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Associations between plasma lipid concentrations and dietary, lifestyle and physical factors in the Oxford Vegetarian Study

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Blood lipids data were available for 3773 subjects from a cohort study of 11 000 individuals, 6000 of whom do not eat meat. The effects of dietary, lifestyle and physical factors on concentrations of total and HDL cholesterol were investigated. Meat, cheese and dietary fibre, with smoking habit and height were found to be significantly related to total cholesterol in men. For women, meat, cheese, dietary fibre and tomatoes, and type of spreading fat were all significantly related to total cholesterol. Current alcohol consumption and body mass index were significantly related to HDL cholesterol concentration for men. The same factors, plus type of spreading fat, were related to HDL cholesterol levels in women. The findings provide further evidence of the hypolipidaemic effect of vegetarian or near vegetarian diets with a high fibre content and limited use of meat and cheese. The exclusion of meat from the diet might result in a 15–25% reduction in CHD risk.

Key words: lipids, cholesterol, diet, lifestyle, vegetarian

Introduction

The importance of reducing mean serum cholesterol concentrations of the whole population for the most effective reduction of rates of coronary heart disease has been elegantly demonstrated (Rose, 1992). An important question, therefore, is what are the best ways of altering dietary habits in order to achieve such a reduction? Analyses of the dietary intake of subjects in a range of studies have shown that specific foods such as oats (Davidson *et al.*, 1991), coffee (Fried *et al.*, 1992), fish and fish oils (Simonsen *et al.*, 1987; Sanders, 1987), soya beans (Carroll, 1991), eggs (Liebman & Bazzarre, 1983), meat (Slattery *et al.*, 1991; Kestin *et al.*, 1989), alcohol (Miller *et al.*, 1988) or a combination of oat and bean products (Anderson & Gustafson, 1988) are related to serum cholesterol levels.

Cohort studies which include a substantial number of vegetarians among their subjects enable researchers to examine a wide range of dietary intakes, particularly with respect to the intake of animal products, fruit and vegetables and dietary fibre. For this reason, they provide a unique opportunity to examine the contribution of these foods to population serum cholesterol levels.

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The Oxford Vegetarian Study is a nationwide prospective cohort study of 6000 subjects who do not eat meat and 5000 control subjects who do. Blood samples were collected from 3800 participants under the age of 70 years in the mid-1980s. We reported analysis of the blood lipids data from 3277 samples by diet group (meat eaters, fish eaters, vegetarians, vegans) in 1987 (Thorogood *et al.*, 1987). Total and LDL cholesterol concentrations were higher in meat eaters than in vegans, with vegetarians and fish eaters having intermediate and similar values. HDL cholesterol concentration was highest in the fish eaters but did not differ among the other groups. However, our previous paper did not examine the effect of lifestyle and dietary factors other than diet group. We report here data from all the 3773 blood samples eventually available for analysis, exploring the relationship between lifestyle and dietary factors and blood lipid levels in subjects with an unusually wide range of dietary practices.

Subjects and methods

The methodology of the Oxford Vegetarian Study and a description of the subjects has been published previously (Thorogood *et al.*, 1987). Briefly, recruitment to the study took place from September 1980 to January 1984. Between April 1984 and January 1986 all participants under 70 years of age were sent a kit consisting of a 10-ml heparinized tube, a syringe and an explanatory letter for their general practitioner, who was asked to take a blood sample and send it to the laboratory for analysis. For reasons of practicality it was not possible to ensure that the samples taken were fasting samples.

Blood samples were obtained from 3800 of the roughly 9700 subjects who were sent a blood kit (exact figures are not available as there is no computerized record of who was sent a blood kit), representing a response rate of about 40%. Nineteen samples were excluded from analysis because the person concerned was a registered cancer patient, pregnant or breast feeding, too old, or because the sample was haemolysed. As a result, blood lipid measurements are available for 3773 subjects. Questionnaire data on sex, date of birth and lifestyle were collected at study entry. A simple food frequency questionnaire with a limited range of individual foods was used to divide participants into five diet groups. The foods in the food frequency questionnaire were chosen as those that, from knowledge available in 1980, might be related to ischaemic heart disease or cancer. This food list forms the basis of the analyses examining the relationship between dietary factors and blood lipids. The questionnaire was not comprehensive and it was therefore not possible to quantify nutrient intake. However, the main sources of dietary fibre intake were included, so an estimate of the intake of dietary fibre was possible using the method of Gear and colleagues (Gear *et al.*, 1979). Age was taken as at 28 February 1985, this being the midpoint of the period of blood sample collection.

