Effect on lipoprotein profile of replacing butter with margarine in a low fat diet: randomised crossover study with hypercholesterolaemic subjects

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Abstract

Objective—To examine the effect on lipid and lipoprotein concentrations when butter or an unsaturated margarine is used for cooking or spreading in a reduced fat diet.

Design—Randomised crossover study with two intervention periods of six weeks' duration separated by a five week washout.

Setting—Community setting in New Zealand.

Subjects—49 volunteers with polygenic hypercholesterolaemia and baseline total cholesterol concentration in the range 5.5–7.9 mmol/l.

Main outcome measures—Concentrations of total and low density lipoprotein, Lp(a) lipoprotein, high density lipoprotein, apolipoprotein B 100, and apolipoprotein A I.

Results—Concentrations of low density lipoprotein cholesterol and apolipoprotein B were about 10% lower with margarine than with butter. Lp(a) lipoprotein and high density lipoprotein cholesterol concentrations were similar with the two diets.

Conclusion—Despite concerns about adverse effects on lipoproteins of trans fatty acids in margarines, the use of unsaturated margarine rather than butter by hypercholesterolaemic people is associated with a lipoprotein profile that would be expected to reduce cardiovascular risk.

Introduction

Dietary recommendations aimed at reducing the risk of coronary heart disease require an appreciable reduction in saturated fatty acids from present intake in most Western countries.^{1,2} A major justification for this recommendation is the association between saturated fatty acids and total and low density lipoprotein cholesterol.³ n-6 Polyunsaturated fatty acids and n-9 monounsaturated fatty acids are widely recommended as replacements for saturated fatty acids.⁴ It has recently become apparent that high intakes of n-6 polyunsaturated fatty acids are associated with decreased concentrations of high density lipoprotein cholesterol⁵ and that trans forms of unsaturated fatty acids may increase the risk of coronary heart disease⁶; possibly by raising total and low density lipoprotein cholesterol and Lp(a) lipoprotein and reducing high density lipoprotein cholesterol.^{7,8} Health professionals and the public have become uncertain about implementing nutrient recommendations and about the optimal sources of dietary fat.

Margarines rich in unsaturated fatty acids are often recommended as replacement fats for butter, which is relatively high in saturated fatty acids. Most margarines contain at least some trans unsaturated fatty acids,⁸ and this has led to the suggestion that their

use might be inappropriate in diets aimed at reducing the lipoprotein mediated risk of coronary heart disease. To assess the effects on lipid and lipoprotein concentrations, we compared butter and a margarine high in monounsaturates used as a source of "hard" fat in the context of an overall reduced fat diet.

Subjects and methods

Fifty six subjects with total cholesterol concentrations in the range 5.5–7.9 mmol/l were recruited by newspaper advertisements and from those who had participated in previous studies. People with familial or secondary hyperlipidaemia or taking drugs known to influence lipid metabolism were excluded; thus most subjects had polygenic hyperlipidaemia. All subjects gave informed consent on the understanding they could withdraw at any time from the study, which was approved by the Ethics Committee (Otago) of the Southern Regional Health Authority. Forty nine subjects (35 women, 14 men), mean age 46.8 (SD 11.6) years, completed the 21 week protocol. Initial mean body mass index was 26.4 (3.8) kg/m². At the time of recruitment mean concentrations of total cholesterol low density lipoprotein cholesterol, and high density lipoprotein cholesterol were 6.5 (0.6), 4.2 (0.7), and 1.3 (0.3) mmol/l.

During a four week run in period participants continued their usual diet and completed a four day food record for assessment of usual food and nutrient intakes. They also completed questionnaires concerning past, present, and family medical history as well as use of medications. At the end of the run in period they were randomised to groups who were asked to follow either the butter diet or the margarine diet for six weeks. After the first experimental period, followed by a five week washout period (during which they were asked to revert to their usual diet), they then followed the alternative diet for a final six week period. Two blood samples were collected after an overnight fast during the last week of the run in period, the last week of the washout period, and the last week of each six week experimental period. Blood specimens were separated by centrifugation at 1500 g. Lipid and lipoprotein concentrations were measured in each blood sample, and apolipoproteins A I and B, Lp(a) lipoprotein, and triglyceride fatty acids were measured in one sample during each period.

