EFFECT OF MONOUNSATURATED FATTY ACIDS VERSUS COMPLEX CARBOHYDRATES ON HIGH-DENSITY LIPOPROTEINS IN HEALTHY MEN AND WOMEN

RONALD P. MENSINK MARTIJN B. KATAN

Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands

Summary The effects of two strictly controlled diets, one rich in complex carbohydrates, the other rich in olive oil, on serum lipids were studied in healthy men and women. Serum cholesterol levels fell on average by 0·44 mmol/l in the carbohydrate group and 0·46 mmol/l in the olive oil group. HDL cholesterol levels fell by 0·19 mmol/l in the carbohydrate group and rose by 0·03 mmol/l in the olive oil group. Serum triglycerides rose by 0·19 mmol/l in the carbohydrate group and fell by 0·06 mmol/l in the olive oil group. The changes in both HDL and triglycerides were larger in men than in women. These results clearly show that the olive-oil-rich diet, unlike the complex-carbohydrate-rich diet, caused a specific fall in non-HDL cholesterol while leaving serum triglyceride levels virtually unchanged.

Introduction

HIGH-density lipoproteins (HDL) may protect against coronary heart disease (CHD),[1,2] and diets chosen for lowering total serum cholesterol should, therefore, not lower HDL cholesterol. Unfortunately, which type of diet will selectively lower atherogenic lipoproteins is not clear. In this respect, the Cretan diet merits attention. The Seven Countries Study[3] showed that the cohort from Crete was something of an anomaly because the incidence of CHD in these middle-aged men was lower than would be expected from their total serum cholesterol levels. This could not be explained by other risk factors for CHD, such as cigarette smoking or high blood pressure. HDL cholesterol levels, however, were not studied. The Cretan men were unique among the study populations in that they combined a low intake of saturated fatty acids with a high intake of total fat because of their liberal use of olive oil.[3] We have earlier suggested that high-fat diets result in high HDL cholesterol levels, irrespective of the fatty-acid composition of the diets.[4] If this is true, then replacement of saturated by monounsaturated fatty acids should lower total serum cholesterol levels, while leaving HDL cholesterol levels unchanged.

We have tested this hypothesis in a strictly controlled dietary experiment with healthy normolipidaemic men and women by comparing the effects on serum lipids of three mixed natural diets—one rich in saturated fat, one rich in olive oil, and one rich in complex carbohydrates and fibre.

Subjects and Methods

Subjects

57 volunteers, mainly students, were selected for the study. They had no history of atherosclerotic disease and all were apparently healthy, as indicated by a medical questionnaire. None had anaemia, glycosuria, proteinuria, or hypertension. Their total serum cholesterol ranged from 3·43 to 7·58 mmol/l (mean 5·07 mmol/l), HDL cholesterol from 0·72 to 2·58 mmol/l (mean 1·38 mmol/l), and serum triglycerides from 0·33 to 2·77 mmol/l (mean 0·98 mmol/l). None received medication known to affect serum lipids for at least 2 months before or during the study. Before the study, all volunteers were given 50 ml of olive oil and two slices of olive-oil-rich bread. When interviewed 4 days later none reported any adverse gastrointestinal or other effect.

The protocol, approved by the ethical committee of the department, was fully explained to the volunteers, who then gave written consent for the study. No monetary inducement was offered except for the free food. Subjects were asked to maintain their usual pattern of activity and not to change their smoking habits or use of oral contraceptives.

During the second week 1 man contracted influenza and withdrew from the study. Data from 8 other subjects were eliminated before the analysis of the results, because of a bout of influenza during the study (1 man in each diet group), changes in smoking habits (1 woman in the carbohydrate group and 1 man in the olive oil group), or weight loss of more than 2·5 kg (1 man and 2 women in the carbohydrate group and 1 man in the olive oil group). Thus, 48 subjects completed the experiment. They were aged between 18 and 59 years (mean 27 years), weighed 53 to 88 kg (mean 71 kg), their height ranged from 160 to 202 cm (mean 177 cm) and their body mass index from 18·4 to 28·4 kg/m^2 (mean 22·6 kg m^2).