Total cholesterol concentrations and concentrations of cholesterol in the various lipoprotein subfractions were measured using a Technicon autoanalyser. Total cholesterol concentration was measured directly by the cholesterol oxidase peroxidase antiperoxidase enzymatic method. Two further aliquots of plasma were taken in order to measure high density lipoprotein and high plus low density lipoprotein concentrations, respectively (Thorogood *et al.*, 1987), and quantities of cholesterol in the various subfractions were calculated by subtraction. Triglyceride concentrations were not measured because blood samples were not collected under fasting conditions. Owing to difficulties with the assay, HDL cholesterol concentration was unavailable for a few subjects. In addition, values of total cholesterol lower than 3.0 mmol/l and values of HDL cholesterol less than 0.6 mmol/l or greater than 2.9 mmol/l were excluded from the analysis as outliers. Although such values may be genuine, they are more likely to be the result of laboratory error such as misreporting the true value. Four observations of total cholesterol, and 19 of HDL cholesterol were excluded for these reasons.

Age was divided into eight groups for analysis: 29 and under, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59 and 60 years and over. Subjects were allocated to one of five diet groups defined as follows: meat eater, fish eater, semi-vegetarian, vegetarian and vegan. Diet groups were defined using the reported weekly consumption of meat, fish, eggs and dairy produce from the questionnaire. Meat eaters reported that

Table 1. List of factors used in the modelling process and their levels

Cigarette smoking habits (never smoked; former smoker; current smoker)
Current weekly alcohol consumption (none; 1–7 units, 8 + units)
Social class (I & II; III–V; other)
Body mass index (quintiles)
Height (quintiles)
Amount of exercise (low; moderate; heavy)
Daily total dietary fibre intake (< 18; 18–24; 25–31; 32 + g)
Daily cereal fibre intake (< 6; 6–10; 11–15; 16 + g)
Daily vegetable fibre intake (< 7; 7.0–9.4; 9.5–11.9; 12.0 + g)
Daily fruit fibre intake (< 2.5; 2.5–3.9; 4.0–5.4; 5.5 + g)
Number of times cheese consumed per week (< 1; 1–4; 5–9; 10 +)
Number of times meat consumed per week (0; < 1–4; 5 +)
Number of times fish consumed per week (0; < 1; 1 +)
Number of times coffee consumed per week (< 1; 1–9; 10 +)
Number of times tea consumed per week (< 1; 1–9; 10 +)
Weekly egg consumption (< 1; 1–5; 6 +)
Daily milk consumption (< 0.5; 0.5; 1.0 + pints)
Number of times green vegetables consumed per week (< 5; 5 +)
Number of times carrots consumed per week (< 1; 1–4; 5 +)
Number of times garlic consumed per week (< 1; 1 +)
Number of times tomatoes consumed per week (< 5; 5 +)
Number of times fresh/dried fruit consumed per week (< 5; 5–9; 10 +)
Number of times nuts consumed per week (< 1; 1–4; 5 +)
Number of times biscuits/cakes consumed per week (< 1; 1–4; 5 +)
Type of fats used on bread (none or unsaturated; saturated)
Type of fats used in frying/grilling (none or unsaturated; saturated)
Type of fats used in baking (none or unsaturated; saturated)
Type of fats used in salad dressings (none; unsaturated or saturated)

A consumption level of ' < 1' includes subjects who never consume the food in question or who consume it less than once a week on average.

they ate meat at least once a week, fish eaters reported eating fish (but not meat) at least once a week, semi-vegetarians reported eating meat or fish less than once a week, vegetarians never ate meat or fish but ate eggs and/or dairy produce, and vegans never ate meat, fish, eggs or dairy produce.