The experimental diets were individually prescribed and based on the energy intake calculated from the self selected diets reported during the run in period. They were designed so that protein, carbohydrate, and fat provided approximately 19%, 55%, and 26% of total energy, respectively. The two experimental diets differed only in the source of "hard fat" used for baking, cooking, and spreading. Total saturated fatty

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acids were calculated to provide 12% and 6% of total energy in the butter and margarine diets respectively and total polyunsaturated fatty acids 3% and 7% (see table 3). Table 1 shows the fatty acid composition of butter and margarine (a standard brand widely available in New Zealand and comparable with margarines made from canola oil available in the United Kingdom, North America, and most other countries). The only other nutrient that differed as a consequence of the differing fat source was cholesterol (32 mg/MJ in the butter diet and 23 mg/MJ in the margarine diet).

Detailed dietary information and reinforcement were provided during personal and telephone interviews initially and at regular periods throughout the study. Individual instruction booklets containing practical details which translated the nutrient requirements of the experiment into advice about food were prepared for each participant. Butter and margarine were provided for the participants and their families. Compliance was measured by four day food records completed during the last week of each experimental period and by examining the fatty acid profile of plasma triglyceride. Participants were encouraged to continue their usual pattern of physical activity and all other aspects of their lifestyle throughout the study.

Cholesterol concentration in plasma and lipoprotein fractions was measured enzymatically with Boehringer kits and calibrators, and triglyceride was measured enzymatically with Roche Diagnostic kits on a Cobas Fara analyser. Coefficient of variation was 1.6% for cholesterol and 3.4% for triglycerides in the Royal Australasian College of Pathologists' quality assurance programme. After precipitation of lipoproteins containing apolipoprotein B, high density lipoprotein cholesterol was measured in the supernatant with phosphotungstate-magnesium chloride solution.⁹ Low density lipoprotein cholesterol was calculated by using the Friedewald formula.¹⁰ Apolipoproteins A I and B were measured by immunoturbimetry with Boehringer kits (coefficient of variation 2.6% and 6% respectively). Lp(a) lipoprotein was determined by two site radioimmunoassay with a commercial kit provided by Pharmacia (Uppsala, Sweden; coefficient

of variation 4%). Plasma lipids were extracted according to the procedure of Bligh and Dyer¹¹ and triglycerides were separated by thin layer chromatography. Triglyceride fatty acids were analysed by gas liquid chromatography.¹² The precision of the fatty acid measurement was determined by repeated analysis of a pooled plasma sample. The coefficient of variation for all fatty acids in the pooled sample ranged from 1% to 4%.

For all variables, measurements made during the last week of each period were not significantly different, so the mean of the two values was used to provide a more reliable measure of values before intervention (end of run in, end of washout) and after intervention. Differences between the changes in measurements before and after intervention on the two diets were compared by analysis of variance with repeated measures. As part of this analysis, the effect of the diet sequence on the relative treatment responses was tested as a diet by sequence interaction term. When no significant effects of sequence on the dietary responses were observed, the data for the butter and margarine periods were pooled over both dietary sequences. The difference between the changes are represented as means with 95% confidence intervals. Statistics for Lp(a) lipoprotein were computed with log transformed data.

Results

Body weight and blood pressure were unchanged throughout the study. Table 2 shows concentrations of plasma lipids, lipoproteins, apolipoproteins, and Lp(a) lipoprotein. Total and low density lipoprotein cholesterol and apolipoprotein B showed an appreciably greater decrease during the margarine diet than the butter diet. The sequence of dietary interventions had no significant effects on these differences between diets ($P=0.416$, $P=0.525$, and $P=0.153$, respectively). All other measurements were similar on the two diets.