Diets and Design

Each diet consisted of conventional mixed solid foods, and menus were changed daily. All subjects consumed a Western-type diet for 17 days. Then two groups were formed, matched for total and HDL cholesterol, triglycerides (as measured on day 1), and sex. Each group consisted of 12 men and 12 women. 3 of the women used oral contraceptives. For the next 36 days one group received a high-carbohydrate, high-fibre diet and the other an olive-oil-rich diet. These diets were formulated to cause the same fall in total serum cholesterol according to the formula of Keys et al.[5] The composition of the diets is given in table I. To maintain the use of normal foodstuffs, the olive oil group received special bread containing 8 g of olive oil/100 g and a special margarine (van den Bergh & Jurgens BV, Rotterdam) made of "new" rapeseed oil, high in oleic and low in erucic acid. Olive oil contributed 75% of monounsaturated fatty acids and the margarine 10%. The high-carbohydrate diet was enriched with bread, pulses, vegetables, potatoes, fruits, and jam.

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TABLE I—MEAN COMPOSITION OF THE DIETS ACCORDING TO DUPLICATE PORTION ANALYSIS PLUS CALCULATED CONTRIBUTION OF FREE-CHOICE ITEMS

Daily intake	Control period	Test period	
	Western diet (n=48)	Carbohydrate-rich diet (n=24)	Olive-oil-rich diet (n=24)
*Energy intake (MJ)**	11·1	11·8	11·1
Protein (% of energy)	13·6	14·1	12·2
Fat (% of energy):			
Total	38·0	22·1	40·6
C12-C16 saturated fatty acids†	13·9	4·1	6·8
Total saturated fatty acids	20·0	6·7	9·8
Monounsaturated fatty acids	12·4	9·3	24·0
Polyunsaturated fatty acids	4·1	5·2	5·1
Carbohydrates (% of energy):			
Total	47·7	62·2	46·0
Monosaccharides and disaccharides‡	25·9	32·4	22·4
Polysaccharides	21·8	29·8	23·6
Alcohol (% of energy)	1·3	1·6	1·2
Cholesterol (mg/day)§	390	390	345
Dietary fibre (g/day)§	42	60	43

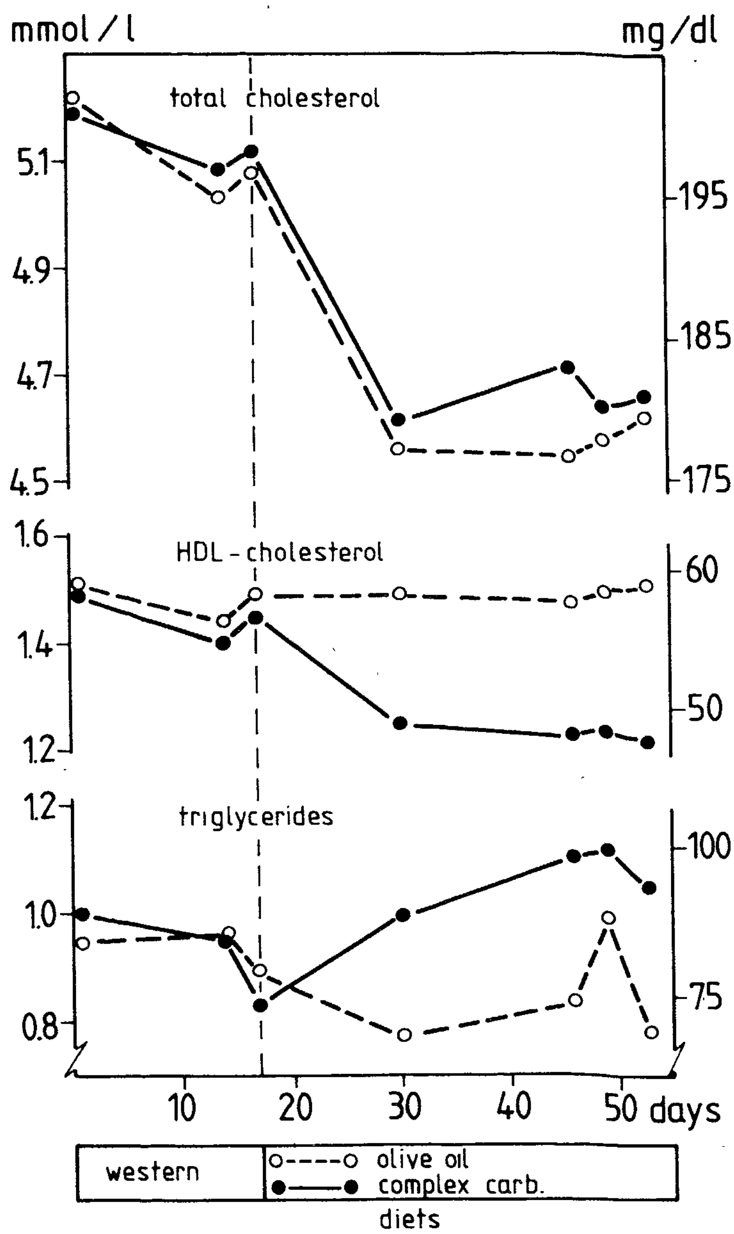
*Individual intakes ranged from 6·1 to 19 9 MJ (1 MJ = 239 kcal) on the Western diet, from 6·3 to 20·5 MJ on the carbohydrate-diet, and from 7·1 to 15·5 MJ on the olive oil diet.
†Saturated fatty acids with chain lengths of 12–16 carbon atoms.[5]
‡Including the natural sugars in fruit, milk, &c.
§For a subject of average energy intake.