Statistical methods

A logarithmic transformation was used to make the lipid concentrations more nearly Gaussian and all the analyses were performed using the transformed variables.

Total and HDL cholesterol concentrations were fitted as a linear function of age and diet group for each sex using the GLIM statistical package (Payne, 1985) to determine whether diet group affects blood lipid levels after allowing for the effects of age and sex. Diet group was then removed from the model and the effects of lifestyle, physical and dietary factors on the blood lipid measurements was examined by adding each factor to the basic model in turn. In this way a set of linear models 'explaining' some of the variation in blood lipid levels was obtained.

The lifestyle, physical and dietary factors used in the modelling process are shown in Table 1. These factors were chosen from an initial screening of the data to determine which dietary and lifestyle factors were associated with total and/or HDL cholesterol concentration.

To explore the relationship between reported lifestyle and dietary factors and blood lipid levels, the effects of adding lifestyle and dietary factors to a model including age group alone were investigated by a stepwise procedure in which the factor with the lowest P value (as determined from the F-to-enter statistic) was added to the model. This model was then the

Table 2. Mean age in years and numbers by sex and diet group

	Men		Women	
Diet	Mean age	No.	Mean age	No.
Meat eater	42.3	553	41.6	791
Fish eater	44.7	43	43.8	93
Semi-vegetarian	41.1	100	41.9	274
Vegetarian	42.6	569	41.3	1216
Vegan	41.3	53	41.4	81
Total		1318		2455

Table 3. Age-adjusted ratios of cholesterol concentration compared with meat eaters by sex and diet group and their standard errors

	Total cholesterol		HDL cholesterol	
Diet	Men	Women	Men	Women
Meat eater	1.00 (—)	1.00 (—)	1.00 (—)	1.00 (—)
Fish eater	0.93 (0.027)	0.96 (0.019)	1.08 (0.039)	1.05 (0.026)
Semi-vegetarian	0.92 (0.019)	0.95 (0.012)	1.04 (0.026)	1.00 (0.015)
Vegetarian	0.92 (0.010)	0.92 (0.008)	1.01 (0.014)	0.99 (0.010)
Vegan	0.84 (0.023)	0.82 (0.017)	1.04 (0.035)	0.98 (0.025)

standard, and the effect of adding other factors to it was investigated by a method analogous to stepwise forward linear regression. The procedure was repeated until none of the F-to-enter values were significant at the 1% level. The first order interaction terms for the factors included in the model, including age interactions, were then added to the model if their F-to-enter value reached the 1% significance level.

Dietary and lifestyle factors are closely interrelated. To explore the relative importance of lifestyle and diet, the potential explanatory factors were divided into lifestyle/physical factors (cigarette smoking habits, current alcohol consumption, amount of exercise, social class, body mass index and height) and dietary factors (all the rest). The model fitting procedure was then repeated, once with the dietary factors entered first followed by the lifestyle/physical factors, and then with the lifestyle/physical factors entered first followed by the dietary factors. The same rule for entering a factor was used as described above.

Observations for which one or more factors were missing were excluded from the model fitting process. However, once the model fitting was completed, the final model was re-fitted excluding only those observations for which one or more of the factors in the model were missing.

Results

Table 2 shows the numbers of men and women in each diet group with their mean ages. The diet groups are well matched for age except that the small group of fish eaters were, on average, about 2 years older.

The mean (SE) total cholesterol concentration in male and female meat eaters was 5.94 (0.053) mmol/l and 5.93 (0.046) mmol/l, respectively, while the equivalent mean (SE) high density lipoprotein concentration was 1.46 (0.015) mmol/l and 1.71 (0.013) mmol/l, respectively. Table 3 shows the age-adjusted ratios of cholesterol concentration for each diet group compared with meat eaters. All other diet groups had lower mean total cholesterol con-