The reported nutrient intakes based on the four day diet records are contrasted with the prescribed diets in table 3. Participants consumed similar amounts of foods from the major food groups during the two experimental periods. On the two diets an average 17 g

Table 1—Fatty acid composition (g/100 g fatty acids) of butter and margarine used in study

	Fatty acid											A1
	C14:1			C16:1			C18:1		C18:2			
	C14			C16:0			C18:0	Cis	Trans*	Cis	Trans*	
		Cis	Trans		Cis	Trans						
Butter	9.5	0.9	0.4	29.2	1.9	0.5	11.9	21.6	3.5	1.9	0.8	0
Margarine	0.0	0.0	0.0	7.5	0.2	0.2	6.2	40.8	12.5	22.7	1.0	1

*Includes all trans isomers.

Table 2—Plasma lipid, lipoprotein and Lp(a) lipoprotein values before and after the two experimental diets. Values are mean (SD) except for Lp(a) lipoprotein, which is given as antilog of mean log (range)

	Butter diet		Margarine diet		Change with margarine diet minus change with butter diet (95% confidence interval)	P value
	Before intervention	After intervention	Before intervention	After intervention		
Total	6.58 (0.77)	6.27 (0.87)	6.48 (0.76)	5.89 (0.79)	0.28 (0.07 to 0.49)	0.011
Low density lipoprotein	4.44 (0.74)	4.21 (0.78)	4.38 (0.74)	3.82 (0.74)	0.32 (0.09 to 0.56)	0.009
High density lipoprotein	1.27 (0.31)	1.26 (0.29)	1.26 (0.30)	1.24 (0.30)	0.01 (-0.04 to 0.07)	0.482
Total triglyceride (mmol/l)	1.91 (1.01)	1.80 (0.99)	1.85 (1.17)	1.85 (1.11)	-0.10 (-0.38 to 0.16)	0.439
Apolipoprotein A I (mg/l)	123.67 (21.30)	119.71 (17.71)	123.08 (17.08)	117.57 (25.79)	1.55 (-7.66 to 11.076)	0.679
Apolipoprotein B 100 (mg/l)	83.49 (11.47)	80.12 (10.12)	87.61 (19.28)	75.67 (11.45)	8.57 (1.39 to 15.74)	0.006
Lp(a) lipoprotein (U/l)	225 (19-1182)	223 (18-1095)	237 (19-1306)	249 (17-1111)		0.605

Table 3—Mean (SD) nutrient intake prescribed for experimental diets and reported in four day food records. Nutrients are expressed as percentage total energy except where indicated

Nutrients	Prescribed intake		Reported intake	
	Butter diet	Margarine diet	Butter diet	Margarine diet
Protein	19	19	18 (4)	17 (3)
Carbohydrate	55	55	52 (7)	57 (8)
Total fat	26	26	27 (6)	26 (6)
Saturated	12	6	13 (3)	8 (3)
Monounsaturated	8	11	8 (2)	10 (3)
Polyunsaturated	3	7	3 (1)	5 (1)
Cholesterol (mg/MJ)	32	23	32 (12)	26 (12)
Fibre (g/l MJ)	4	4	3 (1)	3 (1)
Alcohol	0	0	4 (5)	4 (5)

Table 4—Mean (SD) percentage fatty acid composition of plasma triglycerides

Fatty acid	Butter diet (n=20)	Margarine diet (n=20)
C14:0	3.45 (1.02)	2.71 (0.98)*
C14:1	0.46 (0.17)	0.35 (0.17)*
C16:0	29.29 (3.53)	26.72 (3.75)
C16:1	5.89 (1.51)	5.63 (1.25)
C18:0	3.54 (0.75)	3.62 (1.11)
C18:1	37.03 (3.93)	38.46 (2.90)
C18:2n-6	11.06 (2.77)	13.45 (3.79)*
C18:3n-3	0.97 (0.17)	1.12 (0.31)
C20:4n-6	0.74 (0.17)	0.08 (0.26)
C20:5n-3	0.18 (0.08)	0.17 (0.08)
C22:6n-3	0.62 (0.35)	0.58 (0.26)