In addition to the food supplied, subjects were allowed a limited choice of items (such as alcoholic drinks, fruit, and sweets), free from fat and cholesterol and providing a fixed amount of energy, ranging from 4·6 to 10% of their mean daily energy intake. We have found that this helps subjects to pursue a normal lifestyle and improves their cooperation.

All foodstuffs were weighed. On weekdays at noon, hot meals were served at the department. All other food was provided daily as a package. Food for the weekend and guidelines for its preparation were provided on Fridays. Subjects were asked to record in diaries any sign of illness, medication used, the self-selected food items, and deviations from the diet. Duplicate portions of each diet were collected daily, pooled per diet period, and analysed. These analysed values were combined with the values calculated for the free-choice items (table I). Body-weights without shoes, jackets, and heavy sweaters were recorded twice weekly, and energy intake was adjusted to avoid weight changes. Average body-weight fell by 0·5 kg (range −2·3 to +1·6 kg) in the carbohydrate group and 1·0 kg (range −2·5 to +1·3 kg) in the olive oil group during the study. However, part of this weight-loss could be accounted for by a change to lighter clothes as winter passed into spring. The mean difference in weight-loss between subjects in the two diet groups amounted to 9 g per day. No significant correlations were found between changes in weight and changes in serum lipids, calculated either for all subjects together or for each diet group and sex separately.

Blood Sampling and Analysis

Fasting blood samples were obtained on days 1, 14, 17 (control period) and on days 30, 46, 49, and 53 (test period). Serum was obtained by low-speed centrifugation within 1 h of venepuncture. Sera were checked for the presence of chylomicrons[6] (all samples were negative), stored at −80°C, and analysed for total and HDL cholesterol and triglyceride levels 1 day later (samples of day 1) or at the end of the study (all others). All samples from one subject were analysed within one run. The within-run coefficient of variation for control sera was 0·9% for total cholesterol, 1·8% for HDL cholesterol, and 1·0% for total triglycerides. Accuracy was checked by analysis of serum pools of known value provided by the Centers for Disease Control (Atlanta, GA). Mean bias with regard to CDC



Mean serum total and HDL cholesterol and serum triglyceride concentrations throughout the experiment.

All 48 subjects first received a Western-type diet high in saturated fat for 17 days. For the next 36 days half of the subjects received an olive-oil-rich diet (○) and the other half a diet low in fat and high in complex carbohydrates and fibre (●).

target values was +0·1% for total cholesterol, −3·2% for HDL cholesterol, and −1·5% for total triglycerides.

