Effect of monounsaturated fatty acids on high-density and low-density lipoprotein cholesterol levels and blood pressure in healthy men and women

Promotoren: dr. J.G.A.J. Hautvast,
hoogleraar in de leer van de voeding en de voedselbereiding.

dr. M.B. Katan,
universitair hoofddocent aan de Vakgroep Humane Voeding en bijzonder hoogleraar in de voedingsleer van de mens aan de Katholieke Universiteit te Nijmegen.

Ronald P. Mensink

Effect of monounsaturated fatty acids on high-density and low-density lipoprotein cholesterol levels and blood pressure in healthy men and women

PROEFSCHRIFT

ter verkrijging van de graad van
doctor in de landbouw- en milieuwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van de Plas,
in het openbaar te verdedigen
op vrijdag 23 maart 1990
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen.

ISN: 268204

De studies die in dit proefschrift zijn beschreven zijn financieel ondersteund door de Nederlandse Hartstichting, het Ministerie van Welzijn, Volksgezondheid, en Cultuur, de Stichting Voeding Nederland, en de Commissie van de Europese Gemeenschappen.

STELLINGEN

1. De aanduiding "zuiver plantaardig" op bepaalde harde margarines suggereert ten onrechte dat deze producten "gezond" zouden zijn.

2. Een verlaging van de huidige vetopneming in Nederland is gewenst, ook al zal dit mogelijk gepaard gaan met een verlaging van het HDL-cholesterolgehalte.

3. Het is bepaald niet zeker dat het risico voor coronaire vaatziekten afneemt, indien het HDL-cholesterolgehalte door behandeling met medicijnen of dieet wordt verhoogd.
(Gordon DJ, Rifkind BM. N Engl J Med 1989; 321:1311-1316)

4. Het is niet waarschijnlijk dat de hoeveelheid vet in de voeding of de vetzuursamenstelling een effect heeft op de bloeddruk van gezonde, normotensieve mensen, in dien de opneming van essentiële vetzuren adequaat is.
(O.a. dit proefschrift)

5. Het is onjuist om voedingsadviezen om het serumcholesterolgehalte te verlagen slechts en alleen te baseren van de formule van Keys.
(Keys et al. Metabolism 1965; 14:776-786)

6. Het gebruik van de verhouding meervoudig onverzadigde : verzadigde vetzuren ("P/S-verhouding") in de voeding, die vaak wordt gebruikt als maat voor de invloed van voeding op het serumcholesterolgehalte, is misleidend.

7. Alhoewel de meeste mensen ouderwetse architectuur het mooist vinden, wordt voornamelijk modern gebouwd.

8. De stelling dat de mens dom is, maakt deze bij voorbaat tot een zwakke.

9. Het feit dat voeding een "vrouwelijk" woord is, vormt geen verklaring voor het hoge aantal vrouwelijke studenten aan de vakgroep Humane Voeding: techniek is ook een "vrouwelijk" woord.

Proefschrift R.P. Mensink.
Effect of monounsaturated fatty acids on high-density and low-density lipoprotein cholesterol levels and blood pressure in healthy men and women.
Wageningen, 23 maart 1990.

Aan

mijn ouders

en

Dianne.

Abstract

EFFECT OF MONOUNSATURATED FATTY ACIDS ON HIGH-DENSITY AND LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS AND BLOOD PRESSURE IN HEALTHY MEN AND WOMEN

Thesis, Department of Human Nutrition, Wageningen Agricultural University, Wageningen, the Netherlands, 23 March 1990

Ronald P. Mensink

The purpose of the studies described in this thesis was to examine the effect of monounsaturated fatty acids on the distribution of serum cholesterol over high-density and low-density lipoproteins (HDL and LDL) and on blood pressure in healthy men and women. High levels of LDL cholesterol and blood pressure, and low levels of HDL cholesterol are associated with an increased risk for coronary heart disease.

Three controlled dietary studies were carried out. In the first study it was found that that monounsaturated fatty acids (oleic acid) specifically lowered non-HDL cholesterol when they replaced saturated fatty acids in the diet. In contrast, complex carbohydrates lowered both HDL and LDL cholesterol. The results of the second study indicated that it is immaterial whether saturated fatty acids in the diet are replaced by a mixture of monounsaturated and (n-6)polyunsaturated fatty acids (oleic and linoleic acid) or by (n-6)polyunsaturated fatty acids alone. The two unsaturated-fat-rich diets had the same effect on HDL cholesterol and both lowered the level of LDL cholesterol to the same extent. In the third experiment we found that, when compared with cis monounsaturated fatty acids, trans monounsaturated fatty acids lower HDL cholesterol levels. In addition, trans fatty acids increased the level of LDL cholesterol, although to a lesser extent than did saturated fatty acids.

No effects of specific fatty acids on blood pressure were detected in any of the three experiments.

It is concluded that replacement of fats rich in saturated or trans fatty acids by oils rich oleic or linoleic acid might be helpful for the prevention of coronary heart disease, as far as lipoprotein levels are concerned. Weight gain, however, might be an unwanted side-effect of such high-oil diets.

Contents

Voorwoord

Het presenteren en verdedigen van een proefschrift is een solo-gebeuren. Het proefschrift zelf, echter, is tot stand gekomen dankzij de hulp van velen. Dit voorwoord wil ik dan ook gebruiken om de mensen te bedanken, die mij bij het onderzoek hebben geholpen.

Op de eerste plaats wil ik mijn twee promotoren, Prof. Dr. M.B. Katan en Prof. Dr. J.G.A.J. Hautvast, bedanken. Zij hebben mij geleerd hoe voedings-interventiestudies bij mensen kunnen worden uitgevoerd. De resultaten van deze studies moesten echter ook nog worden uitgewerkt en opgeschreven. Martijn, jij was degene die als eerste mijn manuscripten moest doorwerken. Ik hoop dat ik iets heb opgestoken van jouw heldere onderzoeksvisie en duidelijke manier om resultaten te presenteren.

De Nederlandse Hartstichting ben ik erkentelijk voor de aan mij toegekende beurs in het kader van het Dr. E. Dekker-programma (project D87-002).

Het was iedere keer weer een hele opluchting om te merken dat er belangstelling was om aan onze studies deel te nemen. Ik ben alle deelnemers dan ook dankbaar voor hun enthousiasme en prettige samenwerking.

Het kloppend maken van de nutriëntberekeningen van de voedingen, het samenstellen en bereiden van de menu's, en het afnemen en coderen van de 267 voedingsanamneses werd verzorgd door de diëtisten. Met name wil ik Jeanne de Vries en Anja Severijnen-Nobels bedanken. Zij waren bij alle studies betrokken en zorgden ervoor dat, althans voor het voedingsgedeelte, alles op rolletjes is verlopen.

Het afnemen, opwerken, en verwerken van de vele bloed- en serummonsters, er zij meer dan 4000 buisjes met bloed afgenomen, en de analyses in de voedingsmiddelen werden voornamelijk gedaan door de medewerkers van de Vakgroep: Peter van de Bovenkamp, Jannie Bos, Cock Germing-Nouwen, Truus Kosmeijer-Schuil, Joke Barendse-van Leeuwen, Ans Soffers, Ronald Sperber, Frans Schouten, en Zeeger Kruijswijk, hartelijk dank. Hennie Kapelle heeft geholpen bij de data-invoer gedurende de laatste studie.

Aan dit onderzoeksproject hebben 11 doctoraalstudenten meegewerkt, die ik alle wil bedanken voor zowel hun wetenschappelijke als practische bijdrage: Léon van den Broeke, Edith Feskens, Monique de Groot, Mary-Christel Janssen,

Yvonne de Bont, Corné van Dooren, Gerda Feunekes, Monique Mentjens, Annette Stolwijk, Marian de Louw, en Arnold Timmer.

In alle proeven zijn margarines gebruikt, die speciaal voor onze proeven zijn ontwikkeld en bereid. Ik ben Van den Bergh en Jurgens BV uit Rotterdam, en de Sectie Productontwikkeling van Unilever Research Laboratory uit Vlaardingen erkentelijk dat zij deze taak op zich hebben willen nemen en op een uitstekende wijze hebben volbracht. Het verwerken van deze margarines of van olijfolie door het brood was in de vakbekwame handen van Bakkerij Bouwman en, later, bakkerij Holdinga uit Wageningen.

Het kritisch commentaar van Dr. J.L. Zevenbergen, Dr. U.H.M. Houtsmuller, Dr. J.J. Gottenbos en Dr. O. Korver van Unilever Research Laboratory uit Vlaardingen gedurende de voorbereidingsfase van de studies en de wetenschappelijke discussies over de interpretatie van de resultaten was altijd erg stimulerend.

De hulp van Wilma de Maar en het secretariaat van de Vakgroep, en de medewerkers van de tekenkamer en de afdeling Fotografie van het Biotechnion was onmisbaar. Zonder jullie hulp was dit proefschrift nooit meer geweest dan een bundeling losse blaadjes en wat ruwe schetsen.

De leden van de Ethische Commissie ben ik erkentelijk voor het kritisch beoordelen van de diverse onderzoeksprotocollen.

I am indebted to Prof. C. Aravanis, Mrs B. Christodoulou and Prof. A. Kafatos from Greece. I hope that the two epidemiological studies carried out in Crete, together with the studies described in this thesis, have lead to a better understanding of the virtues of olive oil.

The financial support for the studies by the Netherlands Nutrition Foundation, the Netherlands Ministry of Welfare, Public Health and Cultural Affairs, the Commission of the European Communities, and the Netherlands Heart Foundation is gratefully acknowledged.

Tot slot wil ik Kees de Graaf, Ben Scholte, Peter Zock, Jaap Seidell, en Monique Berns bedanken voor de prettige tijd op de Vakgroep. Het was altijd gezellig om jullie van het werk af te houden.

CHAPTER 1

Introduction

Coronary heart disease is a group of syndromes characterized by a failure of the coronary arteries to transport sufficient blood to the myocardium. It is usually but not always caused by atherosclerotic narrowing of coronary arteries. Large prospective population surveys have shown that a high level of serum total cholesterol, a high blood pressure, and cigarette smoking, are the three major preventable risk factors for coronary heart disease [1].

The aim of this introduction is to describe briefly the relation of serum cholesterol and blood pressure with the risk for coronary heart disease, and to describe the relation of these risk factors with dietary fat intake. The final section of this chapter gives an outline of the experiments described in this thesis.

Serum cholesterol, lipoproteins and coronary heart disease

An elevated level of serum total cholesterol is positively related to the risk of coronary heart disease. Earlier studies suggested that the rates for coronary heart disease are relatively constant for cholesterol levels below 5.7 mmol/L (220 mg/dL) [2], and that above this level the risk for coronary heart disease rises steadily. However, data for the men screened as part of the Multiple Risk Factor Intervention Trial (MRFIT) clearly showed that no such threshold level exists; the relation between the level of serum total cholesterol and the mortality from coronary heart disease is continous and progressive [1]. Unfortunately, women were not included in this study.

In addition to the absolute level of serum total cholesterol the distribution of cholesterol over the various lipoproteins is important. Lipoproteins are the carrier of cholesterol in the blood vessels. The two major cholesterol-transporting classes are low-density lipoprotein (LDL) and high-density lipoprotein (HDL). LDL carries 60 to 70 percent of the total amount of cholesterol in the blood, and HDL 20 to 30 percent. These lipoproteins may have opposite effects on the risk for coronary heart disease. It is generally accepted that LDL cholesterol is atherogenic, while

HDL cholesterol may have an anti-atherogenic effect. In the Helsinki heart study it was shown that by treating hyperlipidaemic men with the drug gemfibrozil the risk for coronary heart disease was significantly reduced. Gemfibrozil lowers LDL cholesterol, but increases HDL cholesterol. It could be demonstrated that the reduction in LDL cholesterol and the increase in HDL cholesterol both contributed, independently of each other, to the observed reduction in coronary heart disease [3]. Similar findings were reported in other intervention trials [4].

As LDL carries the major part of serum cholesterol, the total serum cholesterol is in general a good index for the risk of coronary heart disease.

Lipoproteins are made up of lipids - cholesterol, cholesteryl esters, triglycerides and phospholipids - and proteins, the so-called apolipoproteins. Some studies suggest that concentrations of the apolipoprotein of LDL, apolipoprotein B, and of HDL, apolipoprotein A-I, are better indicators for the risk of coronary heart disease than serum LDL and HDL cholesterol [5, 6]. However, this theory still awaits confirmation.

Blood pressure and coronary heart disease

As with serum total cholesterol levels, the relation of both systolic and diastolic blood pressure with the mortality of coronary heart disease is an ever-increasing function [7]. In addition, systolic blood pressure, and to a lesser extent, diastolic blood pressure, are positively related with the incidence of stroke [8]. In the Framingham study [8] the power of blood pressure to predict the incidence of various diseases was similar in men and women.

Although blood pressure is a risk factor for coronary heart disease, observational studies cannot prove that lowering blood pressure indeed reduces the incidence of coronary heart disease. The Australian Therapeutic trial, however, demonstrated that reducing blood pressure with drugs decreased the mortality and morbidity from cardiovascular disease in men and women [9]. Thus, the relation between blood pressure and coronary heart appears to be causal. However, many other trials could not demonstrate a beneficial effect of treating mild hypertension by drugs on cardiovascular mortality (for a review see O'Kelly et al [10]). This might partly be due to side-effects of the drugs used [10], especially those on serum lipid levels. This observation stresses the importance of lowering blood pressure by diet.

Dietary fat and serum lipoproteins

The level of serum total cholesterol can be influenced by diet [11]. It increases when saturated fatty acids with 12 to 16 carbon atoms are isocalorically substituted for carbohydrates. These conclusions emerged from the metabolic ward studies carried out by several groups of investigators [12-14]. Linoleic acid, the most abundant polyunsaturated fatty acid in the diet, was found to have a cholesterol-decreasing effect, while monounsaturated fatty acids and stearic acid, a saturated fatty acid with 18 carbon atoms, had no effect when they were used to replace carbohydrates. It was thought that the effects of diet on the level of total serum cholesterol were entirely due to changes in the LDL cholesterol fraction [13, 14]. This conclusion was supported by earlier studies of Keys et al [15] which found that no relation existed between diet and the level of HDL cholesterol when different countries were compared. However, both epidemiological and controlled dietary studies [16-18] have shown that replacement of carbohydrates by fat increases the level of HDL cholesterol.

Dietary fat and blood pressure

Some studies have suggested that dietary fat, and in particular the essential polyunsaturated fatty acid linoleic acid, is involved in the regulation of blood pressure in both normotensive and hypertensive subjects [19, 20]. Available data, however, do not exclude the possibility that the ratio of polyunsaturated to saturated fatty acids and/or the total fat intake is more important than the absolute intake of polyunsaturated fatty acids. The results of these studies have proved to be difficult to reproduce in normotensive subjects. Also, results were sometimes less than conclusive [21, 22] and difficult to interpret, as the experimental diets differed not only in their fat composition; the intake of other nutrients which may affect blood pressure, such as sodium, potassium and fiber, changed as well.

Outline of the thesis

Dietary recommendations in the Netherlands advocate a reduction in the intake of saturated fatty acids and of cholesterol, and an increase of polyunsaturated fatty acids and of complex carbohydrates [23]. Overweight subjects are also adviced to decrease their energy intake. Less attention

was given to monounsaturated fatty acids, as these were thought to decrease the level of serum total cholesterol less effectively than do polyunsaturated fatty acids [12-14]. However, fairly little information is available about the effects of various fatty acids on the concentration of cholesterol in the HDL and LDL fraction. It has earlier been suggested [18] that high-fat diets result in high HDL cholesterol levels. If this is true, then replacement of saturated by monounsaturated fatty acids should mainly lower LDL cholesterol, while replacement by carbohydrates might lower both LDL and HDL cholesterol. Thus, the effects of monounsaturated fatty acids on cholesterol metabolism might not be as neutral as they were thought to be. The purpose of the studies described in this thesis were therefore to examine the effect of monounsaturated fatty acids, as compared to other macronutrients, on the distribution of cholesterol over HDL and LDL. In contrast to many other studies, both men and women were involved. The effects on blood pressure were also studied.

In the first study, monounsaturated fatty acids were compared with carbohydrates. According to Keys' equation [13] the two nutrients should have the same effect on serum total cholesterol levels. However, we were interested in the specific effects of these two macronutrients on the cholesterol levels in HDL and LDL. In addition, this enabled us to study the effect of the dietary polyunsaturated to saturated fat ratio on blood pressure at different intakes of total fat.

In the second experiment monounsaturated fatty acids were compared with polyunsaturated fatty acids of the (n-6) family. It is uncertain whether polyunsaturated fatty acids lower the level of LDL cholesterol only, or also that of HDL cholesterol. The results of a study of Mattson and Grundy [24] indicated that the superior cholesterol-lowering effect of polyunsaturated fatty acids over monounsaturated fatty acids was due to a lowering of HDL cholesterol. The effect of increasing the intake of polyunsaturated fatty acids on blood pressure was also studied.

In the final study the effects on serum lipoproteins of oleic acid were compared with those of trans isomers of monounsaturated fatty acids and with those of saturated fatty acids were. Trans isomers of unsaturated fatty acids are found mainly in hydrogenated oils and fats, and are used for the production of certain types of margarines and shortenings. This study also offered us the possibility to examine the effects of these fatty acids on blood pressure.

REFERENCES

1. Stamler J, Wentworth D, Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continous and graded? Findings in 356 222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). JAMA 1986; 256: 2823-2828.
2. Oliver MF. Serum cholesterol - The knave of hearts and joker. Lancet 1981; 2:1090-1095.
3. Manninen V, Elo MO, Frick H, et al. Lipid alterations and decline in the incidence of coronary heart disease in the Helsinki Heart Study. JAMA 1988; 260:641-651.
4. Gordon DJ, Rifkind BM. High-density lipoprotein - The clinical implications of recent studies. N Engl J Med 1989; 321:1311-1316.
5. Avogaro P, Cazzolato G, Bittolo Bon G, Quinci GB. Are apolipoproteins better discriminators than lipids for atherosclerosis?. Lancet 1979; i:901-903.
6. De Backer G, Rosseneu M, Deslypere JP. Discriminative value of lipids and apoproteins in coronary heart disease. Atherosclerosis 1982; 42:197-203.
7. Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361 662 men. Lancet 1986; 2:933-936.
8. Dawber TR. The Framingham Study. Cambridge, Massachusetts and London: Harvard University Press, 1980.
9. Report by the Management Committee. The Australian therapeutic trial in mild hypertension. Lancet 1980; 1:1261-1267.
10. O'Kelly BF, Massie BM, Tubau JF, Szachcic J. Coronary morbidty and mortality, pre-existing silent coronary artery disease, and mild hypertension. Ann Intern Med 1989; 110:1017-1026.
11. Groen J, Tjiong BK, Kamminga CE, Willebrands AF. The influence of nutrition, individuality and some other factors, including various forms of stress, on the serum cholesterol; an experiment of nine months durations in 60 normal human volunteers. Voeding 1952; 13:556-587.
12. Vergroesen AJ, de Boer J. Effecten van meervoudig onverzadigde en andere vetzuren in de voeding. Voeding 1971; 32:278-290.
13. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet IV. Particular saturated fatty acids in the diet. Metabolism 1965; 14:776-786.
14. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 1965; 17:281-295.
15. Keys A, Karvonen MJ, Fidanza F. Serum cholesterol studies in Finland. Lancet 1958; 2:175-178.
16. Knuiman JT, Westenbrink S, van der Heijden L, et al. Determinants of total and high density lipoprotein cholesterol in boys from Finland, the Netherlands, Italy, the Philippines and Ghana with special reference to diet. Hum Nutr Clin Nutr 1983; 37C:237-254.
17. Becker NB, Illingworth R, Alaupovic P, Connor WE, Sundberg EE. Effects of saturated, monounsaturated, and w-6 polyunsaturated fatty acids on plasma lipids, lipoproteins, and apoproteins in humans. Am J Clin Nutr 1983; 37:355-360.
18. Katan MB. Diet and HDL. In: Miller GJ, Miller NE, eds. Metabolic aspects of cardiovascular disease. Vol 3 - Clinical and metabolic aspects of high-density lipoproteins. Oxford: Elsevier 1984; 103-132.

19. Iacono JM, Puska P, Dougherty RM, et al. Effect of dietary fat on blood pressure in a rural Finnish population. Am J Clin Nutr 1983; 38:860-869.
20. Puska P, Iacono JM, Nissinen A, et al. Controlled, randomised trial of the effect of dietary fat on blood pressure. Lancet 1983; i:1-5.
21. Sacks FM, Stampfer MJ, Munoz A, McManus K, Canessa M, Kass EH. Effect of linoleic and oleic acids on blood pressure, blood viscosity, and erythrocyte cation transport. J Am Coll Nutr 1987; 6:179-185.
22. Margetts BM, Beilin LJ, Armstrong BK, et al. Blood pressure and dietary polyunsaturated and saturated fats: a controlled trial. Clin Sci 1985; 69:165-175.
23. Erkelens DW. Cholesterol consensus in the Netherlands. Eur J Clin Nutr 1989; 43:89-96.
24. Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 1985; 26:194-202.

CHAPTER 2

Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women

Ronald P. Mensink, Martijn B. Katan

Lancet 1987; i:122-125

ABSTRACT

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 or 17 mg/dL in the carbohydrate group and by 0.46 mmol/L or 18 mg/dL in the olive oil group. HDL cholesterol levels fell by 0.19 mmol/L or 7 mg/dL in the carbohydrate group and rose by 0.03 mmol/L or 1 mg/dL in the olive oil group. Serum triglycerides rose by 0.19 mmol/L or 17 mg/dL in the carbohydrate group and fell by 0.06 mmol/L or 5 mg/dL in the olive oil group. The changes in both HDL cholesterol and triglycerides were larger in men than in women.

These results clearly show that the olive-oil-rich diet, unlike the diet high in complex carbohydrates, 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 [1, 2], and diets chosen for lowering serum total 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 showed that the cohort from Crete was something of an anomaly because the incidence of coronary heart disease in these middle-aged men was lower than would be

expected from their serum total cholesterol levels [3]. This could not be explained by other risk factors for coronary heart disease, 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 serum total cholesterol levels, while leaving HDL cholesterol levels unchanged.

We have tested this hypothesis in a strictly controlled dietary experiment with healthy normolipidemic 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 fiber.