Table 4. Linear models of cholesterol concentration

Cholesterol	Gender	Model terms
Total	Men	1 + AGEG + MEAT + DAYF + CHEE + SMOK + HTGP (950 obs; $R^2 = 0.21$)
Total	Women	1 + AGEG + MEAT + CHEE + FATS + DAYF + TOMS (1827 obs; $R^2 = 0.28$)
HDL	Men	1 + AGEG + DRNK + BMIG (1299 obs; $R^2 = 0.06$)
HDL	Women	1 + AGEG + FATS + BMIG + DRNK (2362 obs; $R^2 = 0.03$)

1: constant term.
AGEG: age group.
BMIG: body mass index quintile.
CHEE: times cheese eaten per week (< 1, 1–4, 5–9, 10 or more).
DAYF: dietary fibre per day (0–17, 18–24, 25–31, 32 or more g).
DRNK: weekly alcohol intake (none, 1–7 units, 8 or more units).
FATS: type of spreading fats used (none or unsaturated, saturated).
HTGP: height quintile.
MEAT: times meat eaten per week (never, up to 4, 5 or more times).
SMOK: smoking habits (never smoked, former smoker, current smoker).
TOMS: times tomatoes eaten per week (up to 4, 5 or more times).
R^2: fraction of the total variance 'explained' by the fitted model.

centrations than meat eaters. High density lipoprotein cholesterol concentration did not vary with diet group, except that the fish eaters had slightly higher mean values. Fitting cholesterol concentration as a function of age and diet group shows that total cholesterol concentration for both men and women differed significantly by diet group ($P < 0.0001$ in each case). There was no such difference in HDL cholesterol concentration.

Table 4 shows the linear models obtained as a result of the stepwise modelling procedure. There were no significant interactions involving the factors. The factors were entered in the order shown so that, for example, meat was the first factor after age group to be entered into the model for total cholesterol concentration for men. The numbers of observations used to fit each model and the R^2 goodness-of-fit statistic are shown in parentheses.

The directions of the effects in men were as follows:

1. *Total cholesterol concentration* increased with age, meat and cheese consumption, decreased with fibre intake, and was higher for those in the lowest quintile of height and for current cigarette smokers.
2. *High density lipoprotein concentration* increased with alcohol consumption, was inversely related to body mass index, and was unaffected by age.

In women the relationships were as follows:

1. *Total cholesterol concentration* increased with age, meat and cheese consumption, decreased with fibre intake, and was higher among women spreading saturated fats on bread, and among women eating tomatoes five or more times per week.
2. *High density lipoprotein concentration* was inversely related to body mass index, increased with alcohol consumption, was higher among women spreading saturated fats on bread, and was unaffected by age.

The R^2 goodness-of-fit values show that the fitted models explain 20–30% of the variation in total cholesterol concentration but only a few per cent of the variation in HDL cholesterol concentration. Of the 136 fish eaters in the study 125 reported eating fish between one and four times per week, so there was a limited range of consumption. This may explain why frequency of fish consumption was not related to HDL cholesterol concentration, despite a slightly higher level in fish eaters compared to the other diet groups.

Table 5 and Fig. 1 show the percentage change in cholesterol concentration associated with the factors included in the models (Table 4), thus showing the magnitude of the effect of each factor.

The sensitivity of the results to the order in

Table 5. Percentage change in total and HDL cholesterol associated with deviations from the reference level for the factors in the fitted models