Statistical Analysis

To reduce skewness of the distribution of serum lipids and to make the square root of the variance proportional to the individual level rather than being constant, all values were transformed to their natural logarithm. The responses to the carbohydrate-rich or the olive-oil-rich diets were calculated for each subject as the change from the end of the Western diet (mean of days 14 and 17) to the end of the test period (mean of days 46, 49, and 53). The effects of diet, sex, and diet × sex interaction on the response were examined by analysis of variance. Pearson correlation coefficients were computed between the response and the intrinsic lipid level (average of the mean of days 14 and 17 and the mean of days 46, 49, and 53) for both sexes for each diet group.

Results

The changes in serum lipid values during the study are shown in the figure. Total serum cholesterol fell on both

TABLE II—EFFECTS OF A HIGH-CARBOHYDRATE, HIGH-FIBRE DIET AND AN OLIVE-OIL-RICH DIET ON SERUM LIPID CONCENTRATIONS (MEAN ± SD)

	Carbohydrate group		Olive oil group	
	Men	Women	Men	Women
—	(n = 12)	(n = 12)	(n = 12)	(n = 12)
Cholesterol:				
Control period*	5·08 ± 1·15	5·12 ± 0·74	4·74 ± 0·72	5·37 ± 0·60
Test period†	4·65 ± 0·99	4·67 ± 0·61	4·35 ± 0·75	4·83 ± 0·54
Change	−0·43 ± 0·45	−0·45 ± 0·42	−0·39 ± 0·51	−0·54 ± 0·35
HDL cholesterol:				
Control period*	1 30 ± 0·36	1·54 ± 0·31	1·29 ± 0 29	1·65 ± 0·41
Test period†	1·08 ± 0·30	1·37 ± 0 23	1·39 ± 0·27	1·61 ± 0·39
Change‡¶	−0·22 ± 0·10	−0·17 ± 0 12	0·10 ± 0 15	−0·04 ± 0·12
Triglycerides:				
Control period*	0·96 ± 0·56	0·82 ± 0·24	0·99 ± 0·79	0·87 ± 0·32
Test period†	1·27 ± 0·82	0·91 ± 0·73	0·91 ± 0·73	0·82 ± 0·29
Change§‖	0·31 ± 0·35	0·08 ± 0 23	−0·08 ± 0·14	−0·04 ± 0 15

*Two samples per subject were averaged.
†Three samples per subject were averaged.
Statistical comparison between diets, with sexes pooled: ‡$p < 0{\cdot}001$; §$p < 0{\cdot}005$; diet × sex interaction: ¶$p < 0{\cdot}005$; ‖$p < 0{\cdot}1$.

diets and to the same extent. HDL fell by 0·19 mmol/l on the high-carbohydrate, high-fibre diet, and rose by 0·03 mmol/l on the olive-oil-rich diet ($p < 0{\cdot}001$). In the complex-carbohydrate group the mean reduction in HDL cholesterol equalled 51% of the total serum cholesterol decrease in men and 38% in women. Triglycerides rose by 0·19 mmol/l on the carbohydrate-rich diet and fell by 0·06 mmol/l on the olive oil diet ($p < 0{\cdot}005$). The composition of HDL_2 and HDL_3 was not affected by either diet (data not shown). The differences in changes between the diets were more pronounced in men than in women for both HDL cholesterol ($p < 0{\cdot}005$) and triglycerides ($p < 0{\cdot}1$) (table II). The response to diet was unrelated to the intrinsic levels of total or HDL cholesterol or triglycerides.