METHODS

Subjects

Fifty-seven 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 anemia, glucosuria, proteinuria, or hypertension. Their serum total cholesterol ranged from 3.43 to 7.58 mmol/L (mean, 5.07 mmol/L or 196 mg/dL), HDL cholesterol from 0.72 to 2.58 mmol/L (mean, 1.38 mmol/L or 53 mg/dL) and serum triglycerides from 0.33 to 2.77 mmol/L (mean, 0.98 mmol/L or 87 mg/dL). 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 ethics committee of the Department of Human Nutrition, 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 changes in weight 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 indexes 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 control 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. Three of the women used oral contraceptives. For the next 36 days one group received a high-carbohydrate, high-fiber diet and the other group an olive-oil-rich diet. These diets were formulated to cause the same fall in serum total cholesterol according to the formula of Keys et al [5]. The composition of the diets is given in Table 1. To maintain the use of normal foodstuffs, the olive oil group received special bread containing 8 g of olive oil per 100 g and a special margarine (van den Bergh & Jurgens BV, Rotterdam, The Netherlands) made of "new" rapeseed oil, high in oleic and low in erucic acid. Olive oil contributed 75 percent of monounsaturated fatty acids and the margarine 10 percent. The high-carbohydrate diet was enriched with bread, pulses, vegetables, potatoes, fruits and jam.

In addition to the food supplied, subjects were allowed a limited choice of items (such as alcoholic drinks, fruit, and sweets), free of fat and cholesterol and providing a fixed amount of energy, ranging from 5 to 10 percent 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 signs of illness, medications used, the self-selected food items, and deviations from the diet. Duplicate

Table 1. Mean daily intake of energy and compsition of the diets.*

	Type of diet*		
	Control (N=48)	Carbohydrate-rich (N=24)	Olive-oil-rich (N=24)
Energy+			
MJ/day	11.1 ± 2.6	11.8 ± 3.0	11.1 ± 2.6
kcal/day	2653 ± 621	2820 ± 717	2653 ± 621
Protein (% of energy)	13.6	14.1	12.2
Fat (% of energy)	38.0	22.1	40.6
Saturated fatty acids	20.0	6.7	9.8
Lauric acid (C12:0)	1.2	0.3	0.4
Myristic acid (C14:0)	3.0	0.4	0.6
Palmitic acid (C16:0)	9.7	3.4	5.8
Stearic acid (C18:0)	4.1	1.3	2.2
Monounsaturated fatty acids	12.4	9.3	24.0
Polyunsaturated fatty acids	4.1	5.2	5.1
Linoleic acid	3.4	4.3	4.2
P/S ratio¥	0.21	0.78	0.52
Carbohydrates (% of energy)	47.7	62.2	46.0
Mono- 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/MJ)§	35.1	33.1	31.1
Dietary fiber (g/MJ)§	3.8	5.1	3.9

* Based on chemical analysis of duplicate diets. The variation between subjects in the composition of the study diets was negligible; therefore, no standard deviations are given for the nutrients.

\+ Individual intakes ranged from 6.1 to 19.9 MJ on the control diet, from 6.3 to 20.5 MJ on the carbohydrate-rich diet and from 7.1 to 15.5 MJ on the olive-oil-rich diet.

¥ Ratio of polyunsaturated to saturated fatty acids.

§ To convert values for the intake of cholesterol to milligrams and dietary fiber to grams per 1,000 kcal, multiply by 4.184.

portions of each diet were collected daily, pooled per diet period, and analyzed. These analyzed values were combined with the values calculated for the free-choice items (Table 1). 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 by 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 changes in body weights 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 one hour of venipuncture. Sera were checked for the presence of chylomicrons [6] (all samples were negative), stored at -80 °C and analyzed 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 of one subject were analyzed within one run. The within-run coefficient of variation for control sera was 0.9 percent for total, 1.8 percent for HDL cholesterol, and 1.0 percent for total triglycerides. Accuracy was checked by analysis of serum pools of known value provided by the U.S. Centers for Disease Control. Mean bias with regard to target values of the Centers for Disease Control was 0.1 percent for total cholesterol, -3.2 percent for HDL cholesterol, and -1.5 percent 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 diet 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 x sex interaction on the

response were examined by analysis of variance. Pearson's correlation coefficients were computed between the response and the intrinsic lipid level (average of the mean of days 14, 17 and the mean of days 46, 49 and 53) for both sexes for each diet group.

RESULTS

The changes in serum lipid values are given in Figure 1. Serum total

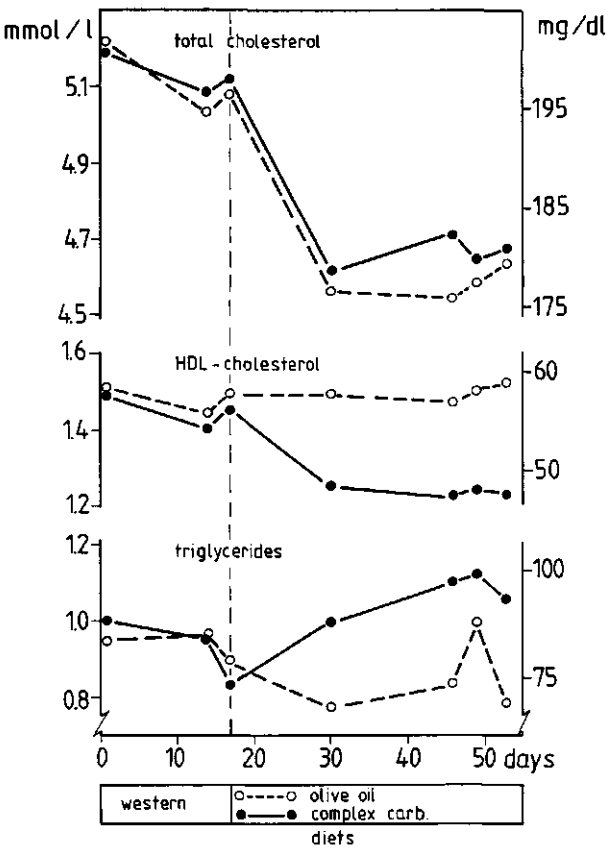

Figure 1. Mean serum concentrations of total, and HDL cholesterol and serum triglycerides during the experiment.

All 48 subjects received a Western-type control diet high in saturated fat for 17 days. For the next 36 days, 24 subjects received an olive-oil-rich diet, and 24 subjects received a diet low in fat and high in complex carbohydrates and fiber.

cholesterol fell on both diets and to the same extent. HDL cholesterol fell by 0.19 mmol/L or 7 mg/dL on the high-carbohydrate, high-fiber diet, and rose by 0.03 mmol/L or 1 mg/dL on the olive-oil-rich diet ($P<0.001$ for the difference in changes between the diet groups). In the complex-carbohydrate group the mean reduction in HDL cholesterol equalled 51 percent of the serum total cholesterol decrease in men and 38 percent in women. Triglycerides rose by 0.19 mmol/L or 17 mg/dL on the carbohydrate-rich diet and fell by 0.06 mmol/L or 5 mg/dL on the olive oil diet ($P<0.005$ for the difference in changes). The differences in changes between the diet groups were more pronounced in men than in women for both HDL cholesterol ($P<0.005$) and triglycerides ($P<0.1$) (Table 2). The response to diet was unrelated to the intrinsic levels of total or HDL cholesterol or triglycerides.

Table 2. Effects of a high-carbohydrate, high-fiber diet and an olive-oil-rich diet on serum lipid concentrations.*

	Carbohydrate group		Olive oil group	
	Men (N=12)	Women (N=12)	Men (N=12)	Women (N=12)
Total 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

* Values are expressed in mmol/L and are means ± SD. For the control period two samples per subject were averaged and for the test period three samples. To convert values for total and HDL cholesterol to mg/dL, multiply by 38.67. To convert values for triglycerides to mg/dL, multiply by 88.54.

Statistical comparison for difference in changes between diet groups with sexes pooled: + $P<0.001$, ¥ $P<0.005$; diet x sex interaction: § $P<0.005$, ¶ $P<0.1$.

DISCUSSION

This controlled study in 48 healthy volunteers showed that diets high in either complex carbohydrates plus fiber or monounsaturated fatty acids caused the same fall in total serum 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 accomodate potential difficulties. In previous studies with volunteers from the same population, adherence to the diets was documented by changes in serum cholesteryl ester fatty-acid composition [7] or colonic function [8]. In the present study compliance with the diets is confirmed by the observed reduction of 0.44 mmol/L or 17 mg/dL for the carbohydrate and 0.46 mmol/L or 18 mg/dL 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-fiber diet HDL cholesterol levels fell by 0.19 mmol/L or 7 mg/dL on average. This observation agrees with the results of Grundy [10], who compared the effects of high-oleic-acid safflower oil and glucose on lipoproteins in obese men, who were between 49 and 69 years old. 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 glucose-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 cholesterol 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 serum total cholesterol) were associated with diets high in

fat and low in complex carbohydrates. Becker et al [13], in a study of male volunteers, compared the effects on plasma lipids of three cholesterol-free formula diets, containing 40 percent 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 optimal level of (n-6)polyunsaturated fatty acids in the diet are therefore needed.

The reduction in HDL cholesterol was observed despite the higher fiber content of the carbohydrate-rich diet. It has been suggested that the fiber content of a diet is positively associated with HDL cholesterol levels [17, 18]. However, the increased fiber 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 triglyceride 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 phenomenon is real and not transient. A difference in the effect of diet on serum triglyceride levels between men and women has been shown [21, 22] and confirmed in the present study. If the difference in effect on HDL cholesterol 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, normolipidemic subjects a specific fall in non-HDL cholesterol, while leaving HDL and triglyceride values unchanged; this contrasts the effects of the high-carbohydrate, high-fiber 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 coronary heart disease.

REFERENCES

1. Schaefer EJ. Clinical, biochemical and genetic features in familial disorders of high density lipoproteins deficiency. Arteriosclerosis 1984; 4:303-322.

2. Brensike JF, Levy RI, Kelsey SF, et al. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI type II coronary intervention study. Circulation 1984; 69:313-324.
3. Keys A, ed. Coronary Heart Disease in Seven Countries. Circulation 1970; 41:Suppl 1.
4. Katan MB. Diet and HDL. In: Miller GJ, Miller NE, eds. Metabolic aspects of cardiovascular disease. Vol 3 - Clinical and metabolic aspects of high-density lipoproteins. Oxford: Elsevier 1984; 103-132.
5. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. Metabolism 1965; 14:776-787.
6. Schinella M, Signori D, Lippi U. Sensitivity and specificity of a new rapid and simple microprocedure for detection of serum chylomicrons. Clin Chim Acta 1982; 141:73-76.
7. Brussaard JH, Katan MB, Groot PHE, Havekes LM, Hautvast JGAJ. Serum lipoproteins of healthy persons fed a low-fat diet or a polyunsaturated fat diet for three months. Atherosclerosis 1982; 42:205-219.
8. Stasse-Wolthuis M, Albers HFF, van Jeveren JAC, et al. Influence of dietary fiber from vegetables and fruits, bran or citrus pectin on serum lipids, fecal lipids and colonic function. Am J Clin Nutr 1980; 33:1745-1756.
9. Anderson JT, Jacobs DR, Foster N, et al. Scoring systems for evaluating dietary pattern effect on serum cholesterol. Prev Med 1979; 8:525-537.
10. Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. New Engl J Med 1986; 314:745-748.
11. Report of a WHO Expert Committee. Prevention of coronary heart disease. WHO Techn Rep Ser no 678, Geneva, 1982.
12. Knuiman JT, Westenbrink S, van der Heijden L, et al. Determinants of total and high density lipoprotein cholesterol in boys from Finland, the Netherlands, Italy, the Philippines and Ghana with special reference to diet. Hum Nutr Clin Nutr 1983; 37C:237-254.
13. Becker NB, Illingworth R, Alaupovic P, Connor WE, Sundberg EE. Effects of saturated, monounsaturated, and w-6 polyunsaturated fatty acids on plasma lipids, lipoproteins, and apoproteins in humans. Am J Clin Nutr 1983; 37:355-360.
14. Shepherd J, Packard CJ, Grundy SM, Yeshurun D, Gotto Jr AM, Taunton OD. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of high density lipoproteins. J Lipid Res 1980; 21:91-99.
15. Vessby B, Boberg J, Gustafsson IB, Karlström B, Lithell H, Östlund-Lindquist AM. Reduction of high density lipoprotein cholesterol and apolipoprotein A-I concentrations by a lipid-lowering diet. Atherosclerosis 1980; 35:21-27.
16. Schwandt P, Janetschek P, Weisweiler P. High density lipoproteins unaffected by dietary fat modification. Atherosclerosis 1982; 44:9-17.
17. Dodson PM, Stocks J, Holdsworth G, Galton DJ. High-fibre and low-fat diets in diabetes mellitus. Br J Nutr 1981; 46:289-294.
18. Flanagan M, Little C, Milliken J, et al. The effect of diet on high density lipoprotein cholesterol. J Hum Nutr 1980; 34:43-45.
19. Hayford JT, Danney MM, Wiebe D, Robert S, Thompson RG. Triglyceride integrated concentrations: effect of variation of source and amount of dietary carbohydrate. Am J Clin Nutr 1979; 32:1670-1678.
20. Sullivan DR, West CE, Katan MB, Kruijswijk Z. An international study on the effect of dietary carbohydrate on atherogenic and protective lipoproteins. In: Beynen AC, Geelen MJH, Katan MB, Schouten JA, eds. Cholesterol metabolism in health and disease: studies in the Netherlands. Ponsen & Looijen, Wageningen 1985:130-136.

21. Beveridge JMR, Jagannathan SN, Ford Connel W. The effect of the type and amount of dietary fat on the level of plasma triglycerides in human subjects in the post absorptive state. Can J Biochem 1964; 42:999-1003.
22. Ernst N, Fisher M, Bowen P, Schaefer EJ, Levy RI. Changes in plasma lipids and lipoproteins after a modified fat diet. Lancet 1980; ii:111-113.

CHAPTER 3

Effect of monounsaturated fatty acids versus complex carbohydrates on serum lipoproteins and apolipoproteins in healthy men and women

Ronald P. Mensink, Monique J.M. de Groot, Léon T. van den Broeke, Anja P. Severijnen-Nobels, Pierre N.M. Demacker, Martijn B. Katan

Metabolism 1989; 38:172-178

ABSTRACT

The effects of a high-carbohydrate, high-fiber diet and an olive-oil-rich diet on the distribution of cholesterol over the various lipoproteins, on serum apolipoproteins, and on the composition of HDL_2 and HDL_3 were studied under strict dietary control. Fourty-eight healthy subjects first consumed a high-saturated-fat diet (20.0 percent of their daily energy intake from saturated fat, 38.0 percent total fat) for 17 days. For the next 36 days, 24 subjects consumed a diet high in complex carbohydrates (9.3 percent monounsaturated fat, 22.1 percent total fat) and the other 24 a high-fat, olive-oil-rich diet (24.0 percent monounsaturated fat, 40.6 percent total fat). The amounts of protein (12 to 14 percent), polyunsaturated fatty acids (4 to 5 percent) and cholesterol (31 to 35 mg/MJ) were similar in all three diets.

Serum cholesterol levels fell by 0.44 mmol/L or 17 mg/dL in subjects consuming the carbohydrate diet and by 0.52 mmol/L or 20 mg/dL for those receiving the olive-oil-rich diet. VLDL cholesterol levels rose by 0.08 mmol/L or 3 mg/dL in the carbohydrate group and fell by 0.08 mmol/L or 3 mg/dL in the olive-oil group ($P<0.05$ for difference in changes between the diet groups). HDL_2 and LDL cholesterol levels fell to the same extent on both diets. HDL_3 cholesterol fell by 0.09 mmol/L or 4 mg/dL on the high-carbohydrate diet and increased by 0.01 mmol/L on the olive oil diet ($P<0.05$). There was no change in the composition of HDL_3, suggesting that the fall was due to a decrease in the total number of circulating particles. Thus, Keys formula, which predicts no change in serum cholesterol when carbohydrate is replaced by oleic acid, holds for LDL cholesterol but not for VLDL and HDL cholesterol, which change in opposite directions. Apolipoprotein A-I levels decreased by 102 mg/L in the carbohydrate group and rose by 26 mg/L in the olive-oil group ($P<0.05$). For apolipoprotein B these values were 5 mg/L and -83 mg/L, respectively. The ratio of apolipoprotein A-I to apolipoprotein B decreased by 0.12, or 10 percent, on the carbohydrate diet and increased by 0.19, or 16 percent, on the olive oil

diet ($P<0.01$).

It is concluded that the lipoprotein risk profile for coronary heart disease was affected more favorably by the olive-oil-rich diet than by the diet high in complex carbohydrates.

INTRODUCTION

High-carbohydrate diets, which are often recommended for the prevention of coronary heart disease [1], lower both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels [2]. The reduction in the atherogenic LDL cholesterol is favorable, but the reduction in HDL cholesterol may be less beneficial in view of its negative relationship with coronary heart disease in epidemiological studies [3]. Diets rich in monounsaturated fatty acids lower serum total cholesterol levels to the same extent as carbohydrate-rich diets. Recent studies, however, have indicated that in contrast to monounsaturates, carbohydrates reduce HDL cholesterol levels [4, 5]. Therefore, it has been suggested that monounsaturates might be helpful for the prevention of coronary heart disease [6]. However, some studies suggest that concentrations of apolipoproteins and of HDL_2 cholesterol might be better indicators for the risk of atherosclerosis than just serum total and HDL cholesterol [7, 8]. The effects of monounsaturates on these variables are unknown as yet. Here we report the effects of an olive-oil-rich and a high-carbohydrate, high-fiber diet on the distribution of cholesterol over the various lipoprotein fractions, on serum apolipoprotein A-I and apolipoprotein B levels, and on the composition of HDL_2 and HDL_3. Some of these data have been reported at a conference [9].

METHODS

Experimental design, subjects and diets

Forty-eight healty men and women participated in this experiment. They were, on average, 27 years old, and weighed 71 kg. Two groups were formed by randomization, with stratification for serum lipids and sex. Each group consisted of 12 men and 12 women. Three women in each group used oral contraceptives. Other baseline characteristics are shown in Table 1.

All subjects first received for 17 days a Western-type control diet high in saturated fat (Figure 1). According to duplicate portion analysis, the

proportion of energy from saturated fatty acids was 20.0 percent, from monounsaturated fatty acids 12.4 percent, and from total fat 38.0 percent. Dietary fiber intake amounted to 3.1 g/MJ. For the next 36 days one group consumed a high-carbohydrate, high-fiber diet (6.7 percent of their daily energy intake from saturated fatty acids, 9.3 percent from monounsaturated

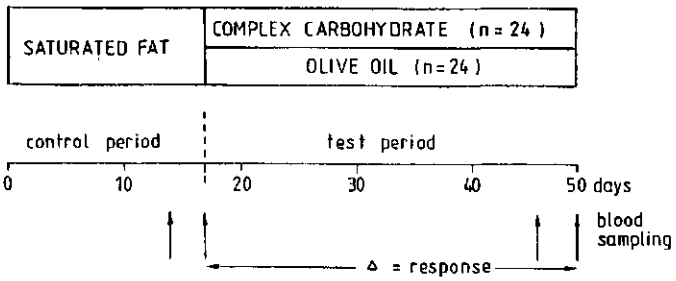

Figure 1. Experimental design.

fatty acids, 22.1 percent from total fat; dietary fiber intake was 5.1 g/MJ), and the other group consumed an olive-oil-rich diet (9.8 percent from saturated fatty acids, 24.0 percent from monounsaturated fatty acids, 40.6 percent from total fat; dietary fiber intake was 3.9 g/MJ). The amounts of

Table 1. Age, physical characteristics, and serum lipids of the subjects 2 months prior to the experiment.*

	Men (N=24)	Women (N=24)	All (N=48)
Age (years)	25.6 ± 8.0	27.9 ± 10.9	26.8 ± 9.5
Height (cm)	184 ± 7.2	171 ± 6.6	177 ± 9.5
Weight (kg)	75.0 ± 6.8	66.7 ± 6.8	70.8 ± 7.9
Body mass index (kg/m^2)	22.3 ± 2.2	23.0 ± 2.3	22.6 ± 2.3
Total cholesterol (mmol/L)	5.00 ± 1.03	5.14 ± 0.79	5.07 ± 0.91
HDL cholesterol (mmol/L)	1.21 ± 0.32	1.55 ± 0.38	1.38 ± 0.39
Triglycerides (mmol/L)	1.08 ± 0.67	0.88 ± 0.31	0.98 ± 0.52

* Values are means ± SD. To convert values for total and HDL cholesterol to mg/dL, multiply by 38.67. To convert values for triglycerides to mg/dL, multiply by 88.54.

protein (12 to 14 percent), polyunsaturated fatty acids (4 to 5 percent), alcohol (1 to 2 percent), and cholesterol (31 to 35 mg/MJ) were similar in all three diets. Mean daily energy intake was 13.5 MJ (3250 kcal) for men and 9.1 MJ (2180 kcal) for women.

Each diet consisted of conventional solid foods, and menus were changed daily. All foodstuffs were supplied individually. On weekdays at noon, hot meals were served at the Department of Human Nutrition. Other foods were provided daily as a package. Food for the weekend and guidelines for its preparation were provided on Fridays. In addition to the food supplied, subjects were allowed a limited choice of items free of fat and cholesterol. For each subject these items provided a fixed amount of energy, which ranged between subjects from 5 to 10 percent of mean daily energy intake. Thus, a limited use of alcoholic beverages was allowed. However, the importance of not changing one's selection of free-choice items between the periods was repeatedly explained and stressed. Differences in alcohol intake between the control and the experimental period were, therefore, small. Six subjects took no alcohol at all during the experiment. Two subjects had only one glass of wine during the control period, and took no alcohol during the experimental period. For all other subjects, the mean daily alcohol intake was comparable during the two dietary periods. Subjects were asked to record in diaries the self-selected items as well as any signs of illness, medications used, and deviations from the diet. Body weights were recorded twice weekly, and energy intake was adjusted when it became evident that a subject was gradually losing or gaining weight. During the study, changes in weight did not exceed 2.5 kg. Average body weight fell by 0.5 kg in the carbohydrate group and 1.0 kg in the olive oil group during the study. Further details have been published [5].