Model term	Men		Women	
	Total (%)	HDL (%)	Total (%)	HDL (%)
Age group (years)				
under 30	Ref	Ref	Ref	Ref
30–34	+ 0.4	− 0.6	+ 2.3	+ 2.1
35–39	+ 3.9	− 1.0	+ 3.1	+ 1.3
40–44	+ 11.6	+ 0.2	+ 6.9	+ 2.4
45–49	+ 12.0	+ 4.1	+ 12.4	+ 3.8
50–54	+ 17.1	+ 2.0	+ 24.3	+ 5.7
55–59	+ 15.2	− 4.3	+ 29.6	+ 2.2
60 +	+ 16.1	− 0.1	+ 31.4	+ 6.1
Smoking history				
Never smoked	Ref	—	—	—
Former smoker	+ 1.3	—	—	—
Current smoker	+ 5.3	—	—	—
BMI quintile				
One (low)	—	Ref	—	Ref
Two	—	− 4.1	—	− 1.1
Three	—	− 4.0	—	− 2.8
Four	—	− 7.0	—	− 2.4
Five (high)	—	− 11.7	—	− 7.2
Height quintile				
One (short)	Ref	—	—	—
Two	− 6.4	—	—	—
Three	− 4.9	—	—	—
Four	− 7.0	—	—	—
Five (tall)	− 6.0	—	—	—
Meat eating (times/week)				
Never	Ref	—	Ref	—
≤ 4	+ 3.6	—	+ 5.3	—
5 +	+ 8.6	—	+ 8.8	—
Daily fibre intake (g)				
≤ 17	Ref	—	Ref	—
18–24	− 0.7	—	− 0.5	—
25–31	− 1.9	—	− 3.4	—
32 +	− 7.4	—	− 4.5	—
Spreading fats				
None or unsaturated	—	—	Ref	Ref
Saturated	—	—	+ 3.1	+ 3.3
Cheese eating (times/week)				
< 1	Ref	—	Ref	—
1–4	+ 6.7	—	+ 5.5	—
5–9	+ 6.4	—	+ 6.6	—
10 +	+ 10.4	—	+ 8.2	—
Tomato eating (times/week)				
≤ 4	—	—	Ref	—
5 +	—	—	+ 2.4	—
Alcohol (units/week)				
None	—	Ref	—	Ref
1–7	—	+ 5.1	—	+ 2.9
8 +	—	+ 12.3	—	+ 6.9

which dietary and lifestyle factors were entered was tested by running the model twice, entering either the lifestyle or the dietary factors first. Entering the dietary factors first did not make any difference, but when the lifestyle factors were entered first a new factor, body mass index, was added to the models for total cholesterol concentration in men and women. Factors that are in the model with either entry order probably exert a real effect on cholesterol concentration. Thus, each of the factors in the models set out in Table 4 are likely to have a real effect on cholesterol concentration. In contrast, the apparent effect of body mass index on total cholesterol concentration in men and women is likely to be due to confounding with the other factors in the model.

Discussion

We have modelled differences in blood cholesterol concentration for various dietary, lifestyle and physical factors. Total cholesterol concentration was positively associated with meat and cheese consumption and negatively associated with dietary fibre intake for both men and women, independent of age. Type of spreading fat was related to both total and HDL cholesterol concentration in women but not in men. We have previously reported a negative association between height and total cholesterol concentration (Thorogood *et al.*, 1989). This relationship was apparent only in men. Cigarette smoking was positively related to total cholesterol levels in men. An unexpected finding was that frequent tomato consumption was associated with raised total cholesterol in women.

HDL cholesterol concentration was positively and strongly associated with alcohol consumption for both men and women as we (Thorogood *et al.*, 1990) and others (Miller *et al.*, 1988; Fraser & Babaali, 1989) have reported previously. HDL cholesterol levels were also inversely associated with body mass index for both genders. There was no relationship between age and HDL cholesterol levels.

Many studies of the relationship between diet and plasma lipid concentration have measured diet by overall nutrient intake (Bolton-Smith *et al.*, 1991; Kushi *et al.*, 1988; Trevisan *et al.*, 1990; Thorogood *et al.*, 1990). Other studies examine the effect of specific foods such as oats (Davidson *et al.*, 1991), coffee (Fried *et al.*, 1992), fish and fish oils (Simonsen *et al.*, 1987; Sanders, 1987), soya beans (Carroll, 1991), eggs (Liebman & Bazzarre, 1983), meat (Slattery *et al.*, 1991; Kestin *et al.*, 1989), alcohol (Miller *et al.*, 1988) or a combination of oat and bean products (Anderson & Gustafson, 1988). Our study explores the relationship between consumption of a range of foods and cholesterol concentration in a manner similar to the analysis of data from the Tromso Heart Study (Jacobsen & Thelle, 1987), a cohort study of over 14 000 free-living inhabitants of the Norwegian town of Tromso. That study found positive associations between total cholesterol levels and high coffee consumption, use of butter or hard margarine, failure to use low fat milk, and a low consumption of bread. HDL cholesterol concentration was virtually independent of dietary factors, in accordance with our findings, while high body mass index was associated with high total cholesterol and low HDL cholesterol. The present study differs from the Tromso Heart Study in that subjects followed a variety of diets, from omnivorous to vegan. The wide range of intake of animal products means that an association between these products and cholesterol concentration is easier to detect.

