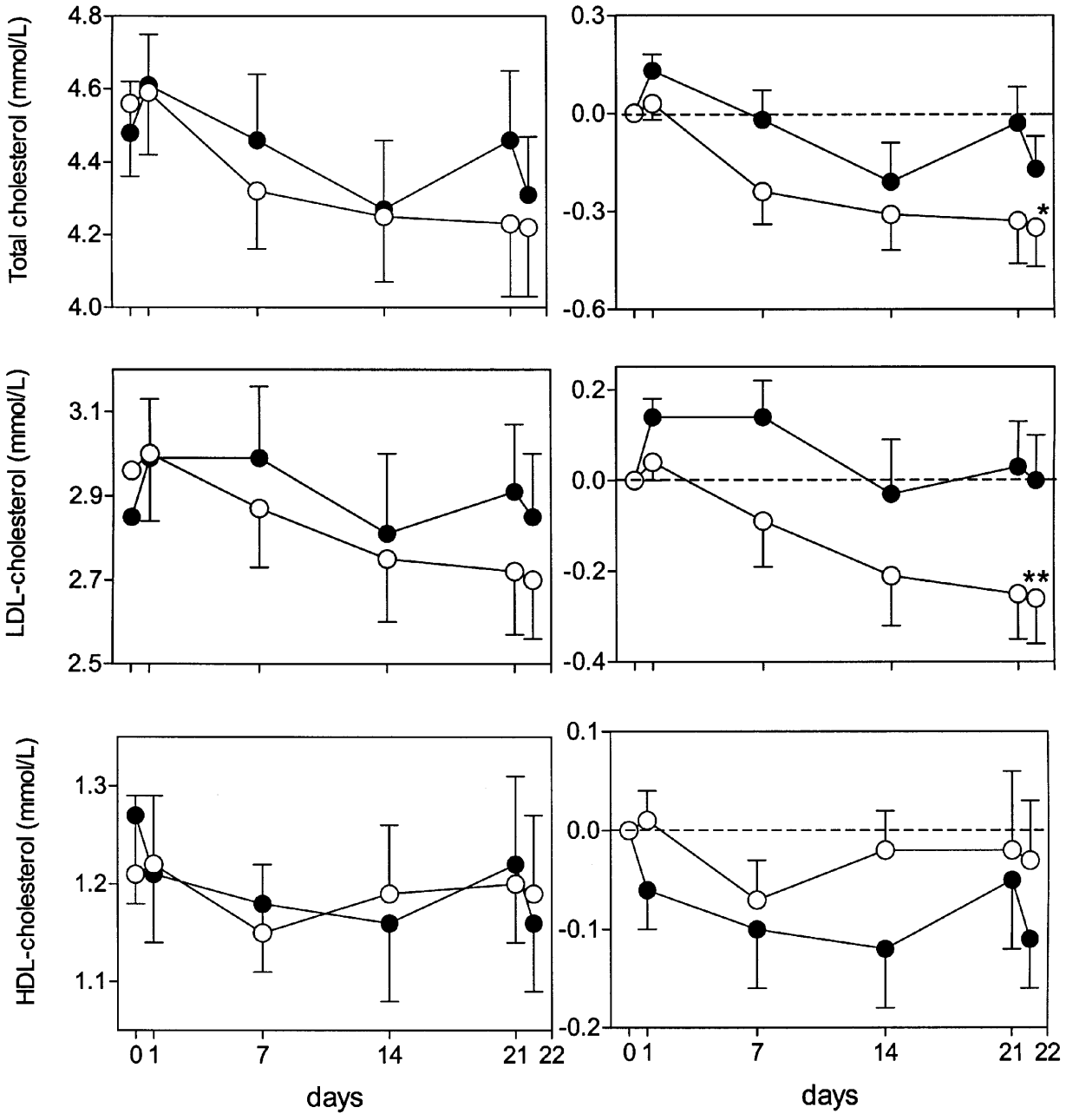

Figure 3 Total, LDL- and HDL-cholesterol during the 3 week control and modified butter-fat treatments. Change from day 0 baseline is shown in the right hand panel. Statistical significance shown for between treatment effects (ANOVA). *$P < 0.05$; **$P < 0.01$, mean ± s.e.m.

maintained within limits of ±2 kg of the baseline weight on both arms of the intervention.