*P < 0.05.

of butter or margarine was used per day for cooking and spreading. Plasma triglyceride fatty acid composition on the two diets is shown for 20 randomly selected participants in table 4. As expected, proportions of myristic acid (C14:0) and palmitic acid (C16:0) were significantly lower and proportions of linoleic acid (C18:2) were significantly higher in plasma triglycerides of subjects during the margarine diet than the butter diet.

Discussion

We compared the effects of butter and of margarine consisting of predominantly unsaturated fatty acids on lipid and lipoprotein concentrations in a group of moderately hypercholesterolaemic subjects. This approach has practical application, having been carried out in an outpatient setting rather than a metabolic ward, and in addition permits conclusions concerning the effects of various fatty acids consumed in the context of a low fat diet.

COMPLIANCE

The subjects were free living individuals and it could be argued that reduced compliance might have influenced the results. However, the participants were all highly motivated and were given detailed and specific dietary advice as well as free supplies of the test fats. Also, the experimental design reduced the possibility that declining enthusiasm over the duration of the study might have accounted for reduced compliance with either experimental diet. Four day dietary records meticulously kept throughout the study showed a high level of compliance. The fatty acid composition of plasma triglyceride in a randomly selected subsample of participants reflected the difference in composition of the major dietary fat sources and provides confirmation of adherence to prescribed diet.

LIPOPROTEIN RISK FACTORS

The findings concerning total and low density lipoprotein cholesterol and apolipoprotein B (the major apoprotein of low and very low density lipoprotein) were unequivocal; appreciably lower concentrations occurred with the margarine diet than the butter diet. These changes occurred in the context of a diet in which total fat was reduced from that usually eaten in Western countries to levels of intake recommended for populations and individuals at high risk of coronary heart disease.¹³ The amounts of butter and margarine were typical of what might be eaten in such a diet. Concentrations of triglycerides, high density lipoprotein cholesterol, apolipoprotein A I (the major apoprotein of high density lipoprotein) and Lp(a) lipoprotein were virtually identical in the two diets. Thus lipoprotein mediated risk of coronary heart disease might be expected to be lower with the margarine than the butter diet.

The suggestion that margarine may have untoward effects on cardiovascular disease and lipoprotein concentrations is based on epidemiological and experimental evidence. Willett and colleagues found an increase in the risk of coronary heart disease during eight years of follow up in subjects whose trans fatty acid intake was in the highest fifth when baseline measurements were made.¹⁴ Furthermore, high intake of foods that were the major sources of trans isomers of fatty acids (margarine, biscuits, cakes, and white bread) was associated with an increased risk of coronary heart disease. Mensink and Katan, and Nestel and colleagues, showed that C18:1 trans fatty acids, which comprise most trans isomers in the Western diet, are associated with a particularly unfavourable lipoprotein profile.^{15,16} Total cholesterol and low density lipoprotein cholesterol concentrations are at least as high as those found with diets rich in cholesterol raising saturated fatty acids. Lp(a) lipoprotein concentrations seem to be appreciably increased when elaidic acid provides around 7% total energy (about twice that in a Western diet). High density lipoprotein cholesterol concentrations may be reduced in association with increased intakes of C18:1 isomers. This constellation of changes might be expected to be associated with a substantial increase in the lipoprotein mediated risk of coronary heart disease. We found a more favourable lipoprotein profile with margarine than butter; concentrations of trans fatty acids in the margarine may have been insufficient to elicit these adverse effects on plasma lipids, and lipoproteins.