Data from the Scottish Heart Health Study (Bolton-Smith *et al.*, 1991) showed a positive relationship between saturated fat intake and total cholesterol concentration among men. Meat and cheese contribute around 30% of the fat intake in an average diet (Gregory *et al.*, 1990), up to half of this being saturated fat, an important determinant of serum cholesterol levels. Data from the CARDIA study (Slattery *et al.*, 1991), a longitudinal study of 5000 young American adults, have also shown a positive association between meat consumption and total cholesterol level, although no such association was found in data from the Tromso Heart Study (Jacobsen & Thelle, 1987) which, however, reported a positive association between butter and hard margarine and whole milk consumption and total cholesterol, suggesting that dairy produce raises blood lipid levels. The negative association between dietary fibre intake and total cholesterol concentration may be a reflection of the cholesterol-lowering properties of some plant foods (Davidson *et al.*, 1991; Carroll, 1991; Anderson & Gustafson, 1988; Singh *et al.*, 1992).

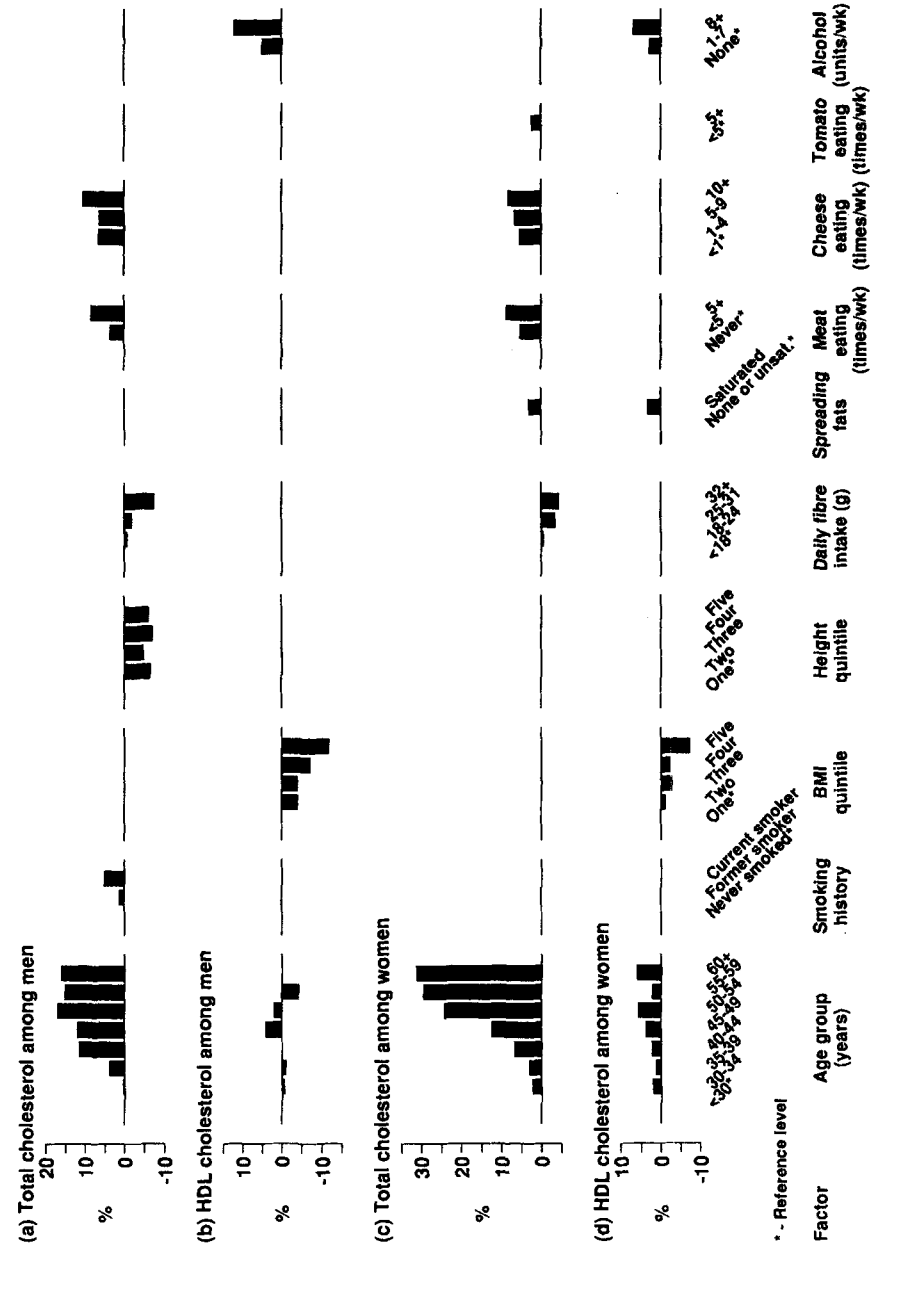

Fig. 1. Percentage change in total and *HDL* cholesterol associated with deviations from the reference level for the factors in the fitted models. Where bars do not appear that factor does not feature in the fitted model.

It has been argued that a 1% reduction in total cholesterol level over a long period translates into a 2–3% reduction in coronary heart disease risk (Gotto *et al.*, 1990). In this case, our results suggest that people who eat meat at least five times per week might expect a 15–25% higher risk of CHD than non-meat eaters. Men who eat cheese at least 10 times weekly might expect a 20–30% increase in CHD risk compared to men who eat little or no cheese, and by a similar comparison, women would expect a 15–25% increase in risk. By contrast, a man eating a high fibre diet (at least 32 g fibre per day) might enjoy a 15–20% lower risk of CHD and a similar woman might enjoy a 10–15% lower risk compared to those eating a more refined diet. Findings from mortality studies of Seventh Day Adventists who eat a range of diets provide some support for these estimates. These studies have shown a positive association between meat intake and coronary heart disease mortality for both men and women (Snowdon, 1988) or for men alone (Fraser *et al.*, 1992), negative associations between both nuts and wholewheat bread consumption and CHD mortality (Fraser *et al.*, 1992), but no association between cheese consumption and any of the mortality endpoints studied.