Discussion

This controlled study in 48 healthy volunteers showed that diets high in either complex carbohydrates plus fibre or monounsaturated fatty acids caused the same fall in serum total cholesterol, compared with a diet high in saturated fatty acids. All diets were tolerated well, and no adverse gastrointestinal or other effects of olive oil were reported. Compliance with the diets was probably very good. Our volunteers were unpaid and highly motivated. All hot meals were eaten in our presence. We tried to elicit comments, and we went to great lengths to accommodate potential difficulties. In previous studies with volunteers from the same population, adherence to the diets was documented by changes in serum cholesterylester fatty acid composition[7] or colonic function.[8] In the present study compliance with the diets is confirmed by the observed reductions of 0·44 mmol/l for the carbohydrate group and 0·46 mmol/l for the olive oil group, which are in excellent agreement with Keys' equation (as formulated by Anderson et al[9]), which predicts changes of 0·50 mmol/l and 0·41 mmol/l, respectively. However, this formula does not distinguish between the effects of diet on different lipoproteins.

HDL cholesterol levels did not change if saturated fatty acids were replaced by monounsaturated fatty acids. In contrast, on the high-carbohydrate, high-fibre diet HDL cholesterol levels fell by 0·19 mmol/l on average. This observation agrees with the results of Grundy,[10] who compared the effects of high-oleic-acid safflower oil and dextrose on lipoproteins in obese subjects aged 49–69. The liquid formula diets used by Grundy[10] were very low in cholesterol and did not provide any starch. We have now shown that this finding may be extended to natural solid mixed diets. Specifically, the deleterious effect of the dextrose formula on HDL cholesterol seen by Grundy[10] can now be extended to a natural low-fat diet high in unrefined carbohydrates of the type recommended for the prevention of coronary heart disease,[11] and there are reasons to suspect that this rise is not transient.[4] Men showed a greater change in HDL than women. Whether this is real or due to chance remains to be established.

Our results agree with other evidence that total fat intake is a major determinant of HDL cholesterol levels. Knuiman et al[12] found in a study of schoolboys from five countries that higher concentrations of HDL cholesterol (and total serum cholesterol) were associated with diets high in fat and low in complex carbohydrates. Becker et al, in a study of male volunteers,[13] compared the effects on plasma lipids of three cholesterol-free formula diets, containing 40% of energy from fat, in which the predominant fatty acids were either saturated, monounsaturated, or polyunsaturated. They found similar mean HDL cholesterol levels on all diets. There are indications that HDL is lowered by diets containing extreme amounts of polyunsaturated fatty acids.[14,15] However, diets with less extreme changes have no influence on HDL.[16] Further studies of the optimum level of (n-6) polyunsaturated fatty acids in the diet are therefore needed.

The reduction in HDL cholesterol was observed despite the higher fibre content of the carbohydrate-rich diet. It has been suggested that the fibre content of a diet is positively associated with HDL cholesterol levels.[17,18] However, the increased fibre content of our carbohydrate-rich diet failed to prevent the decrease in HDL cholesterol.

As in other studies, substitution of fat by carbohydrates raised serum triglycerides values.[7,19] There is much dispute whether this rise is transient. However, a positive correlation between fasting serum triglyceride levels and the percentage intake from carbohydrates in schoolboys from different countries[20] suggests that at least part of this is real and not transient. A difference in the effect of diet on serum triglyceride levels between men and women[21,22] has been confirmed in the present study. If the difference in effect on HDL found by us is also confirmed, then dietary guidelines derived from studies in men might not be appropriate for women.

The olive oil diet, which combined a high intake of total fat with a low intake of saturated fat, caused in our healthy normolipidaemic subjects a specific fall in non-HDL cholesterol, while leaving HDL cholesterol and triglyceride values unchanged; this contrasts with the effects of the high-carbohydrate, high-fibre diet. In view of the supposed anti-atherogenic effect of HDL, reducing total fat intake per se might not be the best way to prevent CHD.

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Correspondence should be addressed to M. B. K.