Figure 3 shows both the absolute change (left-hand panel) and the change relative to d0 (right-hand panel) of total, LDL- and HDL-cholesterol. There was a significant treatment effect in this intervention, such that lipid profile improved on the modified product. Both total- ($P<0.05$) and LDL-cholesterol ($P<0.01$) were significantly reduced on the modified butter when compared with the control butter throughout the 3 week intervention. In addition to the between-treatment effect there was also a significant decrease relative to baseline within both treatments. Total cholesterol decreased by −0.36 mmol/l ($P<0.001$) between baseline and day 22 on the modified butter, and by −0.24 mmol/l ($P>0.01$) on the control butter. When calculated as percentage change from baseline, by day 22 total cholesterol had decreased by −7.9% and −5.3%, respectively. The modified butter also decreased LDL-cholesterol between baseline and the end of the intervention by −0.28 mmol/l (−9.5%, $P<0.01$) and remained virtually unchanged on the control butter (−0.07 mmol/l; −2.4%, $P>0.05$). There was no significant difference in HDL-cholesterol ($P>0.05$) between butter treatments during the 3 week intervention, nor was there a significant change between baseline and end of the intervention on the modified butter treatment ($P>0.05$). There was however a longitudinal decrease in HDL-cholesterol on the control treatment ($P<0.05$). Circulating TG levels were also unaffected when compared across treatments ($P>0.05$; Figure 4), but both modified ($P<0.01$) and control ($P<0.05$) butter arms of the intervention reduced TG over the 3 weeks. There was a trend for total cholesterol : HDL and LDL : HDL ratio to both decrease on the modified butter (TC : HDL, $\delta=-0.18$; LDL : HDL, $\delta=-0.15$), but this did not reach significance ($P>0.05$). There was no significant treatment effect for either of the haemostatic clotting factors measured, fibrinogen and factor VII ($P>0.05$), nor did either variable significantly change relative to baseline during intervention. There were no significant between treatment effects on apo A, apo B, NEFA or serum glucose ($P>0.05$).

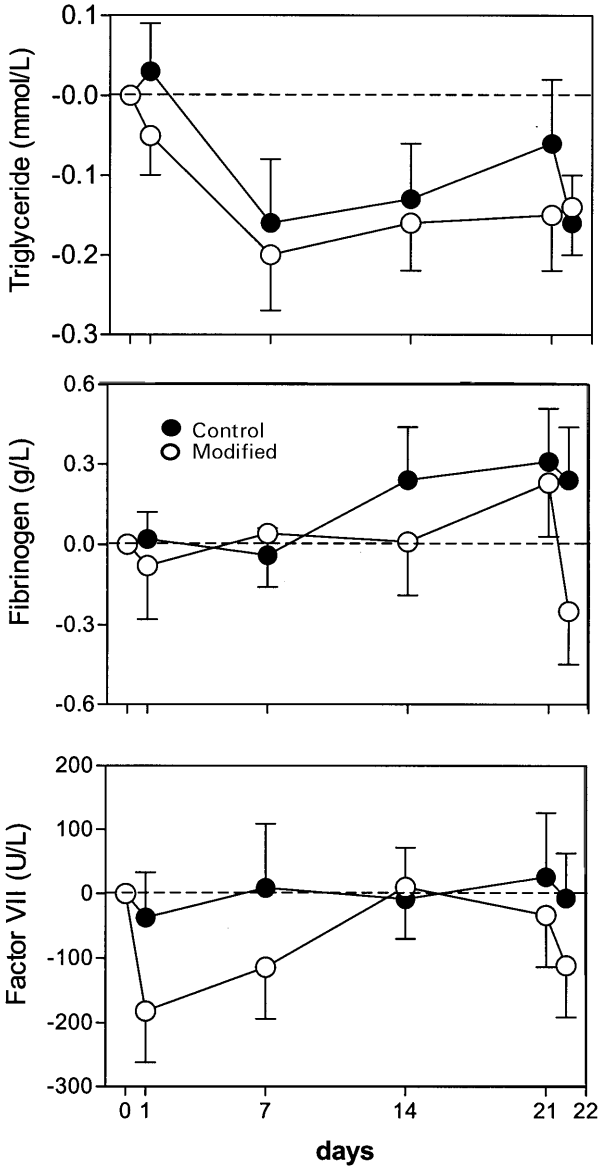

Figure 4 Change in serum triglyceride and haemostatic clotting factors, fibrinogen and factor VII, during the control and modified butter-fat treatments, mean ± s.e.m.

Discussion

This carefully controlled dietary intervention trial has shown that small, but clinically significant, reductions in total- and LDL-cholesterol can rapidly be achieved when a butter fat modified to alter fatty acid profile in favour of mono- and polyunsaturates is introduced into the diet. One of the most important features of the study was the ability to maintain all dietary constituents identical on both arms of the trial. Only butter-fat profile was altered between treatments and hence the significant changes in blood lipid profile can be entirely attributed to the modified butter-fat product.

If the ~8–10% reduction in total- and LDL-cholesterol is converted into reduction in absolute risk of coronary heart disease (CHD) or stroke using criteria set by a trial such as the MR FIT study (Law *et al*, 1994), CHD and stroke risk would be predicted to drop by up to 27 and 24%, respectively. This is a highly significant reduction in clinical risk. It is of considerable importance that this trial has achieved such an improvement in profile in healthy individuals, not only because they may represent the most difficult group of individuals in which to drive changes in circulating lipids, but also because this change represents prevention rather than treatment of cardiovascular disease. The formation of atherosclerotic plaques in Western society may originate in early childhood or adolescence whilst the population is apparently far below the conventional cut-off points for

abnormal lipid profile (Stehbens, 1995). Any nutritional strategy which can ensure prevention rather than cure must underpin current public health policies.