Blood sampling and chemical analyses

Blood samples were collected after an overnight fast at the end of the high-saturated-fat control period on days 14 and 17, and on days 46 and 49, which were 29 and 32 days into the test period (carbohydrate-rich, high-fiber or olive-oil-rich diet). Serum was prepared by low-speed centrifugation within one hour after venipuncture and checked for the presence of chylomicrons by the microcentrifugation technique [10] (all samples were negative). One aliquot was stored at -20 °C for apolipoprotein analysis, and another aliquot was stored at 4 °C for ultracentrifugation. On

day 17, equal volumes of aliquots obtained on days 14 and 17 were pooled per subject, and subjected to density gradient ultracentrifugation in a Ti41 swinging-bucket rotor (Beckman, Palo Alto, CA) for 22 hours at 37,000 rpm [11] after a maximum storage of 3 days at 4 °C. The following density classes (d in g/mL) were then isolated by aspiration: VLDL (d<1.010), IDL (1.010<d<1.019), LDL (1.019<d<1.055), HDL_1 plus lipoprotein(a) (1.055<d<1.075), HDL_2(1.075<d<1.100), HDL_3(1.100<d<1.180), and a bottom fraction (d>1.180). Fractions were stored at -20 °C until analysis. The same pooling and ultracentrifugation procedure was applied to the samples obtained on days 46 and 49. Sera were always randomly assigned to a particular ultracentrifugation run so that the two diet groups were intermixed. Cholesterol concentrations were determined in whole serum and in ultracentrifugal fractions, and triglyceride concentrations in whole serum using enzymatic methods and strict quality control. All samples from one subject were analyzed within one run. The within-run coefficient of variation for control sera was 0.9 percent for total cholesterol and 1.0 percent for total triglycerides. Accuracy was checked by analysis of serum pools of known value provided by the U.S. Centers for Disease Control. Mean bias with regard to target values of the Centers for Disease Control was 0.1 percent for total cholesterol and -1.5 percent for total triglycerides. The mean recovery of cholesterol in the lipoprotein fractions was 93.0 ± 5.3 percent for the control and 93.4 ± 6.5 percent for the test period. Apolipoprotein B was measured in whole serum by radial immunodiffusion using antiserum raised in rabbits. For each subject the two samples obtained at the end of the control diet period or at the end of the carbohydrate-rich or olive-oil-rich diet period were pooled and analyzed in duplicate on the same plate. The areas of the precipitates were quantified with a calibrated gauge. Calibration pools were prepared from a serum pool of known value provided by the Centers for Disease Control (pool #1883). The combined within-day and between-day coefficient of variation for control sera was 3.9 percent.

Aliquots of the HDL fractions obtained after ultracentrifugation were pooled for two or three subjects at a time for detailed analysis of the composition of HDL_2 and HDL_3. Subjects within one diet group were allotted to a pool by chance, except that each pool comprised fractions from either men or women, and that the lipoprotein fractions of women using oral contraceptives were pooled together. In this way, eleven pools (5 for women and 6 for men) were obtained of HDL_2, and another eleven of HDL_3 for each

diet group in each dietary period, for a total of 88 pools. Total cholesterol and triglycerides were measured in the pools by the methods described above. Free cholesterol and phospholipids were determined with an enzymatic method using the test kits and procedures of Boehringer (Mannheim, FRG) (Catalogue nos. 310 328 and 691 884, respectively). Esterified cholesterol was calculated as the difference between total and free cholesterol concentrations. Apolipoprotein A-I and apolipoprotein A-II concentrations were measured in duplicate in the HDL_2 and HDL_3 pools by radial immunodiffusion using antisera raised in rabbits. For apolipoprotein A-I calibration, pools were prepared from a serum pool of known value provided by the Centers for Disease Control (pool #1883). For apolipoprotein A-II calibration, an arbritary pool of sera from healthy donors was used. This pool was assigned an apolipoprotein A-II concentration equal to 0.28 times its apolipoprotein A-I concentration. The factor 0.28 was based on the average ratio of apolipoprotein A-II to apolipoprotein A-I found in normal individuals in eight reports. No apolipoprotein A-II values were available in the standardization program for apolipoprotein for the Centers of Disease Control. The between-day coefficient of variation for control sera was 5.2 percent for apolipoprotein A-I and 7.4 percent for apolipoprotein A-II. The entire pooling procedure was also applied to the bottom fraction, but these pools were analyzed for apolipoprotein A-I and apolipoprotein A-II only. For the control period, 20.1 $\pm$ 4.0 percent of the total apolipoprotein A-I and 4.8 $\pm$ 1.6 percent of the total apolipoprotein A-II was found in the bottom fraction. For the test period, these values were 19.1 $\pm$ 4.7 percent and 4.3 $\pm$ 2.1 percent, respectively.

Pooling was applied because separate analysis of all lipoprotein samples would have produced two HDL_2, two HDL_3, and two bottom fractions for each subject in each diet period, for a total of 576 fractions. A two-stage pooling procedure reduced this to a more manageable number, and the mean values of all variables were still conserved.

Statistical methods

The response to the test diet was calculated per subject as the change from the end of the control diet to the end of the carbohydrate or olive oil diet period. Differences in response of cholesterol levels in the various lipoproteins were examined by analysis of variance with diet, sex, and diet x sex interaction as independent variables. An unpaired t-test was used to

compare responses between diet groups if diet was the only significant variable. Differences in the composition of the HDL subclasses were examined by an unpaired t-test. A possible difference in response between the sexes was not tested, in view of the limited number of observations. Data from 1 pool of 2 males in the olive oil group were eliminated because all the variables measured in this pool deviated by more than twice the standard deviation from the mean values of the other pools from the same diet group. One of these 2 males also showed a highly aberrant total triglyceride value at the end of the experiment (day 49).

RESULTS

Distribution of cholesterol over the lipoprotein fractions

Table 2 shows that serum total cholesterol levels fell by 0.44 mmol/L or 17 mg/dL in the carbohydrate group and by 0.52 mmol/L or 20 mg/dL in the olive oil group. The concentration of cholesterol in the very low-density lipoproteins (VLDL) increased by 0.08 mmol/L or 3 mg/dL on the high-carbohydrate, high-fiber diet and fell by 0.08 mmol/L or 3 mg/dL on the olive-oil-rich diet ($P<0.05$ for the difference in changes between the diet groups). This agrees with the changes in fasting serum triglyceride concentrations, which increased from an initial level of 0.90 ± 0.42 to a final level of 1.09 ± 0.62 mmol/L (80 to 97 mg/dL) in the carbohydrate group and fell from 0.94 ± 0.58 to 0.91 ± 0.59 mmol/L (83 to 81 mg/dL) in the olive oil group ($P<0.01$ for the difference in changes). Both diets caused similar falls in LDL cholesterol levels. In addition, the diet high in carbohydrates caused a decrease in HDL cholesterol. This was due to a decrease in the cholesterol level in both HDL_2 and to HDL_3, although the difference with the olive-oil-rich diet was more marked for the HDL_3 than for the HDL_2 fraction. Changes in lipoprotein cholesterol concentrations were not related to sex, nor was there a significant sex x diet interaction, although for women the changes in serum triglycerides and in HDL and VLDL cholesterol differed less between the diet groups than they did for men.

Table 2. Effects of a high-carbohydrate, high-fiber diet and an olive-oil-rich diet on cholesterol concentrations in the various lipoproteins relative to levels of the same subjects when consuming a high-saturated-fat control diet.*

	Carbohydrate group (N=24)	Olive oil group (N=24)
Total cholesterol		
Control diet	5.15 ± 0.96	5.12 ± 0.76
Change	−0.44 ± 0.46	−0.52 ± 0.47
VLDL		
Control diet	0.34 ± 0.21	0.36 ± 0.34
Change+	0.08 ± 0.15	−0.08 ± 0.25
IDL		
Control diet	0.24 ± 0.16	0.21 ± 0.10
Change	0.01 ± 0.19	0.03 ± 0.10
LDL		
Control diet	2.57 ± 0.67	2.54 ± 0.55
Change	−0.45 ± 0.67	−0.50 ± 0.44
HDL_1 plus Lp(a)		
Control diet	0.24 ± 0.11	0.24 ± 0.09
Change	−0.01 ± 0.07	0.01 ± 0.06
HDL_2		
Control diet	0.47 ± 0.22	0.50 ± 0.24
Change	−0.08 ± 0.17	−0.03 ± 0.10
HDL_3		
Control diet	0.87 ± 0.16	0.87 ± 0.15
Change+	−0.09 ± 0.15	0.01 ± 0.13
Bottom fraction		
Control diet	0.07 ± 0.07	0.05 ± 0.06
Change	0.08 ± 0.08	0.11 ± 0.07

* Values are expressed in mmol/L and are means ± SD. Density ranges (d in g/mL) for the different lipoprotein fractions were: VLDL, $d<1.010$; IDL, $1.010<d<1.019$; LDL, $1.019<d<1.055$; HDL_1 plus Lp(a), $1.055<d<1.075$; HDL_2, $1.075<d<1.100$; HDL_3, $1.100<d<1.180$; and bottom fraction, $d>1.180$. To convert values for cholesterol to mg/dL, multiply by 38.64.

+ Denotes a significant difference in changes between diet groups: $P<0.05$.

Composition of HDL_2 and HDL_3

Changes in the concentrations of the main constituents of HDL_2 were similar on both diets. In contrast, concentrations of apolipoprotein A-I, apolipoprotein A-II, phospholipids, and esterified cholesterol in HDL_3 fell on the high-carbohydrate diet as compared with the olive oil diet (Table 3). Table 4 shows that the ratio of apolipoprotein A-I to phospholipids in HDL_3 decreased by 0.04 g/mmol on the carbohydrate diet and increased by 0.03 g/mmol on the olive oil diet ($P<0.05$ for the difference in changes). The ratio of triglycerides to cholesteryl esters in the HDL_3 fraction increased by 0.02 mmol/mmol in the carbohydrate group and decreased by 0.01 in the olive oil group ($P<0.05$ for the difference in changes). There was no statistically significant change in the ratio of apolipoprotein A-II to free cholesterol or to phospholipids (data not shown).

Table 3. Effects of a high-carbohydrate, high-fiber diet and an olive-oil-rich diet on the composition of HDL_2 and HDL_3 relative to levels of the same subjects when consuming a high-saturated-fat control diet.*

	HDL_2		HDL_3	
	Carbohydrate group (N=11)	Olive oil group (N=10)	Carbohydrate group (N=11)	Olive oil group (N=10)
	mg/L			
Apolipoprotein A-I				
Control period	179 ± 63	213 ± 97	669 ± 81	677 ± 57
Change	-22 ± 38	-22 ± 60	-56 ± 101	64 ± 87¥
Apolipoprotein A-II				
Control period	62 ± 15	74 ± 24	327 ± 27	349 ± 39
Change	4 ± 19	4 ± 20	-4 ± 31	42 ± 49+
Control period	179 ± 63	213 ± 97	669 ± 81	677 ± 57
	mmol/L			
Phospholipids				
Control period	0.36 ± 0.14	0.39 ± 0.16	0.71 ± 0.07	0.70 ± 0.08
Change	-0.05 ± 0.07	-0.03 ± 0.08	-0.03 ± 0.06	0.05 ± 0.08+
Free cholesterol				
Control period	0.10 ± 0.04	0.12 ± 0.05	0.13 ± 0.02	0.14 ± 0.02
Change	-0.02 ± 0.03	-0.01 ± 0.02	-0.00 ± 0.02	0.01 ± 0.02
Cholesteryl esters				
Control period	0.37 ± 0.15	0.42 ± 0.16	0.70 ± 0.07	0.73 ± 0.07
Change	-0.07 ± 0.07	-0.03 ± 0.07	-0.08 ± 0.07	0.02 ± 0.08¥
Triglycerides				
Control period	0.05 ± 0.01	0.04 ± 0.01	0.08 ± 0.02	0.08 ± 0.02
Change	-0.01 ± 0.01	-0.01 ± 0.01	0.00 ± 0.02	-0.01 ± 0.01

* Values are means ± SD. Analyses were done in pools of lipoprotein fractions of two or three subjects at a time. Density limits used were: HDL_2, $1.075<d<1.100$ g/mL; and HDL_3, $1.100<d<1.180$ g/mL. N refers to the number of pools (cf Methods).

Statistical comparison for difference in changes between diet groups: + $P<0.05$, ¥ $P<0.01$.

Table 4. Effects of a high-carbohydrate, high-fiber diet and an olive-oil-rich diet on the ratios of different constituents of HDL_2 and HDL_3 relative to ratios in the same subjects when consuming a high-saturated-fat control diet.*

	HDL_2		HDL_3	
	Carbohydrate group (N=11)	Olive oil group (N=10)	Carbohydrate group (N=11)	Olive oil group (N=10)
Apolipoprotein A-I/ apolipoprotein A-II		g/g		
Control period	2.83 ± 0.51	2.88 ± 0.89	2.05 ± 0.23	1.96 ± 0.30
Change	-0.44 ± 0.51	-0.38 ± 0.64	-0.16 ± 0.20	-0.04 ± 0.10
Apolipoprotein A-I/ free cholesterol		g/mmol		
Control period	1.78 ± 0.32	1.87 ± 0.40	5.05 ± 0.51	5.03 ± 0.58
Change	0.04 ± 0.39	0.00 ± 0.30	-0.42 ± 0.64	-0.06 ± 0.40
Apolipoprotein A-I/ phospholipids		g/mmol		
Control period	0.50 ± 0.06	0.55 ± 0.11	0.95 ± 0.04	0.97 ± 0.07
Change	-0.00 ± 0.05	-0.02 ± 0.09	-0.04 ± 0.09	0.03 ± 0.04+
Free cholesterol/ phospholipids		mmol/mmol		
Control period	0.28 ± 0.02	0.30 ± 0.04	0.19 ± 0.02	0.19 ± 0.01
Change	-0.01 ± 0.04	-0.01 ± 0.03	0.01 ± 0.02	0.01 ± 0.01
Triglycerides/ cholesteryl esters		mmol/mmol		
Control period	0.13 ± 0.03	0.11 ± 0.04	0.11 ± 0.03	0.11 ± 0.03
Change	0.01 ± 0.04	-0.02 ± 0.01	0.02 ± 0.03	-0.01 ± 0.02+
Surface components/ core components¥		g/g		
Control period	1.89 ± 0.14	1.93 ± 0.15	2.93 ± 0.13	2.90 ± 0.13
Change	0.12 ± 0.22	0.05 ± 0.16	0.15 ± 0.15	0.20 ± 0.18

* and +: see Table 3.

¥ The concentration of surface components was calculated as the sum of the concentrations (g/L) of apolipoprotein A-I and A-II, free cholesterol (MW=387), and phospholipids (MW=698) and of core components as that of the concentrations of triglycerides (MW=886), and cholesteryl esters (MW=651).

Apolipoprotein A-I, apolipoprotein A-II, and apolipoprotein B

Table 5 shows that apolipoprotein A-I levels in the d>1.075 g/mL fractions decreased by 102 mg/L on the high-carbohydrate diet and increased by 26 mg/L on the olive oil diet ($P<0.05$ for the difference in changes). Apolipoprotein A-II levels in these fractions also increased in the olive oil group, but did not change in the carbohydrate group ($P<0.05$ for the difference in changes). Serum apolipoprotein B fell on average, by 8.9 percent, in the olive oil group, and showed a slight increase in the

Table 5. Effects of a high-carbohydrate, high-fiber diet and an olive-oil-rich diet on apolipoprotein A-I, apolipoprotein A-II, and apolipoprotein B levels, and the ratio of apolipoprotein A-I to apolipoprotein B relative to levels of the same subjects when consuming a high-saturated-fat control diet.*

	Carbohydrate group (N=11)	Olive oil group (N=10)
Apolipoprotein A-I (mg/L)		
Control diet	1084 ± 153	1089 ± 126
Change+	-102 ± 111	26 ± 107
Apolipoprotein A-II (mg/L)		
Control diet	410 ± 26	443 ± 47
Change+	-1 ± 36	43 ± 56
Apolipoprotein B (mg/L)		
Control diet	942 ± 116	933 ± 162
Change	5 ± 134	-83 ± 137
Apoliprotein A-I/B ratio (mg/mg)		
Control diet	1.17 ± 0.23	1.21 ± 0.32
Change¥	-0.12 ± 0.17	0.19 ± 0.20

* Values are means ± SD. Serum concentration of apolipoprotein A-I and apolipoprotein A-II were calculated as the sum of the concentrations in the HDL_2 (1.075<d<1.100 g/mL), HDL_3 (1.100<d<1.180 g/mL), and bottom fraction (d>1.180 g/mL). N refers to the number of lipoprotein pools of each fraction (cf Methods). Apolipoprotein B was determined in the serum of each individual. These values were then used to calculate the concentrations in whole serum for each pool.

Statistical comparison for difference in changes between diet groups: + $P<0.05$, ¥ $P<0.01$.

carbohydrate group. The difference in changes between the diet groups in apolipoprotein B did not reach statistical significance. The ratio of apolipoprotein A-I to apolipoprotein B decreased by 0.12, or 10 percent, on the carbohydrate diet and increased by 0.19, or 16 percent, on the olive-oil-rich diet. This difference was highly significant ($P<0.01$ for the difference in changes). The ratio of LDL cholesterol to apolipoprotein B ratio decreased from 2.75 ± 0.51 to 2.26 ± 0.36 mmol/g in the carbohydrate group and from 2.73 ± 0.57 to 2.44 ± 0.63 in the olive oil group. The difference in changes between the diet groups was not statistically significant.

DISCUSSION

Cholesterol levels in lipoprotein fractions

In this controlled study with 48 healthy normolipidemic men and women, we have evaluated the effects of an olive-oil-rich and a high-carbohydrate, high-fiber diet on serum lipoprotein components. The major difference in composition between both diets was the levels of total and monounsaturated fat, and of carbohydrate and fiber intake. However, the two diets also differed slightly in the level of saturated fat, which might have confounded a straight comparison between monounsaturated fatty acids and complex carbohydrates.

Both diets reduced LDL cholesterol levels to the same extent. This observation agrees with the results of Grundy [4], who found similar decreases in LDL cholesterol levels when saturated fat in the diet was replaced by either high-oleic-acid safflower oil or by glucose. Extensive experiments have shown that dietary oleic acid does not affect serum total cholesterol when dietary carbohydrate is taken as the reference point [12, 13]. It now appears that this statement can be extended to effects on LDL cholesterol.

VLDL cholesterol was increased by the high-carbohydrate, high-fiber diet. This is in line with the well-known elevating effect of such diets on fasting serum triglycerides [14].

On the high-carbohydrate, high-fiber diet HDL cholesterol fell, but this did not occur on the olive-oil-rich diet. Again, this agrees with Grundy's observations [4] on high-monounsaturated safflower oil versus glucose [4].

Thus, the observed effects are probably due to dietary oleic acid rather than to some unknown factor in olive or safflower oil. The lipoprotein data suggest that neither VLDL nor HDL behave neutrally when dietary oleic acid and carbohydrates are exchanged. The reason that these changes were not detected by Keys et al [12] and Hegsted et al [13] is probably that the cholesterol content of VLDL and HDL change by similar amounts but in opposite directions.

It has been suggested that variations in HDL cholesterol are mostly located in the HDL_2 fraction [15]. This is not confirmed by our results: on the high-carbohydrate diet, cholesterol levels decreased in both HDL_2 and HDL_3, but the changes in HDL_3 were more marked. We have earlier derived an empirical formula for the relationship between HDL_2 and HDL_3 cholesterol with total HDL cholesterol when d=1.100 g/mL is taken as cut-off point between HDL_2 and HDL_3 [16]. This formula predicts that in our subjects the observed fall in HDL cholesterol on the carbohydrate diet is due to a change in HDL_2 of -0.08 and in HDL_3 of -0.07 mmol/L, which compare favorably with the observed values of -0.08 and -0.09 mmol/L, respectively. When density gradient ultracentrifugation is used, d=1.100 g/mL appears to be the proper density limit between the two main fractions of HDL [17] rather than the limit of d=1.125 g/mL used in other studies [18].

The observed changes in lipoprotein cholesterol levels were not significantly related to sex. This appears inconsistent with our previous finding that the decrease in magnesium-dextransulfate-soluble HDL cholesterol on the carbohydrate-rich diet was significantly more pronounced in men than in women [5]. However, the difference in changes in HDL_2 cholesterol between diets was -0.08 mmol/L for men and -0.02 mmol/L for women; for HDL_3 the differences in effect between the diet groups were -0.13 mmol/L for men and -0.08 mmol/L for women. This indicates that changes in HDL cholesterol were indeed more pronounced in men than in women, although not statistically significant.

We are unable to tell whether the use of oral contraceptives affected the observed changes in lipoprotein cholesterol levels, because the number of oral contraceptive users was too small to allow a meaningful comparison with non-users.

The greater decrease in HDL cholesterol for men on diet high in complex carbohydrates was counteracted by a not significantly greater rise in VLDL cholesterol.

HDL subfraction composition and the mechanism of HDL lowering

We analyzed the main components of both HDL subfractions mainly to find an explanation for the fall in HDL cholesterol induced by high-carbohydrate diets. Hopkins and Barter [19] have suggested that a rise in VLDL triglycerides, as observed on high-carbohydrate diets, is the primary cause of this fall in HDL cholesterol. The increased availability of VLDL triglycerides would increase the rate at which the cholesteryl ester transfer protein exchanges cholesteryl ester molecules from HDL for triglycerides from VLDL. The triglyceride molecules acquired in this way by HDL are subsequently hydrolyzed and removed from the circulation, which should result in a decreased diameter of the HDL particle. Our results are in partial agreement with this hypothesis in that we observed a loss of cholesteryl esters from HDL_3 on the high-carbohydrate diet. However, the concentration of other components of HDL_3 also fell, and as a result we did not see an increase in the ratio of surface to core components, which would be expected if the particles had become smaller. The simultaneous decreases in apolipoprotein A-I, apolipoprotein A-II, phospholipids, and esterified cholesterol suggest that the carbohydrate-rich diet caused a decrease in the total number of circulating HDL_3 particles, while the core of these particles became depleted in cholesterylester relative to triglycerides (Tables 2 and 3). This contrasts with the results of Chang et al [20], who reported a shift in the diameter of HDL_3 particles from 4.3 nm to 3.9 nm particles on going from normolipidemic to hypertriglyceridemic patients. It should be noted, however, that the mean increase in triglycerides in our carbohydrate group was only 0.19 mmol/L; this increase might be to small to induce the changes in HDL particle size observed in in vitro experiments [19] and in the cross-sectional study of Chang et al [20].

Apolipoproteins

A reduction in apolipoprotein A-I levels on carbohydrate-rich as compared to high-fat diets has been reported before [21] and confirmed in the present study. Apolipoprotein B levels decreased only on the olive-oil-rich diet and not on the high-carbohydrate, high-fiber diet, although the difference between the two diet groups did not reach statistical significance. There was, however, a marked and highly significant difference in the effect of diet on the ratio of apolipoprotein A-I to apolipoprotein B, with the change

in the olive-oil group being 26 percent more favorable than in the carbohydrate group. It has been suggested that this ratio is superior to any other indicator for the risk of atherosclerotic disease [7, 8].

Conclusion

The subjects who consumed a diet high in olive oil showed lipoprotein patterns associated with a lower risk of coronary heart disease than their counterparts who consumed a high-carbohydrate, high-fiber diet. It remains to be established, however, whether a high-olive-oil diet will still yield the most favorable risk profile when such a diet is consumed for a prolonged time by free-living, coronary-prone populations. Weight gain could be a complication of a high fat intake [22] and might undo the favorable effects observed in our study.