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INADEQUACY OF CHLORPROGUANIL 20 mg PER WEEK AS CHEMOPROPHYLAXIS FOR FALCIPARUM MALARIA IN KENYA

W. M. WATKINS A. D. BRANDLING-BENNETT
A. J. OLOO R. E. HOWELLS
H. M. GILLES D. K. KOECH

Biomedical Sciences Research Centre and Clinical Research Centre, Kenya Medical Research Institute, Nairobi, Kenya; Wellcome Trust Research Laboratories, Nairobi, Kenya; Liverpool School of Tropical Medicine, Liverpool, UK; and Centers for Disease Control, US Public Health Service, Department of Health and Human Services, Atlanta, Georgia, USA

Summary After treatment with chloroquine and pyrimethamine/sulfadoxine, 118 school children aged 6 to 10 years living near the Kenyan coast were enrolled in a malaria chemoprophylaxis study and followed up for 20 weeks. Children were randomly assigned to receive either chlorproguanil 20 mg weekly (n = 78) or placebo (n = 37). The attack rate of *Plasmodium falciparum* infection was 42% in chlorproguanil recipients (39·8 episodes per 1000 person-weeks of prophylaxis) and 73% in placebo recipients (69·2 episodes per 1000 person-weeks, $p < 0·02$). Sensitivity tests on 36 isolates successfully cultured in vitro showed that all 21 isolates from chlorproguanil recipients were resistant to dihydrofolate-reductase inhibitors, whereas only 3 of 15 isolates from the placebo group were resistant ($p < 10^{-6}$). Chlorproguanil in a weekly adult dose of 40 mg does not provide adequate prophylaxis against *P falciparum* in Kenya, probably because drug levels between doses fall below those required to suppress parasites resistant to dihydrofolate-reductase inhibitors.

Introduction

THE biguanides proguanil ('Paludrine') and chlorproguanil ('Lapudrine') have been used as chemoprophylactic agents against malaria since soon after the 1939–45 war. Resistance to the dihydrofolate-reductase inhibitors (DHFRIs), including pyrimethamine and the biguanides, is widespread in Africa[1]—for example, in Kenya.[2] There is cross-resistance between proguanil and pyrimethamine,[3] although cycloguanil, the active metabolite of proguanil, is considerably more active than pyrimethamine against resistant *Plasmodium falciparum* in vitro.[4] In certain locations where the parasite is resistant to proguanil 100 mg daily, a dose of 200 mg has provided effective prophylaxis,[1] and there is limited evidence that this higher dose may afford protection in East Africa.[5] Proguanil 200 mg daily and chlorproguanil 20 mg weekly are now recommended by the World Health Organisation for the prophylaxis of falciparum malaria in areas with chloroquine resistance,[6] although their efficacy remains uncertain in the absence of recent controlled clinical trials.[7] Both the increasing prevalence and degree of chloroquine resistance in Kenya (Brandling-Bennett AD, Watkins WM, Oloo AJ, Spencer HC, Koech DK, unpublished) and the occurrence of serious reactions to pyrimethamine/sulfadoxine taken for prophylaxis[7] point to the urgent need for alternative prophylactic drugs. Since proguanil and chlorproguanil are free from serious side-effects,[8] it is important that their efficacy be investigated.

We chose to investigate chlorproguanil because having to administer a dose only weekly offers logistic advantages for field study, and because chlorcycloguanil, the active metabolite of chlorproguanil, is more active than cycloguanil against *P falciparum*.[9,10] The object of this study was to evaluate a weekly dose of chlorproguanil as prophylaxis against falciparum malaria on the Kenya coast, where there is resistance to both 4-aminoquinolines[11,12] and dihydrofolate reductase inhibitors.[1,13]

Methods

In-vivo tests

The study was conducted at Jilore primary school, near Malindi on the Kenya coast, which is an area of hyperendemic to holoendemic malaria according to Ministry of Health data. The children's parents were fully informed as to the nature of the study and consent to participate was given by parents and teachers. To clear malaria parasites before the start of the study children of both sexes between the ages of 7 and 14 years were enrolled into the study and were treated with pyrimethamine/sulfadoxine (one tablet containing pyrimethamine 25 mg and sulfadoxine 500 mg for children weighing less than 25 kg, 1·5 tablets for those 25–37·5 kg, and 2 tablets for those 37·6–50 kg) and chloroquine (25 mg/kg over 3 days). A week after treatment children were randomly assigned to

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R. P MENSINK AND M. B. KATAN: REFERENCES

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