There have been a number of earlier trials in which ruminant fats (meat and dairy) have been modified (Nestel *et al*, 1973; Nestel & Havenstein, 1974; Hodges *et al*, 1975; Stein *et al*, 1975; Vivian & Fulton, 1975; Brown & de Wolf, 1976) but few where bovine feeding has been used to modify milk fat alone. In the two previous studies (Noakes *et al*, 1996; Tholstrup *et al*, 1998) which have investigated changes in cardiovascular risk achieved by feeding modified dairy products alone, the findings have been equivocal. This is perhaps not surprising when the methodologies employed are more carefully considered. Firstly, only the Danish trial reports that diet was fully controlled (Tholstrup *et al*, 1998), although there was no independent validation of compliance; secondly also only in the Danish trial was dairy fat alone manipulated; thirdly, again in the Danish trial there were important differences between the dairy fat sources given to volunteers, not least of which was the *trans* MUFA content. In the Australian trial (Noakes *et al*, 1996) which was an 8 week community trial of mildly hyperlipidaemic men and women, there was a significant improvement in lipid profile when 20% of dietary fat intake was given as mixed dairy products. The Danish trial (Tholstrup *et al*, 1998) focused on a population of healthy men who were provided with 30% of their total energy intake in the form of a low-saturate modified butter over a 4 week period. As noted above, this was the only trial in which dairy lipids alone were modified. Despite employing a similar method of modified bovine feeding to our current trial, they did not observe any change in plasma lipids or lipoproteins in response to treatment. The authors suggest that this may be due to the high level of *trans* fatty acids in their modified butter, specifically 18 : ln-7, which is likely to be cholesterolaemic in human populations. Certainly *trans* fatty acids, commonly formed during partial hydrogenation, have been shown to increase LDL- and reduce HDL-cholesterol fractions in numerous studies (Ascherio & Willett 1997).

Alteration of the fatty acid profile of dairy, and potentially other high fat products is a novel approach to the problem of dyslipidaemia. Reduction in the total content of dairy-derived fats in a range of products, such as milk and spreads, has long been an important strategy for public health. For example, as predicted from current knowledge of the effects of specific fatty acids on lipids and lipoproteins, low-fat or 'skimmed' milk reduces total- and LDL-cholesterol significantly compared to whole milk containing a higher proportion of saturates. It is interesting to note that the change in fat quality in our current study was able to achieve a reduction in lipid profile of similar magnitude to these fat reduction trials. Steinmetz *et al* (1994) showed a decrease in LDL-cholesterol of 0.19 mmol/l following a 6 week, skimmed milk intervention. A similar study by Rossouw *et al* (1981) had previously shown a decrease of 0.4 mmol/l over 5 weeks when skimmed milk was included in the diet. A number of other studies have shown similar reductions (Maruyama & Ezawa, 1991; Buonopane *et al*, 1992; Estevez-Gonzalez *et al*, 1998). On the modified butter arm of our current study we were able to achieve a decrease in LDL-cholesterol of 0.28 mmol/l across a general 'low-risk' group of the population. It is also of interest that total and LDL-cholesterol were not raised by the period of control butter feeding, which suggests that the level of total and/or saturated fatty acids were no greater during this arm of the intervention than in the normal diet of these subjects.

There are also a number of trials which have investigated the effect of altering the fatty acid profile of spreads, by for example, replacing butter-fat with high mono- or polyunsaturated fat margarines. The lipid lowering effects of the modified butter in our current study compare well with these margarine trials. In an early trial Seppanen-Laakso *et al* (1992) replaced butter with rapeseed oil-containing margarine and observed a significant reduction in LDL-cholesterol of 5.2%. A more recent cross-over trial of regular milk fat, modified (low cholesterol) milk fat and margarine (Jacques *et al*, 1999) showed that margarine decreased LDL-cholesterol by 12%. Wood *et al* (1993) showed a 5% differential in LDL-cholesterol between butter and a high *trans*-PUFA margarine, and in a comparison of butter and margarines with variable *trans* fatty acid contents, LDL-cholesterol decreased by 11, 9 and 5%, respectively, when semi-liquid, soft and stick margarines were incorporated into the diet (Lichtenstein *et al*, 1999) and by 4.9 and 6.7% when *trans* and non *trans* margarines were compared to butter (Judd *et al*, 1998).

In conclusion, incorporation of a butter-fat modified through bovine feeding to replace myristic and palmitic acids predominantly with oleic and linoleic acids resulted in a significant reduction in total- and LDL-cholesterol in healthy adult males without a concomitant decrease in HDL-cholesterol. The magnitude of the improvement in lipid profile compares favourably with the many previous studies which have replaced butter-fat with non-dairy hydrogenated spreads. Such an improvement in cardiovascular risk across the population is predicted to reduce CHD and stroke by approximately one quarter.

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