REFERENCES

1. Anderson JW, Gustafson NJ. High-carbohydrate, high fiber diet. Is it practical and effective in treating hyperlipidemia? Postgrad Med 1987; 82:40-55.
2. Jackson RL, Yates MT, McNerney CA, Kashyap ML. Diet and HDL metabolism: high carbohydrate vs. high fat diets. Adv Exp Med Biol 1987; 210:165-172.
3. Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986; 256:2835-2838.
4. Grundy SM. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. N Engl J Med 1986; 314:745-748.
5. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1987; i:122-125.
6. Grundy SM. Monounsaturated fatty acids, plasma cholesterol, and coronary heart disease. Am J Clin Nutr 1987; 45:1168-1175.
7. Avogaro P, Cazzolato G, Bittolo Bon G, Quinci GB. Are apolipoproteins better discriminators than lipids for atherosclerosis?. Lancet 1979; i:901-903.
8. De Backer G, Rosseneu M, Deslypere JP. Discriminative value of lipids and apoproteins in coronary heart disease. Atherosclerosis 1982; 42:197-203.
9. Mensink RP, Katan MB. In: Paoletti R, Kritchevsky D, Holmes D, eds. Effects of monounsaturated fatty acids versus complex carbohydrates on serum lipoprotein subfractions and apolipoproteins in healthy men and women. Drugs Affecting Lipid Metabolism. Heidelberg: Springer-Verlag, 1987:158-161.
10. Schinella M, Signori D, Lippi U. Sensitivity and specificity of a new, rapid and simple microprocedure for detection of serum chylomicrons. Clin Chim Acta 1984; 141:73-76.

11. Terpstra AHM, Woodward JH, Sanchez-Muniz FJ. Improved techniques for the separation of serum lipoproteins by density ultracentrifugation - Visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum. Anal Biochem 1981; 111:149-157.
12. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet IV. Particular saturated fatty acids in the diet. Metabolism 1965; 14:776-786.
13. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 1965; 17:281-295.
14. Mancini M, Mattock M, Rabaya E, Chait A, Lewis B. Studies of the mechanisms of carbohydrate-induced lipaemia in normal man. Atherosclerosis 1973; 17:445-454.
15. Anderson DW. H.D.L.Cholesterol: The variable components. Lancet 1978; i:819-820.
16. Demacker PNM, Baadenhuysen H, Stuyt PMJ, Van 't Laar A. Studies on the relationship between the cholesterol content in total high density lipoprotein and its subfractions, HDL2 and HDL3 in normo- and hyperlipidemic subjects. Atherosclerosis 1986; 61:225-229.
17. Demacker PNM, van Sommeren-Zondag DF, Stalenhoef AF, Stuyt PM, van 't Laar A. Ultracentrifugation in swinging-bucket and fixed-angle rotors evaluated isolation and determination of high-density lipoproteins subfractions HDL_2 and HDL_3. Clin Chem 1983; 29:656-663.
18. Brussaard JH, Katán MB, Groot PHE, Havekes LM, Hautvast JGAJ. Serum lipoproteins of healthy persons fed a low-fat diet or a polyunsaturated fat diet for three months. Atherosclerosis 1982; 42:205-219.
19. Hopkins GJ, Barter PJ. Role of triglyceride-rich lipoproteins and hepatic lipase in determining the particle size and composition of high density lipoproteins. J Lipid Res 1986; 27:1265-1277.
20. Chang LBF, Hopkins GJ, Barter PJ. Particle size distribution of high density lipoproteins as a function of plasma triglyceride concentration in human subjects. Atherosclerosis 1985; 56:61-70.
21. Schonfeld G, Weidman SW, Witztum JL, Bowen RM. Alterations in levels and interrelations of plasma apolipoproteins induced by diet. Metabolism 1976; 25:261-275.
22. Lissner L, Levitsky DA, Strupp BJ, Kalkwarf HJ, Roe DA. Dietary fat and the regulation of energy intake in human subjects. Am J Clin Nutr 1987; 46:886-892.

CHAPTER 4

Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men

Ronald P. Mensink, Martijn B. Katan

New England Journal of Medicine 1989; 321:436-441

ABSTRACT

Polyunsaturated fatty acids are thought to lower the serum cholesterol level more effectively than monounsaturated fatty acids. It is unclear whether the difference - if any - is due to lowering of the level of high-density lipoprotein (HDL) or low-density lipoprotein (LDL) cholesterol. We therefore placed 31 women and 27 men on a mixed natural diet rich in saturated fat diet (19.3 percent of their daily energy intake from saturated fat, 11.5 percent from monounsaturated fat, and 4.6 percent from polyunsaturated fat) for 17 days. For the next 36 days, they received a mixed diet with the same total fat content, but enriched with olive oil plus sunflower oil ("monounsaturated-fat diet": 12.9 percent saturated fat, 15.1 percent monounsaturated fat, and 7.9 percent polyunsaturated fat) or with sunflower oil alone ("polyunsaturated-fat diet": 12.6 percent saturated fat, 10.8 percent monounsaturated fat, and 12.7 percent polyunsaturated fat).

The serum LDL cholesterol level decreased by 17.9 percent in those on the monounsaturated-fat diet and by 12.9 percent in those on the polyunsaturated-fat diet (95 percent confidence interval for the difference between the effects of the two unsaturated-fat diets, -9.9 to 0.0 percent). In men, the HDL cholesterol level fell slightly on both unsaturated-fat diets. In women, HDL cholesterol levels did not change on either diet. The difference between the sexes in the change was significant.

We conclude that a mixed diet rich in monounsaturated fat was as effective as a diet rich in (n-6)polyunsaturated fat in lowering LDL cholesterol. Both diets lowered the level of HDL cholesterol slightly in men but not in women.

INTRODUCTION

The risk of coronary heart disease rises continuously as serum total and low-density lipoprotein (LDL) cholesterol concentrations increase and falls with increasing levels of high-density lipoprotein (HDL) cholesterol [1, 2]. It is generally accepted that a reduction in the intake of saturated fat will lower the level of LDL cholesterol, but there is disagreement over the type of nutrient that should replace it. Keys et al [3] and Hegsted et al [4] found that replacing of saturated fat with (n-6)polyunsaturated fat in the form of linoleic acid caused a larger decline in total serum cholesterol levels than monounsaturated fatty acids, carbohydrates, or protein. Health authorities in many countries therefore recommend increasing the intake of polyunsaturated fat to 10 percent of energy intake [5, 6] from the usual 4 to 8 percent. However, LDL and HDL lipoprotein fractions were not studied separately in the early trials, and it has been suggested that part of the cholesterol-lowering action of linoleic acid consists of lowering the level of HDL cholesterol [7]. Indeed, Mattson and Grundy showed that HDL cholesterol levels were reduced by regular safflower oil, which is rich in linoleic acid, as compared with safflower oil that was high in the monounsaturated fatty acid oleic acid [8]. Their experiment has been criticized because the amount of linoleic acid in the diet it studied was unrealistically high (28 percent of total energy intake). In addition, their study and other studies [9, 10] have involved mostly men, even though the HDL cholesterol level is also predictive of coronary heart disease in women [11], and the effect of diet on HDL cholesterol levels may differ in men and women [12, 13]. We therefore tested the effects of two diets on the levels of serum lipoproteins. In one, saturated fatty acids were replaced principally by monounsaturated fatty acids, and in the other by polyunsaturated fatty acids. To ensure that any biologically important differences in effects of the two diets on LDL or HDL cholesterol would be detected, both men and women were enrolled in the study, and their diets were strictly controlled.

METHODS

Design and statistical analysis

The trial had a parallel design and consisted of two consecutive periods. In the first, all participants were placed on a diet high in saturated fat (19.3 percent of total energy sources; Table 1), and base-line levels of relevant variables were determined after the serum lipids had stabilized. The subjects were then randomly allocated to one of two test diets (Table 1). One group received a diet rich in oleic acid (the "monounsaturated-fat diet"), and the other group a diet rich in linoleic acid (the "polyunsaturated-fat diet"). After another period of stabilization, the serum lipid levels were measured again. The outcome variables consisted of the changes in lipoprotein levels between the end of the base-line period (mean of days 14 and 17) and the end of the test period (mean of days 50 and 53). For the group as a whole, such changes may have been biased by seasonal effects or other drifts with time. Therefore, the absolute changes observed simultaneously in subjects on both diets may in theory have been artifacts. The study's design allowed the unbiased detection of differences only between the effects of the monounsaturated-fat and the polyunsaturated-fat diets on the outcome variables. A two-sided t-test was used to examine these differences in the changes among those following the two test diets and to examine differences in the responses of women and men within each diet group [14]. To reduce the skewness of the data, the responses of serum lipids, lipoproteins, and apolipoproteins were expressed as a percent rather than an absolute change.

Subjects

Eighty-seven women and men, most of them students, applied for enrollment in the study. One man was on a cholesterol-lowering diet, and two men and one woman did not like dairy products. These four were excluded. The remaining 83 had no history of atherosclerotic disease, and all were apparently healthy, as indicated by a medical questionnaire. None had anemia, glycosuria, proteinuria, or hypertension, and none were taking any medication known to affect serum lipids. Because the study had been designed for only 60 participants, we accepted all 28 eligible men in order to obtain a nearly equal number of men and women. We then added 3 women who were

married to participants and 29 women were selected at random. Nine women were taking oral contraceptive agents.

The protocol and the aim of the study were fully explained to the subjects, who gave their written consent. Approval for the study had been obtained from the ethics committee of the Department of Human Nutrition.

During the first week of the study, one man and one woman withdrew. Thus, data from 58 subjects were analyzed. Their fasting levels of serum lipids before the experiment ranged from 3.68 to 6.12 mmol/L (mean, 4.81 mmol/L or 186 mg/dL) for total cholesterol, from 0.76 to 2.31 mmol/L (mean, 1.34 mmol/L or 52 mg/dL) for HDL cholesterol, and from 0.24 to 3.19 mmol/L (mean, 0.99 mmol/L or 88 mg/dL) for triglycerides. One man had mild hypertriglyceridemia (3.19 mmol/L). The men were between 20 and 48 years old (mean, 25 years). They weighed between 63 and 94 kg (mean, 74 kg), and their body mass indexes ranged from 18.3 to 27.5 kg/m^2 (mean, 21.9 kg/m^2). The women were between 19 and 45 years old (mean, 24 years). They weighed between 53 and 79 kg (mean, 62 kg), and their body mass indexes ranged from 19.1 to 26.6 kg/m^2 (mean, 21.3 kg/m^2). Seven subjects smoked: one man and two women on the monounsaturated-fat diet, and two men and two women on the polyunsaturated-fat diet.

Diets

Before the study began, the participants weighed and recorded their habitual diet for two working days and one weekend day to allow us to estimate their energy and nutrient intake. The food records were coded and the composition of the diets was calculated with use of the Netherlands Nutrient Data Base [15].

The diets consumed during the study consisted of conventional solid foods, and the menus were changed daily. All the participants consumed a control diet high in saturated fat (19.3 percent of total energy intake) for 17 days. They were then divided according to sex and among the women, according to their use of oral contraceptive agents. Half of each group was randomly assigned to the monounsaturated-fat diet (actually a diet enriched with both monounsaturated and polyunsaturated fats), and the others to the polyunsaturated-fat diet. For the next 36 days (days 18 to 53), the diet was changed: 6.5 percent of the total energy intake, which had previously consisted of saturated fat, now consisted of either monounsaturated and (n-6)polyunsaturated fats together or (n-6)polyunsaturated fat alone (Table

1). The monounsaturated-fat diet was enriched with olive oil and the polyunsaturated-fat diet with sunflower oil. In addition, subjects on both diets used a margarine high in linoleic acid. The diets were formulated at 23 levels of energy intake ranging from 5.5 to 16.5 MJ (1310 to 3950 kcal) per day. All the bread for the monounsaturated-fat group and some for the polyunsaturated-fat group contained 8 g of olive oil per 100 g. In the monounsaturated-fat group, olive oil alone contributed 40 percent of the monounsaturated fatty acids; the other 60 percent was provided by the rest of the diet. The intake of other nutrients was kept meticulously constant throughout the study.

All foodstuffs were supplied to the participant individually, as described [12, 16]. However, the participants were allowed to choose a limited number of items, free of fat and cholesterol, which provided 9 to 10 percent of their total daily energy intake. They were urged not to change their selection of these free-choice items between study periods.

The participants were asked to maintain their usual patterns of activity, their smoking habits, and their use of oral contraceptive agents. They recorded in diaries any signs of illness, medications used, the free-choice items chosen, and any deviations from their diet.

Duplicate portions of each diet for an imaginary participant with a daily energy intake of 10 MJ (2390 kcal) were collected, pooled according to diet, and analyzed. The values thus obtained were combined with the values for the free-choice items (Table 1).

Body weights without heavy clothing were recorded twice a week, and energy intake was adjusted when necessary. Over the 53 days of the study, average body weight increased by 0.2 $\pm$ 1.3 kg (range, -3.2 to 2.4 kg) in the monounsaturated-fat group and by 0.2 $\pm$ 1.0 kg (range, -1.8 to 2.2 kg) in the polyunsaturated-fat group. Pearson's correlation coefficients, calculated for each diet group separately, for the relation between changes in weight and changes in all relevant variables ranged from -0.26 (P=0.165) for apolipoprotein B in the polyunsaturated-fat group to 0.30 (P=0.119) for total cholesterol in the monounsaturated-fat group.

Blood sampling and analysis

Venous blood was sampled after an overnight fast on days 1, 14 and 17 (control period), and on days 23, 30, 44, 50 and 53 (test period). Serum was obtained by low-speed centrifugation within one hour of venipuncture, stored

at -80 °C, and analyzed enzymatically at the end of the study to determine the levels of total and HDL cholesterol and triglycerides [17-19]. Before the study began all the subjects were assigned a random number that was then used for labeling their blood and serum tubes. In this way, the technicians who performed the analyses were blinded as to the subjects' diets. All the samples from each subject were analyzed within a single run. The coefficient of variation of control serum samples within one run was 1.4 percent for total cholesterol, 2.5 percent for HDL cholesterol, and 1.9 percent for triglycerides. Accuracy was checked by the analysis of four serum pools of known value provided by the U.S. Centers for Disease Control and, for HDL cholesterol only, of three pools produced by the North-West Lipid Research Clinic [20]. The mean bias with regard to target values of the Centers for Disease Control was 0.01 mmol/L for total cholesterol, -0.07 mmol/L for HDL cholesterol, and -0.05 mmol/L for total triglycerides. Bias with regard to the North-West Lipid Research Clinic's target value for HDL cholesterol was -0.02 mmol/L. The LDL cholesterol concentration was calculated using the Friedewald equation [21].

For each subject, the fatty-acid composition of serum cholesteryl esters was determined in a pool of two serum samples obtained at the end of the control period (days 14 and 17) and in another pool of two samples obtained at the end of the test period (days 50 and 53), as described earlier [22]. Results were expressed as a proportion by weight of all fatty acids detected. The same pooling procedure was used for apolipoprotein analysis. Apolipoprotein A-I was measured in duplicate and apolipoprotein B in triplicate in whole serum, as described [16]. For each subject, samples from the control and test period were analyzed on the same plate. The coefficient of variation of control serum samples within one run was 3.2 percent for apolipoprotein A-I and 4.5 percent for apolipoprotein B.

RESULTS

The total fat and cholesterol intake of the participants did not change during the study (Table 1). Our previous experience had suggested that the actual amount of energy needed to maintain body weight would exceed the amount reported before the experiment by about 15 percent. Therefore, the amount of energy provided at the beginning of the study was already 15 percent higher than the energy intake the participants reported as typical.

Table 1. Mean daily intake of energy and composition of the diets.*

	Type of diet			
	Habitual* (N=58)	Control+ (N=58)	Mono-unsaturated-fat+ (N=29)	Poly-unsaturated-fat+ (N=29)
Energy				
MJ/day	10.1 ± 2.5	11.7	11.8	11.9
kcal/day	2423 ± 602	2796	2820	2844
Protein (% of energy)	14.3 ± 1.8	13.1	13.4	13.1
Fat (% of energy)	35.8 ± 5.6	36.7	37.4	37.6
Saturated fatty acids	14.6 ± 2.9	19.3	12.9	12.6
Lauric acid (C12:0)		1.2	0.7	0.9
Myristic acid (C14:0)		3.2	1.4	1.3
Palmitic acid (C16:0)		9.3	6.8	6.2
Stearic acid (C18:0)		4.1	3.2	3.4
Monounsaturated fatty acids	12.4 ± 2.2	11.5	15.1	10.8
Polyunsaturated fatty acids	6.3 ± 1.9	4.6	7.9	12.7
Linoleic acid	5.5 ± 1.8	4.1	7.7	12.6
P/S ratio¥	0.45 ± 0.2	0.21	0.61	1.00
Carbohydrates (% of energy)	48.6 ± 6.0	49.1	47.8	48.5
Alcohol (% of total energy)§	1.7 ± 2.1	1.2	1.5	1.0
Cholesterol (mg/MJ)¶	31.4 ± 12.6	33.4	35.8	35.3
Dietary fiber (g/MJ)¶	3.1 ± 0.9	4.0	4.1	4.1

* Values were calculated from three-day weighed inventories before the experiment began, and they are means ± SD. No reliable figures for saturated fatty acids with chain lengths of 12, 14, 16, or 18 carbon atoms were available in the food composition table used.

\+ Based on chemical analysis of duplicate diets. The standard deviations for energy intake between subjects during the study were 2.7 MJ for the control diet, 2.8 MJ for the monounsaturated-fat diet, and 2.3 MJ for the polyunsaturated-fat diet. Analysis of ancillary duplicate diets providing 6.5, 10 and 15 MJ per day that were collected during one week of the study showed that the variation between subjects in the composition of the study diets was negligible; therefore, no standard deviations are given for the nutrients.

¥ Ratio of polyunsaturated to saturated fatty acids.

§ Value represents 1.3 percent for the monounsaturated-fat and 1.1 percent for the polyunsaturated-fat diet group.

¶ To convert values for the intake of cholesterol to milligrams and dietary fiber to grams per 1,000 kcal, multiply by 4.184.

Adherence to the diets was confirmed by a lower proportion of oleic and a higher proportion of linoleic acid in the serum cholesteryl esters of those on the polyunsaturated-fat diet than of those on the monounsaturated-fat diet (Table 2). The ratio of oleic (C18:1(n-9)) to linoleic (C18:2(n-6)) acid in the serum cholesteryl esters (mean ± SD) decreased from 0.33 ± 0.04 to 0.29 ± 0.05 in the monounsaturated-fat group and from 0.33 ± 0.04 to 0.21 ± 0.03 in the polyunsaturated-fat group.

Table 2. Changes in the fatty-acid composition of serum cholesteryl esters on the monounsaturated-fat and polyunsaturated-fat diet relative to the baseline control diet high in saturated fatty acids.*

	Monounsaturated-fat group (N=29)			Polyunsaturated-fat group (N=29)		
	Control	Test	Change	Control	Test	Change
	gram per 100 gram fatty acids					
C14:0	1.2 ± 0.3	0.7 ± 0.2	−0.5 ± 0.3	1.1 ± 0.4	0.6 ± 0.1	−0.5 ± 0.3
C16:0	10.9 ± 0.6	10.0 ± 0.8	−0.8 ± 0.7	10.7 ± 0.7	9.7 ± 0.6	−1.1 ± 1.0
C16:1(n-7)	4.1 ± 0.6	2.7 ± 0.6	−1.4 ± 0.7	4.0 ± 0.5	2.4 ± 0.4	−1.6 ± 0.6
C18:0	0.8 ± 0.2	0.7 ± 0.1	−0.1 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	−0.1 ± 0.2
C18:1(n-9)	17.9 ± 1.2	17.2 ± 2.0	−0.8 ± 1.9+	17.9 ± 1.2	13.3 ± 1.6	−4.6 ± 1.6
C18:2(n-6)	54.8 ± 2.5	59.0 ± 3.6	4.2 ± 3.3+	54.6 ± 2.9	63.4 ± 2.7	8.7 ± 3.3
C18:3(n-3)	0.8 ± 0.2	0.4 ± 0.2	−0.4 ± 0.3	0.9 ± 0.2	0.4 ± 0.1	−0.5 ± 0.2
C18:3(n-6)	0.7 ± 0.4	0.6 ± 0.3	−0.1 ± 0.4	0.8 ± 0.4	0.7 ± 0.3	−0.2 ± 0.5
C20:4(n-6)	5.9 ± 0.9	6.5 ± 1.2	0.6 ± 1.0	6.2 ± 1.1	6.7 ± 1.3	0.5 ± 1.3
Others	2.8 ± 0.5	2.2 ± 0.6	−0.6 ± 0.7	3.0 ± 0.9	2.2 ± 0.5	−0.8 ± 0.7

* Values are means ± SD.
\+ Denotes a significant difference in changes between diet groups ($P<0.0001$).

The courses of the serum lipid values over time are shown in Figure 1, and the values at the end of each period are shown in Table 3. The total serum cholesterol levels fell by 14.1 percent (0.72 mmol/L or 28 mg/dL) in those on the monounsaturated-fat diet, which was more than the decrease of 9.7 percent (0.51 mmol/L or 20 mg/dL) in those on the polyunsaturated-fat diet (95 percent confidence interval for the difference in percent changes between the diet groups, -8.6 to -0.3 percent). The LDL cholesterol level

fell by 17.9 percent (0.59 mmol/L or 23 mg/dL) in those on the monounsaturated-fat diet and by 12.9 percent (0.45 mmol/L or 17 mg/dL) in those on the polyunsaturated-fat diet (95 percent confidence interval for the difference, -9.9 to 0.0 percent). The changes in the level of LDL cholesterol were similar in women and men. Because total and LDL cholesterol

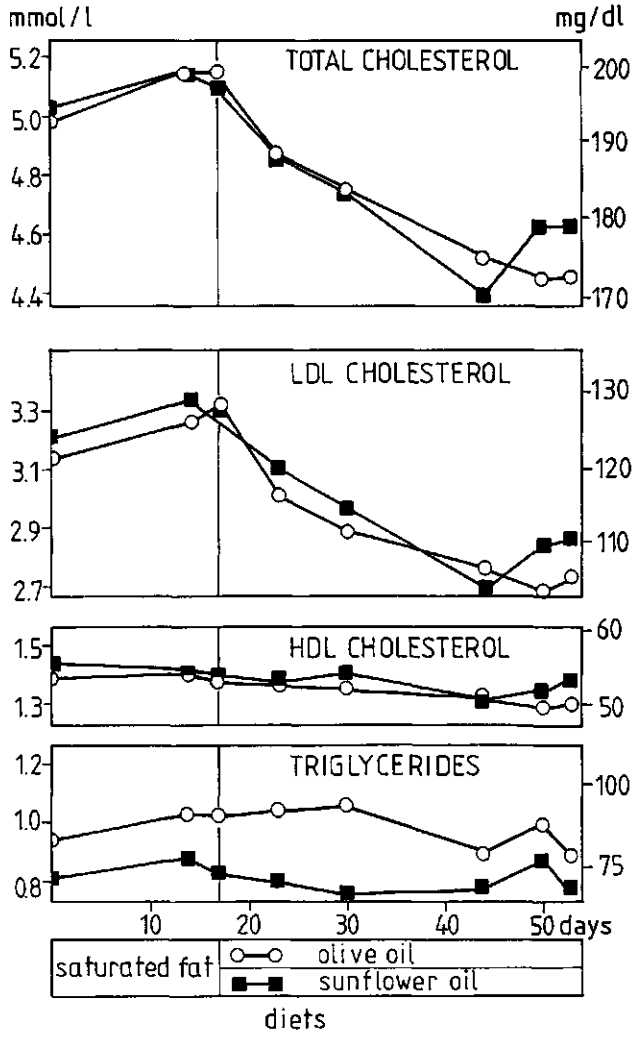

Figure 1. Mean serum concentrations of total, LDL and HDL cholesterol and of serum triglycerides throughout the experiment.