COMPARISON WITH OTHER STUDIES

Our study is the first to compare the effects of butter and unsaturated margarine with a fatty acid composition similar to that available in many countries, in a diet designed to lower plasma lipid concentrations in subjects with mild to moderate hypercholesterolaemia. The other study comparing the effects of butter and margarine on lipids and lipoproteins has limited practical application: normolipidaemic subjects were studied and the total fat content of the experimental diets (37-39% total energy) was appreciably greater than that recommended for individuals and populations at high risk of coronary heart disease. Furthermore, the previous work studied butter, butter enriched with monounsaturated and polyunsaturated fatty acids, and margarines with either a very high content of trans fatty acids or none at all—all products which, except for butter, are not widely available.¹⁷ Nevertheless the findings are broadly in agreement with our own and strengthen our conclusion that margarine is an appropriate replacement fat for butter in the diet of individuals with raised cholesterol concentrations.

Key messages

- Unsaturated margarine is associated with a more favourable lipoprotein profile than butter when the two fats are compared in the context of a low fat diet
- These findings suggest that the relatively small amounts of trans fatty acids that are typically present in unsaturated margarine have no adverse effects on lipid and lipoprotein concentrations
- The effects of unsaturated margarine on oxidation of low density lipoproteins are still to be examined
- Nevertheless, unsaturated margarine seems to be an appropriate substitute for saturated fat in diets recommended to people with hyperlipidaemia

REPLACEMENT OF SATURATED FAT

The need to reduce those saturated fatty acids (myristic and palmitic acids) which have been shown to raise total and low density lipoprotein cholesterol¹⁸ is now widely accepted. This applies especially to populations and individuals at high risk of coronary heart disease because of raised concentrations of total and low density lipoprotein cholesterol.⁴ Lower concentrations of total and low density lipoprotein cholesterol can be achieved by reducing intake of high fat dairy products, palm oil and products rich in this source of palmitic acid, and fatty meat.¹⁹ Oils rich in oleic acid (a monounsaturated fatty acid with a cis configuration) and linoleic acid (an n-6 polyunsaturated fatty acid) are suitable substitutes, provided n-6 polyunsaturated fatty acids do not supply more than 10% total energy, as larger amounts may be associated with reduced concentrations of high density lipoprotein cholesterol.^{20,21}

For many years, patients with hypercholesterolaemia have been advised to replace butter with margarine in their diet.²² However, recent information in the scientific as well as lay media concerning the untoward effects of trans fatty acids has created uncertainty in the minds of health professionals with regard to this recommended dietary change. Margarine might be associated with increased oxidation of low density lipoprotein because of the relatively high content of polyunsaturated fatty acids, and this possibility warrants further study. However, our findings confirm that trans fatty acids in margarine do not offset the beneficial effects of an unsaturated margarine on plasma lipid and lipoprotein concentrations in subjects

with mild hypercholesterolaemia. Thus it seems reasonable to conclude that such products are preferable to butter in the dietary management of dyslipidaemia and may be further improved by reducing their trans fatty acid content to zero.

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Conflict of interest: None.

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ONE HUNDRED YEARS AGO

THE EMBARRASSMENT OF GUY'S HOSPITAL.

The authorities of Guy's Hospital are now making a determined attempt to raise a sum of £500,000 for investment, to make good the depreciation in income from the endowments, which almost wholly consist of agricultural property. That an appeal from a great institution like Guy's is a matter of national importance is evidenced by the warm interest which the Prince of Wales is taking in the movement for re-endowment, and H.R.H. will preside on May 20th next at the only festival dinner ever held in connection with this hospital. A feature of the

movement of much interest, more especially to the medical profession, is the manner in which old Guy's men and present students are rallying to the support of their Alma Mater. Already Guy's men in practice have influenced several munificent donations, while it is believed that the majority are doing something. The present members of the Medical School have voluntarily organised themselves into a collecting committee, and, if Providence helps those who help themselves, then it may safely be prophesied that Guy's Hospital will not only maintain its present position, but also reopen its closed beds. (*BMJ* 1896;i:682.)