Assuming a 2–3% reduction in CHD risk arising from a 1% *increase* in HDL cholesterol concentration (Gotto *et al.*, 1990), men consuming eight or more units of alcohol per week might enjoy a 25–35% lower risk of CHD than those consuming no alcohol at all. Similarly, women who drink regularly might expect a 15–20% decrease in CHD risk compared with teetotal females. These extrapolations are consistent with a prospective study of male health professionals, which showed a CHD risk reduction of 27% or more among those consuming more than 15 g alcohol (equivalent to one drink) per day compared with non-drinkers (Rimm *et al.*, 1991), and a smaller case–control study which showed that all except the heaviest drinkers had at least a 40% reduction in the risk of both fatal and non-fatal CHD compared with those who had never drunk more than once a month (Jackson *et al.*, 1991). However, it should be noted that a significant reduction in CHD mortality does not necessarily translate into a significant reduction in total mortality owing to the adverse effects of alcohol over a range of diseases. Thus, in a study of more than 276 000 middle-aged American men, only those consuming two or less drinks per day had a significantly lower total mortality compared with non-drinkers, while those consuming four or more drinks daily had a significantly higher relative risk (Boffetta & Garfinkle, 1990).

This analysis is based on single cholesterol measurements. Therefore, it is not possible to estimate within-individual variation in cholesterol concentration. If repeat measurements were available cholesterol concentration could be determined more precisely and our models might be expected to explain a greater proportion of the total variance. However, despite the limitations of the methodology and, in particular, the crude food frequency questionnaire which was used, our findings make biological sense. Future data on mortality within the cohort study will enable us to test our estimates further.

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