All 58 subjects first received a diet high in saturated fatty acids for 17 days. For the next 36 days 29 subjects received a diet enriched in both monounsaturated and (n-6)polyunsaturated fatty acids and 27 subjects a diet enriched in polyunsaturated fatty acids alone.

Table 3. Effects of the monounsaturated-fat and the polyunsaturated-fat diets on serum lipids and lipoproteins levels.*

	Men		Women		All	
	Mono-unsaturated-fat diet (N=14)	Poly-unsaturated-fat diet (N=13)	Mono-unsaturated-fat diet (N=15)	Poly-unsaturated-fat diet (N=16)	Mono-unsaturated-fat diet (N=29)	Poly-unsaturated-fat diet (N=29)
Total cholesterol (mmol/L)						
Control period	4.78 ± 1.01	5.27 ± 0.74	5.47 ± 0.59	4.99 ± 0.72	5.14 ± 0.88	5.11 ± 0.73
Test period	4.03 ± 0.95	4.59 ± 0.75	4.78 ± 0.67	4.61 ± 0.62	4.42 ± 0.89	4.60 ± 0.67
Percent change	-15.8 ± 7.9	-12.8 ± 8.1	-12.6 ± 6.7	-7.1 ± 7.8	-14.1 ± 7.4	-9.7 ± 8.3
95% Confidence interval	-9.4 to 3.3		-10.8 to 0.0+		-8.6 to -0.3+	
LDL cholesterol (mmol/L)						
Control period	3.05 ± 0.79	3.62 ± 0.68	3.53 ± 0.65	3.09 ± 0.61	3.30 ± 0.75	3.33 ± 0.68
Test period	2.52 ± 0.73	3.06 ± 0.66	2.89 ± 0.58	2.73 ± 0.52	2.71 ± 0.67	2.87 ± 0.60
Percent change	-17.7 ± 8.1	-15.3 ± 9.1	-18.1 ± 7.6	-11.0 ± 12.1	-17.9 ± 7.7	-12.9 ± 10.9
95% Confidence interval	-9.2 to 4.4		-14.6 to 0.4		-9.9 to 0.0+	
HDL cholesterol (mmol/L)						
Control period	1.25 ± 0.30	1.27 ± 0.24	1.51 ± 0.29	1.50 ± 0.37	1.38 ± 0.32	1.41 ± 0.34
Test period	1.08 ± 0.25	1.15 ± 0.25	1.47 ± 0.27	1.52 ± 0.40	1.28 ± 0.32	1.35 ± 0.38
Percent change	-12.6 ± 14.5¥	-9.0 ± 12.3¥	-1.5 ± 11.1	1.2 ± 7.0	-6.9 ± 13.8	-3.4 ± 10.9
95% Confidence interval	-14.3 to 7.1		-9.5 to 4.1		-10.0 to 3.0	
HDL/LDL cholesterol ratio						
Control period	0.43 ± 0.15	0.37 ± 0.11	0.45 ± 0.14	0.50 ± 0.14	0.44 ± 0.15	0.44 ± 0.14
Test period	0.46 ± 0.15	0.40 ± 0.13	0.53 ± 0.15	0.58 ± 0.19	0.49 ± 0.15	0.50 ± 0.19
Percent change	6.3 ± 14.9¥	7.7 ± 11.1	21.2 ± 17.9	15.4 ± 15.7	14.0 ± 17.9	11.9 ± 14.2
95% Confidence interval	-11.9 to 9.1		-6.6 to 18.1		-6.4 to 10.5	
Triglycerides (mmol/L)						
Control period	1.05 ± 0.64	0.84 ± 0.42	0.94 ± 0.28	0.87 ± 0.24	0.99 ± 0.48	0.86 ± 0.33
Test period	0.95 ± 0.52	0.84 ± 0.53	0.93 ± 0.36	0.80 ± 0.21	0.94 ± 0.43	0.82 ± 0.38
Percent change	-2.5 ± 25.2	-2.8 ± 16.9	1.9 ± 39.3	-5.5 ± 20.2	-0.2 ± 32.7	-4.3 ± 18.5
95% Confidence interval	-16.9 to 17.4		-15.3 to 30.1		-9.9 to 18.0	

* Values are means ± SD. Because of the skewness of the data the difference between the means of the control (days 14 and 17) and test period (days 50 and 53) does not equal the mean of the percent change. To convert values for total, HDL, and LDL cholesterol to mg/DL, multiply by 38.67. To convert values for triglycerides to mg/dL, multiply by 88.54.

+ Denotes a significant difference in changes between diet groups ($P<0.05$).

¥ Denotes a significant difference in changes between women and men within a diet group ($P<0.05$).

levels in the participants on the polyunsaturated-fat diet dipped on day 44 of the study, we also calculated the response to the test diets as the change between the end of the control period and day 44. The 95 percent confidence interval for the difference in percent changes between the monounsaturated-fat and polyunsaturated-fat diets was then -3.9 to 7.5 percent for total cholesterol and -4.5 to 9.1 percent for LDL cholesterol levels. Thus, even the deliberate selection of the most favorable data point did not make (n-6)polyunsaturated fats more efficacious than monounsaturated fats in lowering cholesterol levels.

During the test period, the level of HDL cholesterol fell in the men on both diets, but it remained virtually unchanged in the women. The difference between the sexes in the change was significant for both the monounsaturated-fat and the polyunsaturated-fat group ($P<0.05$).

The diets' effects on the levels of triglycerides were slight and did not differ between the monounsaturated-fat and the polyunsaturated-fat groups (Table 3). The ratio of HDL to LDL cholesterol increased by 14.0 percent in the subjects on the monounsaturated-fat diet and by 11.9 percent in those on the polyunsaturated-fat diet.

As with the LDL cholesterol level, the decline (13.6 percent) in the serum apolipoprotein B level in those on the monounsaturated-fat diet was greater than the decline (7.4 percent) in those on the polyunsaturated-fat diet (95 percent confidence interval for difference, -11.1 to -1.2 percent; Table 4). The changes in the level of apolipoprotein A-I paralleled those in the level of HDL cholesterol. The ratio of apolipoprotein A-I to apolipoprotein B increased by 11.2 percent in subjects on the monounsaturated-fat diet and by 4.4 percent in those on the polyunsaturated-fat diet.

In the final two weeks of the study, two men and one woman following the monounsaturated-fat diet and two men following the polyunsaturated-fat diet reported that they had an influenza-like illness, which lasted from one to three days and was accompanied by a lack of appetite. In these subjects, the level of HDL cholesterol had fallen between the end of the baseline and the end of the test period by 27.9 percent (range, 23.2 to 39.8 percent) and the level of LDL cholesterol by 24.8 percent (range, 11.1 to 32.1 percent). When these subjects were excluded from the analyses, the 95 percent confidence interval for the difference in percent changes between the monounsaturated-fat and polyunsaturated-fat groups was -8.3 to -0.5 percent ($N=53$) for total cholesterol, -10.6 to -0.6 percent for LDL cholesterol, and -11.6 to -0.9 percent for apolipoprotein B. The mean decrease in the HDL cholesterol level

Table 4. Effects of the monounsaturated-fat and the polyunsaturated-fat diets on serum apolipoprotein A-I and apolipoprotein B levels.*

	Men		Women		All	
	Mono-unsaturated-fat diet (N=14)	Poly-unsaturated-fat diet (N=13)	Mono-unsaturated-fat diet (N=15)	Poly-unsaturated-fat diet (N=16)	Mono-unsaturated-fat diet (N=29)	Poly-unsaturated-fat diet (N=29)
Apolipoprotein A-I (mg/L)						
Control period	1247 ± 144	1240 ± 121	1329 ± 144	1389 ± 202	1289 ± 147	1322 ± 184
Test period	1090 ± 109	1127 ± 139	1343 ± 119	1379 ± 211	1221 ± 171	1266 ± 220
Percent change	-11.8 ± 10.1¥	-8.9 ± 8.6¥	1.5 ± 7.2	-0.6 ± 6.6	-4.9 ± 10.9	-4.3 ± 8.5
95% Confidence interval	-10.4 to 4.5		-3.0 to 7.1		-5.8 to 4.5	
Apolipoprotein B (mg/L)						
Control period	1010 ± 248	1087 ± 217	1081 ± 153	1001 ± 147	1047 ± 204	1040 ± 183
Test period	872 ± 253	1002 ± 226	942 ± 171	927 ± 157	908 ± 214	960 ± 191
Percent change	-14.3 ± 8.0	-7.8 ± 7.4	-12.9 ± 9.4	-7.0 ± 12.3	-13.6 ± 8.6	-7.4 ± 10.2
95% Confidence interval	-12.6 to -0.4+		-13.9 to 2.2		-11.1 to -1.2+	
Apo A-I/apo B						
Control period	1.31 ± 0.36	1.19 ± 0.31	1.26 ± 0.26	1.42 ± 0.29	1.28 ± 0.31	1.32 ± 0.31
Test period	1.35 ± 0.43	1.19 ± 0.37	1.47 ± 0.29	1.53 ± 0.35	1.41 ± 0.36	1.38 ± 0.39
Percent change	3.8 ± 16.9¥	-0.7 ± 11.7	18.0 ± 16.9	8.6 ± 15.1	11.2 ± 18.1	4.4 ± 14.3
95% Confidence interval	-7.0 to 16.2		-2.4 to 21.1		-1.8 to 15.3	

* Values are means ± SD. Because of the skewness of the data the difference between the means of the control (days 14 and 17) and test period (days 50 and 53) does not equal the mean of the percent change.

\+ Denotes a significant difference in changes between women and men within a diet group ($P<0.05$).

¥ Denotes a significant difference in changes between diet groups ($P<0.05$).

was then 9.3 percent in the 12 men on the monounsaturated-fat diet and 6.0 percent in the 11 men on the polyunsaturated-fat diet.

DISCUSSION

In this controlled study with 58 volunteers, we did not find that linoleic acid was superior to oleic acid in lowering levels of cholesterol. The amount of polyunsaturated fat in our monounsaturated-fat diet (7.9 percent of total energy intake) represents approximately the upper limit of what is currently consumed by most people. The amount in the polyunsaturated-fat diet (12.7 percent) would represent a substantial increase.

Keys et al have concluded from a large series of studies in middle-aged men that polyunsaturated fatty acids are more effective in decreasing serum cholesterol levels than either monounsaturated fatty acids or carbohydrates [3]. Their findings, as summarized in Keys' formula [3], predict that in our study the decrease in the serum total cholesterol level in subjects on the polyunsaturated-fat diet should have exceeded that in subjects on the monounsaturated-fat diet by 0.18 mmol/L (7 mg/dL). Hegsted and coworkers summarized the results of their experiments in another formula [4], which predicts a difference in change between the two diets of 0.23 mmol/L (9 mg/dL). Our results do not support these conclusions; at the end of our study the decrease in serum total and LDL cholesterol levels was greater in those on the monounsaturated-fat diet than in those on the polyunsaturated-fat diet. Changes in the level of apolipoprotein B paralleled those in the level of LDL cholesterol. The original data of Hegsted et al [4] include one experiment in which olive oil and safflower oil produced the same total cholesterol level. More recently, Mattson and Grundy showed that in normotriglyceridemic patients oleic acid and linoleic acid were equally effective in reducing the LDL cholesterol level [8]. However, the fatty-acid composition of the liquid-formula diets used by Mattson and Grundy [8] was very extreme: the ratio of polyunsaturated to saturated fat was as high as 2.1 in the diet rich in monounsaturated fat and 6.5 in the diet rich in polyunsaturated fat. We have shown that their conclusion is also valid for mixed solid diets with a more realistic composition of fatty acids.

The polyunsaturated-fat diet did not reduce the levels of HDL cholesterol or apolipoprotein A-I more effectively than the monounsaturated-fat diet. This finding is in contrast with the results of Mattson and Grundy [8], but

it agrees with a large body of experimental evidence [23-25] suggesting that different classes of fatty acids have comparable effects on the level of HDL cholesterol. In the studies that demonstrated a decline in the HDL cholesterol level, the intake of polyunsaturated fat was very high and the ratio of polyunsaturated to saturated fat ranged from 2.0 to 6.5 [8, 26-28]. In addition, some of the studies involved other changes in the diet, such as an increase in carbohydrate, a decrease in cholesterol intake or both - all of which are known to lower the HDL cholesterol level [29-31]. We therefore conclude that increasing the intake of (n-6)polyunsaturated fatty acids, as compared with oleic acid, in the quantities recommended by some health agencies does not have an unfavorable effect on HDL cholesterol levels.

Previous studies of dietary fatty acids and lipoproteins have been limited largely to males. We studied both women and men because coronary heart disease is not exclusively a disease of men. We found no evidence that fluctuations in lipid levels during the menstrual cycle made women less suitable subjects. In our study, the systematic effects of the menstrual phase on serum lipid levels tended to be offset by the fact that each woman was in a different phase of the cycle at any given stage of the experiment. Also, in spite of the menstrual cycle, lipid levels varied no more in the women than in the men: the standard deviations of the mean responses observed in the women were similar to those in the men (Tables 3 and 4).

When saturated fat was removed from the diet, the levels of HDL cholesterol and apolipoprotein A-I decreased in the men as compared with the women. This effect was observed in those following both test diets. In the 23 men who were free of intercurrent illness, the absolute decline in both diet groups was between 0.07 and 0.11 mmol/L (3 and 4 mg/dL). Such a change may have been caused in part by factors outside the experiment; we cannot conclude that it was specifically due to the removal of saturated fat from the diet. However, the difference in the change between men and women cannot be explained by base-line drift. We have earlier observed that the effects of diet on HDL levels may be sex-specific [12], and this reinforces the need for more dietary studies in women.

Although we compared olive oil and sunflower oil, there is no reason to limit our conclusions to these sources of fat. The triglycerides of a limited number of fatty acids make up about 99 percent of the weight of most vegetable oils, and in the amounts consumed in our experiment the minor components of olive and other oils do not have major effects on serum lipid levels [32].

Our findings suggest that as far as lipoprotein levels are concerned, it is immaterial whether saturated fatty acids are replaced by a mixture of monounsaturated and polyunsaturated fats or by polyunsaturated fats alone; both diets will lower the level of LDL cholesterol, and both will have the same effect on the HDL cholesterol level, as long as extremely large amounts of polyunsaturated fats (i.e., more than 13 percent of total energy intake) are avoided. We must note, however, that our conclusion that polyunsaturated fats are not superior to monounsaturated fats in lowering total cholesterol levels disagrees with the outcomes of many trials conducted in the 1950s and 1960s by various investigators [3, 4]. The findings of more recent studies, though suggestive, are not yet sufficient to reject the earlier data.

REFERENCES

1. Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361 662 men. Lancet 1986; 2:933-936.
2. Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousdian S, Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA 1986; 256:2835-2838.
3. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet IV. Particular saturated fatty acids in the diet. Metabolism 1965; 14:776-786.
4. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 1965; 17:281-295.
5. Consensus Conference. Lowering blood cholesterol to prevent heart disease. J Am Med Ass 1985; 253:2080-2088.
6. Erkelens DW. Cholesterol consensus in the Netherlands. Eur J Clin Nutr 1989; 43:89-96.
7. Katan MB. Diet and HDL. In: Miller GJ, Miller NE, eds. Metabolic aspects of cardiovascular disease. Vol 3 - Clinical and metabolic aspects of high-density lipoproteins. Oxford: Elsevier, 1984:103-132.
8. Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 1985; 26:194-202.
9. Becker N, Illingworth DR, Alaupovic P, Connor WE, Sundberg EE. Effects of saturated, monounsaturated, and w-6 polyunsaturated fatty acids on plasma lipids, lipoproteins, and apoproteins in humans. Am J Clin Nutr 1983; 37:355-360.
10. Grundy SM, Nix D, Whelan MF, Franklin L. Comparison of three cholesterol lowering diets in normolipidemic men. J Am Med Ass 1986; 256:2351-2355.
11. Abbott RD, Wilson PWF, Kannel WB, Castelli WP. High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction. The Framingham Study. Arteriosclerosis 1988; 8:207-211.
12. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1987; i:122-125.

13. Crouse III JR. Gender, lipoproteins, diet and cardiovascular risk. Lancet 1989; i:318-320.
14. Snedecor GW, Cochran WG,. Statistical methods. 7th ed. Ames: Iowa State University Press, 1980.
15. Commissie UCV. UCV Tabel. The Hague: Voorlichtingsbureau voor de Voeding, 1985.
16. Mensink RP, de Groot MJM, van den Broeke LT, Severijnen-Nobels AP, Demacker PNM, Katan MB. Effects of monounsaturated fatty acids versus complex carbohydrates on serum lipoproteins and apoproteins in healthy men and women. Metabolism 1989; 38:172-178.
17. Röschlau P, Bernt E, Gruber W. Enzymatische bestimmung des gesamt-cholesterins im serum. Z Klin Chem Klin Biochem 1974; 12:403-407.
18. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982; 28:1379-1388.
19. Sullivan DR, Kruijswijk Z, West CE, Kohlmeier M, Katan MB. Determination of serum triglycerides by an enzymatic method not affected by free glycerol. Clin Chem 1985; 31:1227-1228.
20. Warnick GR, Clapshaw P. Availibility of plasma with target values for certain lipids. Clin Chem 1987; 33:2323-2324.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifugation. Clin Chem 1972; 18:499-502.
22. Glatz JFC, Soffers AEMF, Katan MB. Comparison of the fatty acid composition of serum cholesteryl esters and of erythrocyte membranes as indicators of linoleic acid intake in man. Am J Clin Nutr 1989; 49:269-276.
23. Brussaard JH, Dallinga-Thie G, Groot PHE, Katan MB. Effects of amount and type of dietary fat on serum lipids, lipoproteins and apolipoproteins in man. A controlled 8-week trial. Atherosclerosis 1980; 36:515-527.
24. Schonfeld G, Patsch W, Rudel LL, Nelson C, Epstein M, Olson RE. Effects of dietary cholesterol and fatty acids on plasma lipoproteins. J Clin Invest 1982; 69:1072-1080.
25. Weisweiler P, Janetschek P, Schwandt P. Influence of polyunsaturated fats and fat restriction on serum lipoproteins in human. Metabolism 1985; 34:83-87.
26. Vega GL, Groszek E, Wolf R, Grundy SM. Influence of polyunsaturated fats on composition of plasma lipoproteins and apolipoproteins. J Lipid Res 1982; 23:811-822.
27. Schaefer EJ, Levy RI, Ernst ND, Van Sant FD, Brewer Jr HB. The effects of low cholesterol, high polyunsaturated fat, and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. Am J Clin Nutr 1981; 34:1758-1763.
28. Shepherd J, Packard CJ, Grundy SM, Yeshurun D, Gotto Jr AM, Taunton OD. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. J Lipid Res 1980; 21:91-99.
29. Ernst N, Fisher M, Bowen P, Schaefer EJ, Levy RI. Changes in plasma lipids and lipoproteins after a modified fat diet. Lancet 1980; ii:111-113.
30. Lewis B, Hammett F, Katan M, et al. Towards an improved lipid-lowering diet: additive effects of changes in nutrient intake. Lancet 1981; ii:1310-1313.
31. Tan MH, Dickinson MA, Albers JJ, Havel RJ, Cheung MC, Vigne JL. The effect of a high cholesterol and saturated fat diet on serum high-density lipoprotein-cholesterol, apoprotein A-I, and apoprotein E levels in normolipidemic humans. Am J Clin Nutr 1980; 33:2559-2565.

32. Miettinen TA. Dietary squalene related to cholesterol and lipoprotein metabolism. In: Fidge NH, Nestel PJ, eds. Atherosclerosis VII. Amsterdam: Elsevier,1986: 671-674.

CHAPTER 5

Effect of dietary trans fatty acids on levels of high-density and low-density lipoprotein cholesterol in healthy women and men

Ronald P. Mensink, Martijn B. Katan

Submitted for publication

ABSTRACT

Trans fatty acids are consumed in large amounts as hydrogenated oils and fats, but their effects on lipoprotein levels are unknown. Thirty-four women and 25 men therefore consumed three mixed natural diets of identical nutrient composition, except for 10 percent of their daily energy intake, which was provided as either oleic acid, trans isomers of oleic acid, or saturated fatty acids, mainly palmitate. The three diets were each consumed for three weeks, in random order.

On the trans-fatty-acid diet subjects' high-density lipoprotein (HDL) cholesterol levels fell by 0.17 mmol/L or 7 mg/dL compared with values on the diet high in oleic acid ($P<0.017$; 95 percent confidence interval, 0.13 to 0.20 mmol/L). HDL cholesterol on the saturated-fat diet was the same as on the oleic-acid diet. Low-density lipoprotein cholesterol increased by 0.37 mmol/L or 14 mg/dL on the trans-fatty-acid diet ($P<0.017$; 95 percent confidence interval for the difference with the oleic acid diet, 0.28 to 0.45 mmol/L) and by 0.47 mmol/L or 18 mg/dL on the saturated-fat diet ($P<0.017$; 95 percent confidence interval, 0.39 to 0.55 mmol/L). The effects of the diets on lipoprotein levels did not differ between women and men.

We conclude that the effect of trans fatty acids on the serum lipoprotein profile is at least as unfavorable as that of the cholesterol-raising saturated fatty acids.

INTRODUCTION

Recent studies have emphasized the importance of monounsaturated fatty acids in the diet for lowering the level of the atherogenic low-density lipoprotein (LDL) cholesterol [1, 2]. These studies have focused on oleic

acid, a monounsaturated fatty acid with the cis configuration. However, many foods als contain trans fatty acids. These are unsaturated fatty acids in which the carbon moieties on the two sides of a double bond point in opposite directions. Most natural fats and oils contain only cis double bonds, in which the carbon moieties lie at the same side (Figure 1). Trans

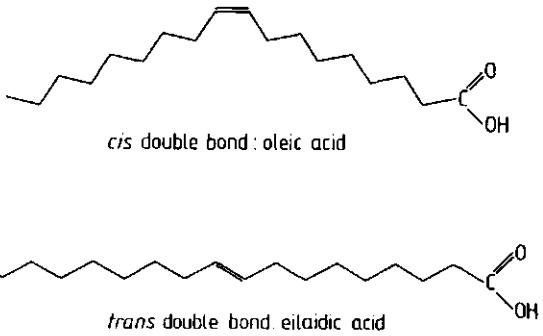

Figure 1. Configuration of cis and trans fatty acids.

Cis double bonds produce a bend in the molecule which impairs crystallization and keeps the oil liquid. In order to convert vegetable and marine oils into fats of various plasticities, edible fat manufacturers straighten out the cis unsaturated fatty acids by converting them to trans isomers or saturated fatty acids, in a process called hydrogenation.

fatty acids are found in small amounts in ruminant fats: milk fat has from 4 to 8 percent of its double bonds in the trans configuration [3]. Much larger amounts are found in certain types of margarines, margarine-based products, shortenings, and frying fats [4, 5]. These trans fatty acids are formed when vegetable and marine oils, rich in polyunsaturated fatty acids, are hardened by a process called hydrogenation to produce fats that have the firmness and plasticity desired by food manufacturers and consumers [6]. The average intake of trans fatty acids is estimated to be 8 to 10 g per day or 6 to 8 percent of total daily fat consumption in the USA [7], and 17 g per day in the Netherlands [8]. Intakes may be much higher in subjects eating large amounts of hard brick-type fats and margarines, or foods prepared with or fried in such fats. The pressure to reduce the use of saturated tropical fats like palm oil might cause an increase in the consumption of trans fatty acids, because for the edible fat industry they are the the most viable alternative to saturated fatty acids for the production of semi-solid and solid fats.

The most abundant species of trans fatty acids in the diet are elaidic acid and its isomers [7], which are fatty acids with eighteen carbon atoms and one double bond (Figure 1). Two studies suggested that that these trans fatty acids elevate serum cholesterol levels relative to their cis isomer oleic acid [9, 10]. However, this effect was not confirmed in a third study [11]. Effects on LDL and HDL cholesterol levels have never been reported. We have therefore studied the effects of trans fatty acids - specifically, elaidic acid and its isomers - on serum lipoproteins and apoproteins in healthy women and men.

METHODS

Subjects

Fourty-eight women and 27 men, most of them students, applied for the study. None of them had a history of atherosclerotic disease, and all were apparently healthy, as indicated by a medical questionnaire. None suffered from anemia, glycosuria or proteinuria. One woman was being treated for hypertension and took a beta-adrenergic blocking agent; none of the other subjects received medication known to affect serum lipids. The protocol and aim of the study were fully explained to the subjects, who gave their written consent. No reward was given, except for the free food. Prior approval for the study was obtained from the ethics committee of the Department of Human Nutrition.

As only 62 subjects could participate, we accepted all 27 men. We then added one woman married to a participant, and selected another 34 women by chance. This included the hypertensive woman; she did not change the dosage of her medicine throughout the study. All men were accepted in order to balance numbers of men and women as much as possible. Before the start of the study one woman and two men withdrew. Thus, 25 men and 34 women started out on the study, and all finished it successfully. Pre-experimental fasting serum lipids ranged from 3.40 to 7.15 mmol/L (mean, 4.75 mmol/L or 184 mg/dL) for total cholesterol, from 0.63 to 2.38 mmol/L (mean, 1.30 mmol/L or 50 mg/dL) for HDL cholesterol, and from 0.36 to 2.54 mmol/L (mean, 0.96 mmol/L or 85 mg/dL) for triglycerides.

The men were between 19 and 52 years old (mean, 25 years), they weighed between 65 and 87 kg (mean, 75 kg), and their body mass indexes ranged from

18.4 to 25.4 kg/m^2 (mean, 22.0 kg/m^2). The women were between 19 and 57 years old (mean, 26 years) and weighed between 54 and 90 kg (mean, 64 kg); their body mass indexes ranged from 17.4 to 29.7 kg/m^2 (mean, 22.0 kg/m^2). Eight women used oral contraceptives, and two women, but none of the men, smoked.

Design and statistical analysis

Each participant consumed each of three different diets during three consecutive periods, each of three weeks duration. One diet was high in oleic acid, another high in trans isomers of oleic acid, and the third high in saturated fat, notably lauric acid (C12:0) and palmitic acid (C16:0). Before the study, subjects were categorized according to sex, and females also according to use of oral contraceptives. Subjects were then randomly divided into six groups in such a way that each group had a nearly identical number of subjects from each category. Each group now received the diets in a different order (Figure 2). In this way variation due to residual effects of the previous diet or to drift of variables with time could be eliminated [12].

The data were analyzed with the General Linear Models procedure of the Statistical Analysis System (SAS) [13]. When the analysis indicated a significant effect of diet ($P<0.05$), the Bonferroni method was used to compare the diets pairwise. As this involved three simultaneous comparisons, the upper limit of statistical significance was set at one third of the customary 0.05, i.e. 0.017. All P-values are two-tailed.

Diets

Before the study, subjects weighed and recorded their habitual food intake for two working days plus one weekend day, in order to enable us to estimate each person's energy and nutrient intake. The food records were coded and the composition of the diets calculated using the 1986 edition of the Netherlands Nutrient Data Base [14].

Diets used during the study consisted of conventional solid foods, and menus were changed daily on a three-weekly cycle. The nutrient content of the three test diets was similar, except for 10 percent of total energy, which was provided by either oleic acid, trans isomers of oleic acid, or saturated fatty acids. The oleic-acid group received special bread enriched

with olive oil, and a margarine made of a variety of sunflower oil high in oleic acid (TRISUN). This oleic-acid-rich sunflower oil made up 85 percent of the fat phase of this margarine, and lightly hydrogenated palm oil and palmkernel oil each made up 7.5 percent. The high-oleic-acid sunflower oil contributed 45 percent of all monounsaturated fatty acids in the oleic acid diet, olive oil contributed 21 percent, and low-erucic acid rapeseed oil 10

Table 1. Fatty acid composition of the margarines used in the three diets.

	Oleic-acid diet	Trans-fatty-acid diet	Saturated-fat diet
	grams per 100 g of fatty acid		
Saturated fatty acids	22.5	14.8	44.0
Lauric acid (C12:0)	2.7	0.2	14.2
Myristic acid (C14:0)	1.5	0.1	5.4
Palmitic acid (C16:0)	6.6	4.4	15.4
Stearic acid (C18:0)	9.6	8.0	6.2
Monounsaturated fatty acids	71.1	75.1	48.6
Cis-C18:1	70.7	32.4*	47.9
Trans-C18:1	0.3	42.7+	0.6
Polyunsaturated fatty acids	6.5	9.0	7.8
Linoleic acid (Cis-cis-C18:2)	6.2	8.7	7.4
Others	0.1	1.2	0.0

* Distribution of positional cis-isomers: C18:1(n-10), 8.8%; C18:1(n-9), 16.3%; C18:1(n-8), 3.1%; C18:1(n-7), 2.7%; C18:1(n-6), 1.3%; C18:1(n-5), 0.6%;C18:1(n-4), 0.3%.

\+ Distribution of positional trans-isomers: C18:1(n-12), 0.3%; C18:1(n-11), 0.7%; C18:1(n-10), 4.4%; C18:1(n-9), 12.9%; C18:1(n-8), 10.3%; C18:1(n-7), 9.1%; C18:1(n-6), 6.1%.

percent. For the trans-fatty-acid diet, the same high-oleic-acid sunflower oil was isomerized in such a way as to convert half of the oleic acid molecules to the trans configuration, while avoiding isomerization of linoleic acid (Table 1). This hydrogenated fat was subsequently mixed with

the unaltered oleic-acid-rich sunflower oil plus regular sunflower oil plus low-erucic acid rapeseed oil so as to prepare a margarine and a shortening high in trans fatty acids. This shortening was also used to prepare a special bread containing 8 g of shortening per 100 g. The saturated-fat diet included another special margarine and shortening, this time high in lauric and palmitic acid (Table 1). These fats were made out of lightly hydrogenated palm oil and palmkernel oil plus some oleic-acid-rich sunflower oil. The special fats and margarines were developed and manufactured by the Unilever Research Laboratory, Vlaardingen, The Netherlands. Other typical items consumed during the study were bread, full-fat cheese, low-fat meat for sandwiches, milk or yoghurt (low-fat varieties were used for the oleic-acid and trans-fatty-acid diet), fruit, cookies, jam or honey, potatoes, cooked vegetables, salad garnished with egg-yolk, gravy, and occasionally an egg.

Diets were formulated at twenty-eight levels of energy intake, ranging from 5.5 to 20.0 MJ per day. The intake of protein, carbohydrates, alcohol, cholesterol and dietary fiber did not differ between diets. In addition, the consumption of the various saturated and polyunsaturated fatty acids was the same on the trans-fatty-acid diet as on the oleic-acid-diet (Table 2).

All foodstuffs were weighed out for each individual subject. On weekdays at noon, hot meals were served at the department and eaten in our presence. All other food was provided daily as a package. Food for the weekend and guidelines for its preparation were provided each Friday. In addition to the foods supplied, subjects were allowed a limited number of food items, free of fat and cholesterol. These "free-choice" items were listed and accorded points corresponding to their energy value, with one point equalling 41.8 kJ (10 kcal). Each subject was required to consume daily a constant number of points that varied slightly with energy intake and ranged from 7 to 11 percent of the total daily energy intake. Subjects were urged not to change their selection of free-choice items between diet periods.

Subjects were asked to maintain their usual pattern of activity and not to change their smoking habits or use of oral contraceptives. They recorded in diaries any signs of illness, medications used, the free-choice items selected, and any deviations from their diets. Inspection of the diaries did not reveal any deviations from the protocol which might have affected the results.

For each of the three diets, complete duplicate portions for one imaginary participant with a daily energy intake of 10 MJ (2390 kcal) were collected for each diet during each of the three periods. The duplicates

were stored at -20 °C, and pooled and analyzed after the study. The free-choice items consumed were coded and their composition calculated using the Netherlands Nutrient Data Base [14]. The analyzed values of the duplicate diets were combined with the calculated values for the free-choice items (Table 2).

Body weights without shoes, jackets or heavy sweaters were recorded twice weekly, and energy intake was adjusted when necessary. Over the 63 days of the study average body weight decreased by 0.1 ± 1.0 kg (range, -2.2 to 2.7 kg) with no effect of specific diets.

Blood sampling and analysis

Before the study all subjects had been assigned a random number which was used for labelling blood and serum or plasma tubes. In this way the technicians who performed the chemical analyses were blinded as to the subjects' diet sequence.

Blood was sampled after an overnight fast on days 1, 18 and 21 (Period I), on days 39 and 42 (Period II), and on days 60 and 63 (Period III). Serum was obtained by low-speed centrifugation, stored at -80 °C, and analyzed enzymatically for total and HDL cholesterol and triglycerides at the end of the study [15, 16]. All samples from one subject were analyzed within one run. The coefficient of variation of control sera within one run was 0.9 percent for total cholesterol, 1.3 percent for HDL cholesterol, and 1.7 percent for total triglycerides. Accuracy was checked by analysis of three serum pools of known value provided by the U.S. Centers for Disease Control and, for HDL cholesterol only, of three pools produced by the North-West Lipid Research Clinic [17]. Mean bias with regard to target values of the U.S. Centers for Disease Control was 0.01 mmol/L for total cholesterol, and 0.08 mmol/L for total triglycerides. Mean bias with regard to the North-West Lipid Research Clinic target value for HDL cholesterol was -0.06 mmol/L. The LDL cholesterol concentration was calculated using the Friedewald equation [18]. The two lipoprotein values obtained at the end of each dietary period were averaged for data analyses.

For each subject, the fatty acid composition of erythrocyte membranes was determined in duplicate in samples obtained at the end of each dietary period (days 21, 42, and 63), as described earlier but now using a capillary Sil-88 column [19]. Results were expressed as proportion by weight of all fatty acids detected. Equal volumes of the two plasma samples obtained at

the end of each dietary period were pooled. Apolipoprotein A-I was measured by immunoturbidimetry [20], using sheep antisera. Apolipoprotein B was measured in plasma in all samples by radial immunodiffusion using antiserum raised in rabbits [21]. All samples of one subject were analyzed in duplicate on the same plate and the results averaged per diet period. The coefficient of variation within one run was 2.5 percent for apolipoprotein A-I and 3.9 percent for apolipoprotein B.

RESULTS

The mean daily intake of energy and nutrients of the subjects on the the three experimental diets are given in Table 2. Total fat and cholesterol intake did not differ between diets during the study. The percent of total energy from oleic acid decreased from 23.0 percent on the oleic-acid diet to 12.6 percent on the trans-fatty-acid and to 12.8 percent on the saturated-fat diet. It was replaced by either trans fatty acids - specifically, elaidic acid and its positional isomers - or by saturated fatty acids alone.

The changes in the the fatty acid composition of the erythrocyte membranes during the three dietary periods confirmed dietary adherence. On the oleic-acid diet the ratio of cis-C18:1 to trans-C18:1 in erythrocytes was 17.1 $\pm$ 6.3 (mean $\pm$ SD), on the trans-fatty-acid diet it was 4.6 $\pm$ 1.2, and on the saturated-fat diet it was 14.5 $\pm$ 4.9.

Compared with the oleic-acid diet (Table 3), serum total cholesterol increased by 0.26 mmol/L (10 mg/dL) on the trans-fatty-acid diet (95 percent confidence interval for difference with the oleic-acid diet, 0.17 to 0.35 mmol/L; $P<0.017$), and by 0.54 mmol/L (21 mg/dL) on the saturated-fat diet (95 percent confidence interval, 0.45 to 0.63 mmol/L; $P<0.017$). The difference between the the trans-fatty-acid diet and the saturated-fat diet of -0.28 mmol/L (11 mg/dL) was also highly significant (95 percent confidence interval, -0.18 to -0.37 mmol/L; $P<0.017$). Compared to the oleic-acid diet HDL cholesterol decreased by 0.17 mmol/L (7 mg/dL) on the trans-fatty-acid diet (95 percent confidence interval for difference with the oleic-acid diet, 0.13 to 0.20 mmol/L; $P<0.017$), but it did not change on the saturated-fat diet. The HDL cholesterol lowering effect of trans fatty acids was evident in each of the six diet sequences and in fifty-four out of the fifty-nine subjects (Figure 2). The changes in apolipoprotein A-I

Table 2. Mean daily intake of energy and composition of the diets.*

	Type of diet*		
	Oleic-acid-rich	Trans-fatty-rich	Saturated-fat
Energy			
MJ/day	11.6 ± 2.8	11.5 ± 2.7	11.4 ± 2.9
kcal/day	2780 ± 669	2751 ± 650	2734 ± 703
Protein (% of energy)	13.1	13.3	14.0
Fat (% of energy)	39.6	40.2	38.8
Saturated fatty acids	9.5	10.0	19.4
Lauric acid (C12:0)	0.5	0.4	3.4
Myristic acid (C14:0)	0.5	0.7	2.7
Palmitic acid (C16:0)	4.7	4.3	8.1
Stearic acid (C18:0)	3.0	3.6	3.5
Monounsaturated fatty acids	24.1	24.2	14.7
Cis-C18:1	23.0	12.6	12.8
Trans-C18:1	0.0	10.9	1.8
Total trans+	0.0	11.0	0.8
Polyunsaturated fatty acids	4.6	4.6	3.4
Linoleic acid	4.0	4.2	2.9
P/S ratio¥	0.48	0.46	0.15
Carbohydrates (% of energy)	46.3	45.6	46.1
Mono- and disaccharides	26.2	25.3	24.0
Polysaccharides	19.9	20.2	22.0
Alcohol (% of energy)	1.1	0.9	1.3
Cholesterol (mg/MJ)§	35.0	31.9	33.6
Dietary fiber (g/MJ)§	4.1	4.1	4.3

* Based on chemical analysis of duplicate diets. Each value represents the mean of three different periods, during which each diet was consumed by a different third of the subjects. Differences in composition of the study diets between periods and between subjects were negligible; therefore, no standard deviations are given for the nutrients. Specially prepared experimental fats (Table 1) provided 25 percent of total fat intake in the oleic-acid diet, 65 percent in the trans-fatty-acid diet, and 39 percent in the saturated-fat diet.

\+ Determined by infra-red spectroscopy and expressed in terms of elaidic acid. Trans fatty acids in the saturated-fat diet were largely derived from milk and cheese; those in the trans-fatty-acid diet were derived almost entirely from the experimental hydrogenated fat.

¥ Ratio of polyunsaturated to saturated fatty acids.

§ To convert values for the intake of cholesterol to milligrams and dietary fiber to grams per 1,000 kcal, multiply by 4.184.

parallelled those in HDL cholesterol (Table 4). LDL cholesterol was 0.37 mmol/L (14 mg/dL) higher on the trans-fatty-acid diet (95 percent confidence interval, 0.28 to 0.45 mmol/L; P<0.017), and 0.47 mmol/L (18 mg/dL) higher on the saturated-fat diet (95 percent confidence interval, 0.39 to 0.55 mmol/L; P<0.017) than on the oleic-acid diet (Table 3). The plasma apolipoprotein B level was highest on the trans-fatty-acid and lowest on the oleic-acid diet (P<0.017; Table 4). The ratio of LDL to HDL cholesterol was

Table 3. Serum lipids and lipoproteins after three weeks' consumption of diets high in oleic acid, in trans-monounsaturated fatty acids, or in saturated fatty acids.*

	Oleic-acid diet	Trans-fatty-acid diet	Saturated-fat diet
Total Cholesterol			
Men	4.23 ± 0.72	4.47 ± 0.77+	4.79 ± 0.82+¥
Women	4.63 ± 0.57	4.90 ± 0.64+	5.15 ± 0.59+¥
All	4.46 ± 0.66	4.72 ± 0.72+	5.00 ± 0.71+¥
LDL cholesterol			
Men	2.59 ± 0.61	2.93 ± 0.65+	3.05 ± 0.66+¥
Women	2.73 ± 0.48	3.12 ± 0.58+	3.20 ± 0.50+
All	2.67 ± 0.54	3.04 ± 0.61+	3.14 ± 0.57+¥
HDL cholesterol			
Men	1.24 ± 0.29	1.10 ± 0.23+	1.28 ± 0.28+¥
Women	1.55 ± 0.32	1.37 ± 0.27+	1.53 ± 0.31¥
All	1.42 ± 0.32	1.25 ± 0.29+	1.42 ± 0.32¥
Triglycerides			
Men	0.86 ± 0.42	0.99 ± 0.50	1.00 ± 0.63
Women	0.78 ± 0.29	0.91 ± 0.30+	0.91 ± 0.32+
All	0.81 ± 0.35	0.94 ± 0.40+	0.94 ± 0.47+

* Values are expressed in mmol/L and are means ± SD. Each diet was fed for three weeks to each of the 25 men and 34 women, in random order. To convert values for total, HDL, and LDL cholesterol to mg/dL, multiply by 38.67. To convert values for triglycerides to mg/dL, multiply by 88.54.

+ Denotes a significant difference with the diet high in oleic acid: P<0.017.

¥ Denotes a significant difference with the diet high in trans fatty acids: P<0.017.

2.02 on the oleic-acid diet, 2.58 on the trans-fatty-acid diet, and 2.34 on the saturated-fat diet: all three values are significantly different from each other ($P<0.017$). The ratio of total cholesterol to HDL cholesterol increased from 3.31 on the oleic-acid diet to 3.96 on the trans-fatty-acid diet and to 3.68 on the saturated-fat diet. These values are also significantly different from each other ($P<0.017$). The ratio of apolipoprotein A-I to apolipoprotein B was 1.46 ± 0.32 on the oleic-acid diet, 1.19 ± 0.27 on the trans-fatty-acid diet, and 1.32 ± 0.31 on the saturated-fat diet. These values are significantly different from each other ($P<0.017$). The level of triglycerides was 0.13 mmol/L (12 mg/dL) higher on both the trans-fatty-acid diet and the saturated-fat diet than on the

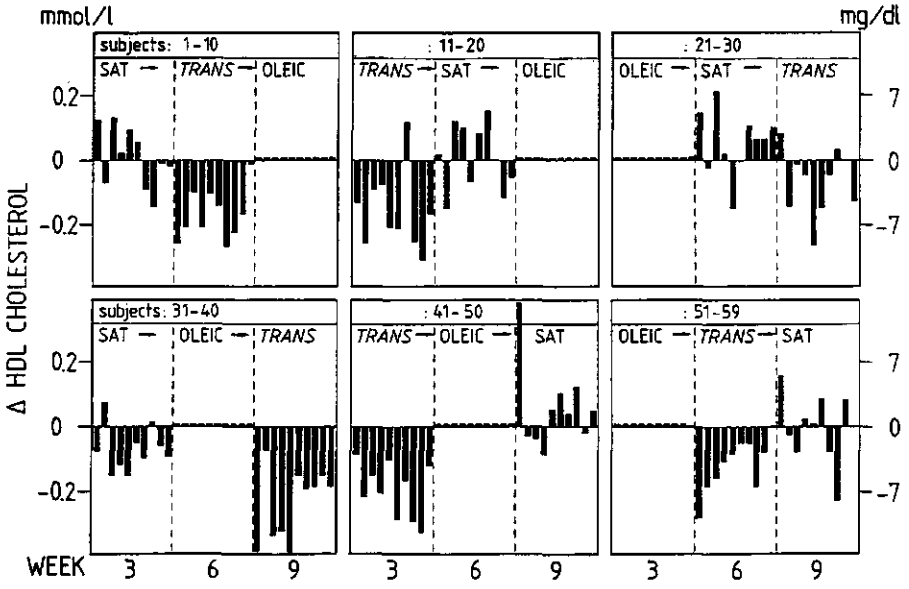

Figure 2. Individual changes in HDL cholesterol levels on diets high in trans fatty acids or saturated fat, relative to a diet high in oleic acid.

Each diet was fed to each subject for three weeks in random order.

oleic-acid diet (95 percent confidence interval, 0.06 to 0.20 mmol/L; $P<0.017$).

The ratio of apolipoprotein B to LDL cholesterol was 356 ± 43 mg/mmol on the oleic-acid diet, 357 ± 48 on the trans-fatty-acid diet, but 334 ± 35 on the saturated-fat diet, which was significantly lower than the other two values. The ratio of apolipoprotein A-I to HDL cholesterol was 965 ± 129 on

the oleic-acid diet and 960 ± 112 on the saturated-fat diet and increased to 1009 ± 127 on the trans-fatty-acid diet. This value was significantly higher than the other two ratios ($P<0.017$).

All these effects were observed to a similar extent in men and women.

Table 4. Plasma apolipoprotein A-I and apolipoprotein B levels after three weeks' consumption of diets high in oleic acid, in trans-monounsaturated fatty acids, or in saturated fatty acids.*

	Oleic-acid diet	Trans-fatty-acid diet	Saturated-fat diet
Apolipoprotein A-I			
Men	1204 ± 160	1129 ± 154+	1247 ± 186¥
Women	1423 ± 173	1313 ± 179+	1407 ± 179¥
All	1330 ± 199	1235 ± 191+	1339 ± 197¥
Apolipoprotein B			
Men	934 ± 214	1069 ± 237+	1030 ± 247+
Women	944 ± 118	1073 ± 156+	1055 ± 141+
All	940 ± 164	1071 ± 193+	1045 ± 192+¥

* Values are expressed in mg/L and are means ± SD. Each diet was fed for three weeks to each of the 25 men and 34 women in random order.
+ Denotes a significant difference with the diet high in oleic acid: $P<0.017$.
¥ Denotes a significant difference with the diet high in trans fatty acids: $P<0.017$.

DISCUSSION

The few previous studies that specifically examined the effects of trans fatty acids on total serum cholesterol yielded conflicting results. Mattson et al [11] did not find a hypercholesterolemic effect of trans-C18:1 relative to its cis isomer oleic acid. Two studies of Vergroesen et al [9, 10], however, suggested that trans isomers of oleic acid have a cholesterol-raising effect, though less so than saturated fatty acids. The present study confirmed this: we found that trans fatty acids were hypercholesterolemic relative to oleic acid, and that their effect is about half as strong as that of a mixture of lauric, myristic and palmitic acid.

However, the effect of trans fatty acids on the lipoprotein risk profile was much more unfavorable than suggested by the modest rise in the total serum cholesterol concentration. The level of LDL cholesterol was increased, but the level of HDL cholesterol was decreased, and as a result the ratio of LDL to HDL cholesterol was even higher on the trans-fatty-acid diet than on the saturated-fat diet. To our knowledge these effects have not been reported before. Laine et al [22] studied the effects of lightly hydrogenated soybean oil on serum lipoproteins in healthy men and women. They found that unhydrogenated soybean oil decreased LDL cholesterol levels by 0.52 mmol/L (20 mg/dL) as compared to hydrogenated soybean oil. The level of saturated fatty acids was the same in the two diets, but polyunsaturated fat intake was 7 percent of energy lower and the intake of trans fatty acids 3 percent higher on the hydrogenated soybean oil diet. The hydrogenated oil did not decrease HDL cholesterol levels in that study [22], but in view of the multiple changes in fatty acid intake this does not necessarily contradict our finding.

We have previously postulated that all classes of fatty acids, more or less equally, increase the level of HDL cholesterol when substitued for carbohydrates in the diet [23]. The present study shows that trans fatty acids might be an exception: replacement of 10 percent of energy from oleic acid or saturated fat by trans fatty acids decreased HDL cholesterol levels by 0.17 mmol/L or 7 mg/dL (Table 3), which is even more than the fall in HDL cholesterol seen when fat is replaced by carbohydrates [24]. The fall in apolipoprotein A-I on the trans-fatty-acid diet was less than that in HDL cholesterol. This may point to a specific loss of the lighter, cholesterol-rich HDL_2 particles. HDL_2 is thought to be more protective against atherosclerosis than the heavier HDL_3 particle, but this theory still awaits confirmation [25].

Apolipoprotein B levels were somewhat higher on the trans-fatty-acid diet than on the saturated-fat diet, but considerable lower on the oleic-acid diet. There are indications that the atherogenicity of LDL particles increases with the ratio of apolipoprotein B to cholesterol in these particles [26]. This ratio was lower on the saturated-fat diet than on the diets high in cis or trans monounsaturates. Both the reproducibility and the biological significance [27] of this observation remain to be established.

Responses to diet were similar for men and women. We have earlier reported that men showed a greater change in HDL cholesterol levels than women when saturated fatty acids were removed from the diet [2]. The main

difference with the previous study was that in the present study the level of stearic acid in the diet did not change between the oleic-acid and the high-saturated fat diet. Whether this might explain the different results needs further investigation.

Both the trans-fatty-acid diet and the saturated-fat diet increased serum triglyceride levels, compared to the oleic-acid diet. In this respect, too, results from other studies are conflicting. Mattson et al [11] did not find a triglyceride-elevating effect of trans relative to cis monounsaturates. Anderson et al [28], however, found higher triglyceride levels on a diet high in trans isomers of mono- and polyunsaturated fatty acids, compared to a diet high in butter fat.

In this study we have specifically examined trans fatty acids with eighteen carbon atoms and one double bond, which are the major class of fatty acids in the diet [7]. We do not know whether our results might be extended to trans fatty acids with a higher number of carbon atoms, such as those formed upon the hydrogenation of fish oil.

The design and the number and variety of subjects employed in our study, as well as the internal consistency of the results, make it highly implausible that our findings on trans fatty acids and serum lipoproteins are due to chance. Therefore, the specific trans-fatty-acid diet employed in our study is likely to lower HDL cholesterol and elevate LDL cholesterol in most groups of adult men and women with normal to mildly elevated cholesterol levels. However, our findings do not apply unreservedly to other hydrogenated fats and oils rich in trans fatty acids. Although the spectrum of trans fatty acids in our experimental diet was quite similar to that of hydrogenated soybean oil [29] - the major source of trans fatty acids in the American diet - subtle but important differences in composition might theoretically exist between the hydrogenated fat used by us and that used in actual food production. Nevertheless, for the time being it would seem prudent for patients at increased risk for atherosclerosis to avoid high intakes of trans fatty acids.

REFERENCES

1. Mattson FH, Grundy SM. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J Lipid Res 1985; 26:194-202.

2. Mensink RP, Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. N Engl J Med 1989; 321:436-441.
3. Parodi PW. Distribution of isomeric octadecenoic fatty acids in milk fat. J Diary Sci 1976; 59:1870-1873.
4. Slover HT, Thompson RH Jr, Davis CS, Merola GV. Lipids in margarines and margarine-like products. JAOCS 1985; 62:775-786.
5. Enig MG, Pallansch LA, Sampugna J, Keeney M. Fatty acid composition of the fat in selected food items with emphasis on trans components. JAOCS 1983; 60:1788-1795.
6. Wiedermann LH. Margarine and margarine oil, formulation and control. JAOCS 1978; 55:823-829.
7. FASEB Report of the ad hoc Committee of the Federation of American Societies for Experimental Biology - 1985. Health aspects of dietary trans fatty acids. Senti FR, ed. Contract Number FDA 223-83-2020.
8. Brussaard JH. Hoeveel transvetzuren bevat de Nederlandse voeding?. Voeding 1986; 47:108-111.
9. Vergroesen AJ. Dietary fat and cardiovascular disease: possible modes of action of linoleic acid. Proc Nutr Soc 1972; 31:323-329.
10. Vergroesen AJ, Gottenbos JJ. In: Vergroesen AJ, ed. The role of fats in human nutrition. New York: Academic Press, 1975:1-41.
11. Mattson FH, Hollenbach EJ, Kligman AM. Effect of hydrogenated fat on the plasma cholesterol and triglyceride levels of man. Am J Clin Nutr 1975; 28:726-731.
12. SAS Institue Inc. SAS User's guide: Statistics Version 5 Edition. Cary, NC: SAS Institue Inc., 1985.
13. Snedecor GW, Cochran WG,. Statistical methods. 7th ed. Ames: Iowa State University Press, 1980.
14. Commissie UCV. UCV Tabel. The Hague: Voorlichtingsbureau voor de Voeding, 1985.
15. Warnick GR, Benderson J, Albers JJ. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin Chem 1982; 28:1379-1388.
16. Sullivan DR, Kruijswijk Z, West CE, Kohlmeier M, Katan MB. Determination of serum triglycerides by an enzymatic method not affected by free glycerol. Clin Chem 1985; 31:1227-1228.
17. Warnick GR, Clapshaw P. Availibility of plasma with target values for certain lipids. Clin Chem 1987; 33:2323-2324.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifugation. Clin Chem 1972; 18:499-502.
19. Glatz JF, Soffers AE, Katan MB. Fatty acid composition of serum cholesterylesters and of erythrocyte membranes as indicators of linoleic acid intake in man. Am J Clin Nutr 1989; 49:269-276.
20. Irish GR, Ledue TB, Barrantes DM. Measurement of apolipoprotein A-I in serum with the Technicon RA-1000 system. Clin Chem 1987; 33:1266.
21. Mensink RP, de Groot MJM, van den Broeke LT, Severijnen-Nobels AP, Demacker PNM, Katan MB. Effects of monounsaturated fatty acids versus complex carbohydrates on serum lipoproteins and apoproteins in healthy men and women. Metabolism 1989; 38:172-178.
22. Laine DC, Snodgrass CM, Dawson EA, Ener MA, Kuba K, Jr Frantz ID. Lightly hydrogenated soy oil versus other vegetable oils as a lipid-lowering dietary constituent. Am J Clin Nutr 1982; 35:683-690.
23. Katan MB. Diet and HDL. In: Miller NE, Miller GJ, eds. Clinical and metabolic aspects of high-density lipoproteins. Metabolic aspects of cardiovascular disease. Vol. 3. Amsterdam: Elsevier, 1984:103-131.

24. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1987; i:122-125.
25. Miller NE. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. Am Heart J 1987; 113:589-597.
26. Sniderman A, Shapiro S, Marpole D, Skinner B, Teng B, Kwiterovich PO. Association of coronary atherosclerosis with hyperapobetalipoproteinemia (increased protein but normal cholesterol levels in human plasma low density (ß) lipoprotein). Proc Natl Acad Sci 1980; 77:604-608.
27. Swinkels DW, Demacker PNM, Hendriks JCM, Brenninkmeijer BJ, Stuyt PMJ. The relevance of a protein-enriched low density lipoprotein as a risk factor for coronary heart disease in relation to other known risk factors. Atherosclerosis 1989; 77:59-67.
28. Anderson JT, Grande F, Keys A. Hydrogenated fats in the diet and lipids in the serum of man. J Nutr 1961; 75:388-394.
29. Sampugna J, Pallansch LA, Enig MG, Keeney M. Rapid analysis of trans fatty acids on SP-2340 glass capillary columns. J Chromatogr 1982; 249:245-255.

CHAPTER 6

Effect on blood pressure of two diets differing in total fat but not in saturated and polyunsaturated fatty acids in healthy volunteers

Ronald P. Mensink, Mary-Christel Janssen, Martijn B. Katan

American Journal of Clinical Nutrition 1988; 47:976-980

ABSTRACT

The effects of a low-fat, carbohydrate-rich and a high-fat, olive-oil-rich diet on blood pressure were studied under strict dietary control. Forty-seven healthy, normotensive men and women were fed a diet high in saturated (20.0 percent of their daily energy intake) and total fat (38.0 percent) for 17 days. Twenty-four subjects then received a low-fat, high-carbohydrate diet (22.1 percent total fat), the other 23 subjects a high-fat, olive-oil-rich diet (24.0 percent oleic acid, 40.6 percent total fat) for 36 days. Both test diets had the same level of saturated fatty acids (7-10 percent) and linoleic acid (4 percent).

Systolic blood pressure fell by 2.3 mm Hg and diastolic by 4.7 mm Hg in the carbohydrate group and by 2.7 mm Hg and 4.4 mm Hg in the olive oil group, respectively (the difference in changes between the diets groups not significant).

These results suggest that a high-fat diet rich in monounsaturated fatty acids has no deleterious effect on blood pressure in healthy normotensive subjects in comparison with a low-fat, carbohydrate-rich diet.

INTRODUCTION

Dietary fat may be involved in the regulation of blood pressure. Animal studies showed that deprivation of linoleic acid caused an increase of systolic blood pressure [1, 2]. However, results from human studies were less conclusive. Epidemiological studies found a relationship between certain fatty acids and blood pressure [3, 4], but this was not confirmed [5, 6]. Clinical studies suggested that increasing intake of linoleic acid

lowers blood pressure [7, 8]. Because linoleic acid can be converted into prostaglandins, which in turn influence blood pressure [9], it is tenable to attribute an effect of dietary fat on blood pressure to an increased intake of linoleic acid. However, available data do not exclude the possibility that blood pressure might be affected by the total amount of fat in the diet or by the ratio of polyunsaturated to saturated fatty acids rather than by the intake of polyunsaturates itself. We compared the effects of a low-fat, carbohydrate-rich diet and a high-fat, olive-oil-rich diet on blood pressure in healthy, non-obese, normotensive volunteers. By having the same level of saturated and polyunsaturated fatty acids in both diets, with only oleic acid intake different, blood pressure effects of total fat intake can be differentiated from possible effects caused by particular saturated or polyunsaturated fatty acids.

METHODS

Subjects

Fifty-seven healthy men and women entered this strictly controlled dietary experiment. None was hypertensive (diastolic blood pressure > 95 mm Hg and systolic > 150 mm Hg), as judged on two separate occasions two months prior to the study, or received medication known to affect blood pressure immediately prior to or during the study. Data from nine subjects were eliminated before the analysis of the results because of departures from the protocol. For one male subject no blood pressure measurements were made because he was not available at the time of the measurements. Thus, data for 47 participants were processed. They were between 18 and 59 years of age (mean 27 years) and weighed between 53 and 88 kg (mean, 71 kg), their height ranged from 160 to 202 cm (mean, 177 cm) and their body mass indexes from 18.9 to 28.4 kg/m^2 (mean, 22.7 kg/m^2).

Diets and design

The main purpose of the experiment was to test the effects of a low-fat, carbohydrate-rich diet and a high-fat, olive-oil-rich diet on serum lipids. Details of this study have been previously published [10].

The experiment was carried out for 55 days from 27 January to 21 March

1986. Before the experiment subjects were asked to weigh and record their food intake on three separate days including one weekend day. Foods were coded and nutrients calculated using the 1985 edition of the computerized Netherlands Nutrient Data Base [11].

During the experiment all subjects first consumed a Western-type control diet high in total and saturated fat for 17 days. Then two groups were formed by randomization with stratification for serum lipids and sex. One group (carbohydrate group, 12 men and 12 women) received a low-fat, carbohydrate-rich diet and the other group (olive oil group, 11 men and 12 women) received a high-fat, olive-oil-rich diet for the next 36 days. Three women in each diet group used oral contraceptives. The base-line values (mean $\pm$ SD) were 5.19 $\pm$ 1.12 (201 mg/dL) and 5.22 $\pm$ 0.86 mmol/L (202 mg/dL) for serum total cholesterol, 1.49 $\pm$ 0.38 (58 mg/dL) and 1.51 $\pm$ 0.35 mmol/L (58 mg/dL) for HDL cholesterol, 1.00 $\pm$ 0.58 (89 mg/dL) and 0.95 $\pm$ 0.49 (84 mg/dL) mmol/L for triglycerides, and 22.8 $\pm$ 2.17 and 22.6 $\pm$ 2.26 kg/m^2 for body mass indexes for the carbohydrate and the olive oil groups, respectively.

Each diet consisted of conventional, mixed solid foods and menus were changed daily. The amount of food necessary to meet each individual's energy requirement was weighed out. Body weights were recorded twice weekly, and energy intake adjusted when necessary. Weight changes did not exceed 2.5 kg over the 8 weeks of the experiment and were on average -0.5 kg in the carbohydrate group and -1.2 kg in the olive oil group. All food was provided except for some free-choice items that were free of fat and cholesterol. Free-choice items were accorded points corresponding to their energy value, with one point equalling 41.8 kJ (10 kcal). Each subject was required to consume an exact number of points, which varied with total energy intake and ranged from 5 to 10 percent of total energy intake. Typical choices were an apple (6 points), orange juice (5 points), or a glass of beer (8 points). The importance of not changing one's selection of free-choice items between periods was repeatedly explained and stressed. Subjects recorded in diaries their free-choice items, the amount of coffee used, and any deviations from the protocol. Coffee consumption was not different between the two diet groups nor did it change during the study. For each of the three diets duplicate portions for one imaginary participant with a daily energy intake of 10 MJ (2390 kcal) were collected and stored at -20 °C for later analysis.

Measurements

Blood pressure was measured with an automatic sphygmomanometer with recorder (Copal UA-251, Adquipment Medical BV, Rotterdam, The Netherlands) on two occasions before and once a week during the experiment except for week 1. Diastolic pressure was recorded at Korotkoff phase V. Subjects were asked not to perform physical activity or to eat or smoke one hour prior to the blood pressure measurements. Three measurements at one-minute intervals were made at each session before the experiment and four measurements per session during the experiment. Measurements were made on the left arm with the subject in the sitting position in a quiet room after at least 5 minutes rest. Three trained investigators, who were unaware of the diet group of a participant, performed the measurements. Measurements for one person were generally made at the same time of the day by the same person with the same sphygmomanometer. Serum total cholesterol was determined twice before the experiment, twice at the end of the control period, and three times at the end of the test period. Blood pressure and serum total cholesterol were also measured 7 weeks after the experiment in 8 men and 10 women of the carbohydrate group, and in 10 men and 9 women of the olive oil group. The other 10 subjects were not available at that time.

The duplicate portions of each diet period were mixed thoroughly and then freeze-dried. The ash content and the moisture level [12] were determined and then the material was stored at -20 °C. Aliquots were analyzed for protein [13], total fat [14], the proportions of individual fatty acids after saponification and methylation [15], dietary fiber [16], and cholesterol [17]. Carbohydrate was calculated by difference. Sodium, potassium, calcium and magnesium were determined by atomic absorption photometry [18] in the freeze-dried material after it had been wet-ashed and neutralized. The mean composition of the diets was calculated according to the duplicate portion analysis plus calculated contribution of the free-choice items.

Statistical methods

The first blood pressure measurement of each session was discarded and the other measurements were averaged. The response of blood pressure to the low-fat, carbohydrate-rich diet or the high-fat, olive-oil-rich diet was calculated per subject as the change from the end of the Western diet period (week 3) to the end of the test period (week 8). Pearson's correlation

coefficients were computed between the response and the average systolic and diastolic blood pressure level over the course of the experiment (mean of weeks 3 plus 8) for both sexes per diet group. Body weights were averaged per week and the change was calculated as the difference between week 8 and week 1. Differences in effects on blood pressure of the test diets were examined by analysis of variance with diet, sex, and diet x sex interaction as independent variables. An unpaired t-test was used if diet was the only significant variable. Changes within each diet group were examined by analysis of variance with sex as the independent variable. A paired t-test was used if sex had no significant effect. In addition, blood pressures 7 weeks after the experiment were compared with week 8, and cholesterol levels with pre-experimental values by the same techniques [19].

RESULTS

Nutrient intake

The mean daily intake of energy and the composition of the diets during the pre-experimental and the study period are shown in Table 1. Individual energy intakes ranged from 6.1 to 19.9 MJ (1460 to 4760 kcal) on the Western-type diet, from 6.3 to 20.5 MJ (1510 to 4900 kcal) on the low-fat, carbohydrate-rich diet, and from 7.1 to 15.5 MJ (1700 kcal to 3700 kcal) on the high-fat, olive-oil-rich diet. The proportion of energy from saturated fatty acids increased from 15.0 percent during the pre-experimental to 20.0 percent during the Western diet period. The intake of monounsaturated and polyunsaturated fatty acids decreased slightly. The intake of dietary fiber increased on average by 8 g per day during the Western diet period. The daily energy intake from saturated fatty acids decreased by 13.3 percent on the low-fat, carbohydrate-rich diet and by 10.2 percent on the high-fat, olive-oil-rich diet. This decrease was compensated for by an increased intake of carbohydrates in the one group and of monounsaturated fatty acids in the other group. The proportion of energy from alcohol between subjects ranged from 0.0 to 7.3 percent on the Western-type diet, from 0.0 to 8.2 percent on the low-fat, carbohydrate-rich diet and from 0.0 to 7.3 percent on the high-fat, olive-oil-rich diet. The mean daily intake of fiber increased by on average 18 g per day on the low-fat, carbohydrate-rich diet. The intake of sodium, potassium, calcium and magnesium was higher on the

Table 1. Mean daily intake of energy and composition of the diets.*

	Type of diet			
	Habitual* (N=45)	Control+ (N=47)	Carbohydrate-rich+ (N=24)	Olive-oil-rich+ (N=23)
Energy				
MJ/day	10.8 ± 2.9	11.1	11.8	10.9
kcal/day	2581 ± 693	2653	2820	2605
Protein (% of energy)	14.2 ± 2.7	13.6	14.1	12.2
Fat (% of energy)	37.3 ± 4.7	38.0	22.1	40.6
Saturated fatty acids	15.0 ± 2.0	20.0	6.7	9.8
Monounsaturated fatty acids	14.2 ± 2.7	12.4	9.3	24.0
Polyunsaturated fatty acids	5.6 ± 1.3	4.1	5.2	5.1
Linoleic acid	4.6 ± 1.3	3.4	4.3	4.2
P/S ratio¥	0.39 ± 0.1	0.21	0.78	0.52
Carbohydrates (% of energy)	47.1 ± 6.0	47.7	62.2	46.0
Alcohol (% of energy)	1.4 ± 2.0	1.3	1.6	1.2
Cholesterol (mg/MJ)§	31.0 ± 12.4	35.1	33.1	31.7
Dietary fiber (g/MJ)§	3.1 ± 0.9	3.8	5.1	3.9
Sodium (mmol/MJ)§	11.5 ± 3.7	14.1	14.3	12.8
Potassium (mmol/MJ)§	9.2 ± 2.0	9.5	10.3	8.3
Calcium (mmol/MJ)§	3.3 ± 1.0	2.7	2.7	2.2
Magnesium (mmol/MJ)§¶		1.7	1.9	1.7

* Values were calculated from three-day weighed inventories before the experiment began, and they are means ± SD.

\+ Based on chemical analysis of duplicate diets. The standard deviations for energy intake between subjects during the study were 2.6 MJ for the control diet, 3.0 MJ for the carbohydrate-rich diet, and 2.6 MJ for the olive-oil-rich diet. The variation between subjects in the composition of the study diets was negligible; therefore, no standard deviations are given for the nutrients.

¥ Ratio of polyunsaturated to saturated fatty acids.

§ To convert values for the intake of cholesterol to milligrams, dietary fiber to grams, and sodium, potassium, calcium and magnesium to mmol per 1,000 kcal, multiply by 4.184.

¶ No figures for magnesium were available in the food composition table used. Thus, values on the study diets are without the minor contribution of magnesium from free-choice items.

low-fat, carbohydrate-rich diet than on the Western-type and the high-fat, olive-oil-rich diets.

Blood pressure

Changes in blood pressure were not related to sex, sex x diet interaction, or the average absolute blood pressure averaged over the duration of the trial. Therefore, results were pooled per diet group. Time courses of blood pressure values are shown in Figure 1. At randomization the groups were not matched for blood pressure. The higher mean blood pressure in the carbohydrate group throughout the study period is therefore not due

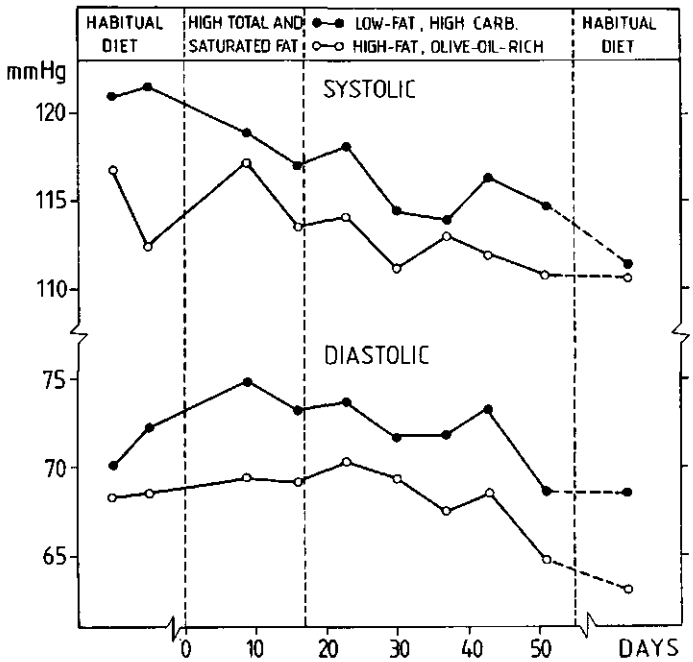

Figure 1. Mean systolic and diastolic blood pressure before, during, and after the experiment.

During the experiment, all 47 subjects first consumed a Western-type control diet high in total and saturated fat for 17 days. For the next 36 days, 24 subjects received a low-fat, high-carbohydrate diet and 23 subjects a high-fat, olive-oil-rich diet with the same level of saturated and polyunsaturated fat. Post-experimental values are based on 18 subjects in the carbohydrate group and 19 in the olive oil group only. Mean levels in these subjects before and during the trial differed only slightly from those in the full diet groups (Table 2).

Table 2. Mean systolic and diastolic blood pressure levels before, during, and after the experiment, and the changes during the experiment.*

	All subjects		Subsample of subjects*	
	Carbohydrate group (N=24)	Olive oil group (N=23)	Carbohydrate group (N=18)	Olive oil group (N=19)
Systolic				
Pre-experimental	121.3 ± 10.6	114.6 ± 10.9	119.2 ± 8.1	114.7 ± 11.9
Experimental				
Control diet	117.1 ± 10.2	113.6 ± 10.4	115.1 ± 8.4	113.8 ± 10.9
Test diet	114.8 ± 11.2	110.9 ± 10.3	113.3 ± 9.9	111.0 ± 11.0
Change	-2.3 ± 9.2	-2.7 ± 6.3¥	-3.7 ± 9.7	-2.8 ± 6.4
Post-experimental			111.4 ± 10.4	110.6 ± 10.5
Diastolic				
Pre-experimental	71.2 ± 10.6	68.5 ± 5.8	72.3 ± 9.3	68.6 ± 6.3
Experimental				
Control diet	73.2 ± 8.4	69.2 ± 7.6	73.1 ± 7.8	68.7 ± 8.3
Test diet	68.5 ± 10.7	64.8 ± 7.8	68.6 ± 11.0	65.3 ± 7.8
Change	-4.7 ± 9.0+	-4.4 ± 6.9§	-4.5 ± 9.6	-3.4 ± 6.1¥
Post-experimental			68.6 ± 11.0	63.0 ± 9.6

* Values are expressed in mm Hg and are means ± SD.
+ The 37 subjects for whom post-experimental measurements were available.
Denotes a significant difference in changes relative to control values: ¥ $P<0.05$, § $P<0.01$.

to diet but to the innately higher blood pressures of some subjects. Mean blood pressures for both diet groups during the pre-experimental and study period are shown in Table 2. The blood pressure changes for the two groups were the same. The systolic pressure of 37 subjects measured 7 weeks after the experiment was on average 1.1 ± 10.0 mm Hg lower and diastolic pressure 1.2 ± 9.4 mm Hg lower than the values at the end of the test period (changes not significant) and 5.9 ± 7.2 mm Hg and 4.7 ± 8.7 mm Hg lower than the pre-experimental values. Repeating the full statistical analyses with only these 37 subjects produced results comparable with those for the full group

(Table 2).

Serum total cholesterol levels 7 weeks after the experiment equalled pre-experimental values (change -0.02 ± 0.50 mmol/L, not significant). This suggests that subjects indeed had returned to their habitual diets and that the fall in blood pressure from pre-experimental to post-experimental measurements was not due to changes in dietary habits.

No significant correlations were found between changes in weight and changes in blood pressure for either diet group (data not shown).

DISCUSSION

An effect of fat-modified diets on blood pressure was reported by several authors. An increased intake of polyunsaturated and a decreased intake of saturated fatty acids lowered both systolic and diastolic blood pressure in middle-aged normotensive subjects [20, 21]. Because both the amount of fat and the fatty-acid composition of the diets had changed in these studies, it is not possible to attribute the observed effects to a single dietary component. We did not find an effect on blood pressure of a high-fat, olive-oil-rich diet relative to a low-fat, carbohydrate-rich with the same level of saturated and polyunsaturated fatty acids. This suggests that total fat is not a determinant of blood pressure in young normotensive volunteers and that the observed effects of fat-modified diets on blood pressure are attributable to an increase in linoleic acid intake. However, this conclusion is contradicted by several experiments. In the experiment of Puska et al [22] blood pressure was lowered on a low-fat diet with only a slight change in polyunsaturated fatty acids. An effect of polyunsaturated fatty acids on blood pressure was not demonstrated in other studies with normotensive subjects [23, 24]. However, note that our subjects were young, non-obese, and normotensive, which may make it difficult to alter blood pressure. It has been shown that a diet known to lower blood pressure in middle-aged subjects [22] does not modify blood pressure in children [25].

Blood pressures were lower on both test than on the Western-type diet high in saturated fatty acids, which could be interpreted as a beneficial effect in general of diets low in saturated fatty acids. Puska et al [22] also noted an equal reduction in blood pressure on two low-fat diets with virtually the same level of saturated fat but with a different ratio of polyunsaturated to saturated fatty acids. However, blood pressure levels

measured 7 weeks after our study did not differ from values at the end of the study and were appreciably lower than pre-experimental values even though serum total cholesterol levels had returned to pre-experimental values. This suggests that the decrease in blood pressure in both groups during the trial might have been a habituation effect - a well-known phenomenon in blood pressure studies [26] - although this cannot be ascertained from the present study. Because there was no control group, it is possible that both diets had the same effect on blood pressure. However, results obtained so far are not uniform, and more well-controlled studies are needed before any firm conclusions can be drawn.

Changes in body weight might also influence changes in blood pressure [26]. However, changes in weights were small in this experiment and did not correlate with changes in blood pressure. From chemical analysis of duplicate portions the intake of sodium, potassium, calcium, magnesium and dietary fiber was slightly higher in the carbohydrate group. All these dietary factors might influence blood pressure [28-32]. Except for sodium, the intakes of all these dietary factors were more favorable with respect to blood pressure in the carbohydrate group. However, there was still no excess fall in blood pressure in this group. This reinforces our conclusion that a high-monounsaturated fat intake does not increase blood pressure in comparison with a low-fat, carbohydrate-rich diet.

In our experiment the standard deviation of the individual change in blood pressure from week 3 to week 8 was about 8 mm Hg. With 24 persons per diet group the statistical power for detecting a true difference between the diets of 2 mm Hg is only 22 percent and of 4 mm Hg is 55 percent. Thus we might have missed a small effect on blood pressure purely by chance. However, we may still conclude that the favorable effects on serum lipids [10] of a high-fat, olive-oil-rich diet as compared to a low-fat, carbohydrate-rich diet are not counteracted by an unfavorable effect on blood pressure in healthy, normotensive subjects.

REFERENCES

1. Düsing R, Scherhag R, Glänzer K, Budde U, Kramer HJ. Dietary linoleic acid deprivation: effects on blood pressure and PGI_2 synthesis. Am J Physiol 1983; 244:H228-H233.
2. Hoffmann P, Block HU, Beitz J, et al. Comparative study of the blood pressure effects of four different vegetable fats on young, spontaneously hypertensive rats. Lipids 1986; 21:733-737.

3. Oster P, Arab L, Schellenberg B, Heuck CC, Mordasini R, Schlierf G. Blood pressure and adipose tissue linoleic acid. Res Exp Med (Berl) 1979; 175:S287-S291.
4. Berry EM, Hirsch J. Does dietary linolenic acid influence blood pressure. Am J Clin Nutr 1986; 44:336-340.
5. McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. Science 1984; 224:1392-1398.
6. Fehily AM, Milbank JE, Yarnell JWG, Hayes TM, Kubiki AJ, Eastham RD. Dietary determinants of lipoproteins, total cholesterol, viscosity, fibrinogen, and blood pressure. Am J Clin Nutr 1982; 36:890-896.
7. Stern B, Heyden S, Miller D, Latham G, Klimas A, Pilkington K. Intervention study in high school students with elevated blood pressures. Dietary experiment with polyunsaturated fatty acids. Nutr Metab 1980; 24:137-147.
8. Comberg HU, Heyden S, Hames CG, Vergroesen AJ, Fleischman AI. Hypotensive effect of dietary prostaglandin precursor in hypertensive man. Prostaglandins 1978; 15:193-197.
9. Heinemann HO, Lee JB. Prostaglandins and blood pressure control. Am J Med 1976; 61:681-695.
10. Mensink RP, Katan MB. Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. Lancet 1987; i:122-125.
11. Commissie U.C.V. U.C.V. Tabel. The Hague: Voorlichtingsbureau voor de Voeding, 1985.
12. Osborne DR, Voogt P. The analysis of nutrients in foods. London: Academic Press, 1978.
13. Williams S, ed. Official methods of analysis of the association of official analytical chemists. 14th ed. Arlington, VA: AOAC Inc, 1984.
14. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226:497-509.
15. Metcalfe LD, Schmitz AA, Pelka JR. Rapid preparation of fatty acid esters from lipids for gas chromotographic analysis. Anal Chem 1966; 38:514-515.
16. Prosky L, Asp NG, Furda J, de Vries JW, Schweizer TF, Harland BF. Determination of total dietary fiber in foods, food products and total diets: interlaboratory study. J Assoc Off Anal Chem 1984; 67:1044-1053.
17. Van de Bovenkamp P, Katan MB. Cholesterol content of chicken skin. J Food Sci 1981; 46:291.
18. Analytical methods for atomic absorption spectrophotometry. Norwalk: Perkin- Elmer Cooperation, 1976.
19. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames: Iowa State University Press, 1980.
20. Iacono JM, Puska P, Dougherty RM, et al. Effect of dietary fat on blood pressure in a rural Finnish population. Am J Clin Nutr 1983; 38:860-869.
21. Puska P, Iacono JM, Nissinen A, et al. Controlled, randomised trial of the effect of dietary fat on blood pressure. Lancet 1983; i:1-5.
22. Puska P, Iacono JM, Nissinen A, et al. Dietary fat and blood pressure: an intervention study on the effects of a low-fat diet with two levels of polyunsaturated fat. Prev Med 1985; 14:573-584.
23. Lemkbe A, Greggersen H, Kay HW, Rathjen G, Steinicke F. Dietary linoleate and blood pressure. Milchwissenschaft 1985; 40:329-333.
24. Margetts BM, Beilin LJ, Armstrong BK, et al. Blood pressure and dietary polyunsaturated and saturated fats: a controlled trial. Clin Sci 1985; 69:165-175.

25. Vartiainen E, Puska P, Pietinen P, Nissinen A, Leino U, Uusitalo U. Effects of dietary fat modifications on serum lipids and blood pressure in children. Acta Paediatr Scand 1986; 75:396-401.
26. Burstyn PG. Variability in blood pressure measurements. Am J CLin Nutr 1982; 36:379.
27. Borkan GA, Sparrow D, Wisniewski C, Vokonas PS. Body weight and coronary disease risk: patterns of risk factor change associated with long-term weight change. The Normative Aging Study. Am J Epidemiol 1986; 124:410-419.
28. Beard TC, Cooke HM, Gray WR, Barge R. Randomised controlled trial of a no-added-sodium diet for mild hypertension. Lancet 1982; ii:455-458.
29. Khaw KT, Thom S. Randomised double-blind cross-over trial of potassium on blood pressure in normal subjects. Lancet 1982; ii:1127-1129.
30. Belizan JM, Vilar J, Pineda O, et al. Reduction of blood pressure with calcium supplementation in young adults. JAMA 1983; 249:1161-1165.
31. Dyckner T, Wester PO. Effect of magnesium on blood pressure. Br Med J 1983; 286:1847-1849.
32. Wright A, Burstyn PG, Gibney MJ. Dietary fibre and blood pressure. Br Med J 1979; 2:1541-1543.

CHAPTER 7

Effect of a monounsaturated versus a polyunsaturated fatty-acid-enriched diet on blood pressure in normotensive women and men

Ronald P. Mensink, Annette M. Stolwijk, Martijn B. Katan

Submitted for publication

ABSTRACT

The effect on blood pressure of monounsaturated and (n-6)polyunsaturated fatty acids was studied in normotensive subjects under strict dietary control. For 17 days 31 women and 27 men received a control diet providing 19.3 percent of their daily energy intake from saturated fat. Then subjects were randomized over two test diets: one diet provided 15.1 percent of energy from monounsaturated and 7.9 percent from polyunsaturated fat ("monounsaturated-fat" diet), and the other diet provided 10.8 percent monounsaturated and 12.7 percent from polyunsaturated fat ("polyunsaturated-fat" diet). Saturated fat intake was now 12.8 percent on both test diets.

The mean blood pressure level at the end of the control period was 116/69 mm Hg for the monounsaturated-fat group and 117/73 mm Hg for the polyunsaturated-fat group. After five weeks on the test diet, the blood pressure level was 115/67 mm Hg for the monounsaturated-fat group and 117/72 mm Hg for the polyunsaturated-fat group (the difference in changes between the two unsaturated-fat diets was not significant).

These findings suggest that linoleic acid, when providing more than 7.9 percent of energy intake, does not influence blood pressure relative to oleic acid in normotensive women and men in high-fat diets.

INTRODUCTION

Elevated levels of blood pressure and of serum total cholesterol are important causes of premature coronary heart disease [1]. In addition, mild hypertension is a major risk factor for stroke in both women and men [2]. To reduce serum cholesterol levels a reduction in dietary total and saturated

fat intake is recommended. The type and amount of dietary fat, however, may also influence blood pressure. Some controlled trials have suggested that a cholesterol-lowering linoleic-acid-enriched diet reduces blood pressure, even in healthy, normotensive subjects [3, 4]. Several other studies, however, failed to confirm this [5, 6]. On the other hand, epidemiological studies did find a negative association between the percentage of polyunsaturated fatty acids in adipose tissue and blood pressure [7, 8]. Even if certain diets rich in polyunsaturated fatty acids do lower blood pressure levels, it is not possible to point out exactly the dietary factor responsible for the hypotensive effect: in addition to an increase in the intake of linoleic acid, the intake of other nutrients like total fat, potassium, and fiber also changed in the studies discussed above [3, 4].

We have tested whether two diets differing in unsaturated fatty acids but not in total fat had an effect on blood pressure in normotensive men and women. Two alternatives to the high-saturated-fat diet that is frequently eaten by affluent populations were compared. One was high and the other moderately high in linoleic acid; the difference between the two diets was made up with oleic acid. The intake of other nutrients was kept constant throughout the study and could therefore not have confounded the results.

METHODS

Subjects

Volunteers were recruited through a local newspaper, through a university newspaper and through posters in university buildings. Subjects were eligible if they (i) had no history of atherosclerotic disease or hypertension, (ii) had no anemia, glycosuria, or proteinuria, (iii) were not using any prescribed medicine, (iv) were healthy as judged by a physician from a medical questionnaire, and (v) expressed no dislikes for any of the foodstuffs provided during the study. Out of 87 applicants, 83 subjects met these criteria. As we only needed 60 people, we accepted all 28 men and 3 of their spouses; we then added another 29 women by chance. Nine women used oral contraceptives. Four of the women and 3 of the men were smokers. Table 1 shows some of their baseline characteristics.

Experimental protocol

The main purpose of the experiment was to compare the effects of a diet rich in monounsaturated fatty acids and a diet rich in polyunsaturated fatty acids on serum lipids. Details of this study have been published [9]. All subjects were studied simultaneously for 55 days from 4 October to 27 November 1987. The first part of the study consisted of a 17-day control period, during which all subjects received a diet high in saturated fatty acids. The subjects were then categorized according to sex, and females also according to use of oral contraceptives. From each category one half of subjects, chosen at random, received for the next 36 days a diet enriched in monounsaturated plus polyunsaturated fatty acids (the "monounsaturated-fat" diet) and the other half a diet enriched in (n-6)polyunsaturated fatty acids alone (the "polyunsaturated-fat" diet). Energy intake from saturated fat was reduced by 6.5 percent. During the study the intake of other nutrients did not change.

Each diet consisted of conventional, mixed solid foods, and menus were changed daily. The amount of food necessary to meet each individual's energy requirement was weighed out. Body weights were checked by us twice weekly, and energy intake was adjusted when necessary. All food was provided, except for some free-choice items, free of fat and cholesterol. Free-choice items were accorded points corresponding to their energy value, with one point equalling 41.8 kJ (10 kcal). Each subject was required to consume a constant number of points per day, which varied with total energy intake and corresponded with 9 to 10 percent of the total energy intake. Typical choices were an apple (6 points), orange juice (5 points) or a glass of beer (8 points). In our experience, the free-choice system helps subjects to reconcile the rigid requirements of participation in a trial with their social life and personal preferences. The importance of not changing one's selection of free-choice items between periods was repeatedly explained and stressed. Subjects recorded in diaries their free-choice items, the amount of coffee used, and any deviations from the protocol. For each of the three diets, duplicate portions for one imaginary participant with a daily energy intake of 10 MJ (2390 kcal) were collected and stored at -20 °C for later analysis.

The design and execution of the study were explained to the subjects and informed consent was obtained. Prior approval was obtained from the ethics committee of the Department of Human Nutrition. Subjects were asked not to

change their smoking habits, use of oral contraceptive, or their physical activity pattern during the study.

Measurements

Before the experiment subjects were asked to weigh and record their food intake on three separate days, including one weekend day. Foods were coded and the composition of the diets calculated using the 1985 edition of the Netherlands Nutrient Data Base [10] to enable us to estimate their energy and nutrient intake. As it was known from our previous studies that such records tend to underestimate actual energy needs by some 15 percent, the starting levels of energy intake were set at 15 percent over stated habitual intake.

Blood pressure was measured during the morning hours with an automatic sphygmomanometer with recorder (Takeda Medical UA-751, Adquipment Medical BV, Rotterdam, The Netherlands) on two occasions before and once a week during the experiment except for week 1. Blood pressure was also measured 11 weeks after the experiment in all subjects except for one man from each group who were out of town at that time. Systolic pressure was recorded at Korotkoff phase I and diastolic pressure at Korotkoff phase V. Subjects were asked not to perform physical activity or to eat or smoke from one hour prior to the blood pressure measurements. Four measurements were made at each session with the subject in the sitting position in a quiet room, after approximately 5 min of rest. Two trained investigators performed the measurements. Measurements on one subject were always made by the same investigator with the same sphygmomanometer and generally at the same time of the day to eliminate effect of diurnal variation on blood pressure as much as possible.

The fatty-acid composition of serum cholesteryl esters was determined at the end of the control period and again at the end of the test period as described [11]. Serum total cholesterol was determined as reported previously [9].

The duplicate portions of each diet period were mixed thoroughly and then freeze-dried. The ash content and the moisture level [12] were determined and then the material was stored at -20 °C. Aliquots were analyzed for protein [13], total fat [14], the proportions of individual fatty acids [15], dietary fiber [16], and cholesterol [17]. Available carbohydrate was

calculated by difference. Sodium, potassium, calcium and magnesium were determined by atomic absorption spectrophotometry [18] in the freeze-dried material after it had been wet ashed and neutralized. The mean composition of the diets was calculated from the duplicate portion analysis plus the calculated contribution of the free-choice items.

Statistical methods

The first blood pressure measurement of each session was discarded and the other measurements were averaged. The response of blood pressure to the monounsaturated-fat diet or to the polyunsaturated-fat diet was calculated per subject as the change from the end of the control period (week 3) to the end of the test period (week 8). Thus each subject was studied on two different diets. Body weights were averaged per week and the change calculated as the difference between week 8 and week 1. An unpaired t-test was used to examine differences in effects on blood pressure of the two test diets relative to the uniform control diet. In addition, blood pressures obtained 11 weeks after the completion of the experiment were compared with week 8, and cholesterol levels with pre-experimental values, using a paired t-test [19]. All P-values are two-tailed.

RESULTS

Subjects

One woman and one man withdrew during the first week of the study. The base-line characteristics of the remaining 58 subjects are presented in Table 1. Inspection of the diaries showed that in the last two weeks of the study, two men and one woman on the monounsaturated-fat diet and two men on the polyunsaturated-fat diet had been sick for one to three days. In addition, one man on the monounsaturated-fat diet reported that he had not always consumed the prescribed amount of energy from the free-choice items.

Nutrient intake

Table 2 shows that the proportion of energy from protein, fat, carbohydrates and alcohol was essentially the same on the habitual diets of

Table 1. Age, height, weight, and body mass index of the subjects 6 to 4 weeks before the experiment, at the time they were eating their habitual self-chosen diets.*

	Monounsaturated-fat diet		Polyunsaturated-fat diet	
	Men (N=14)	Women (N=15)	Men (N=13)	Women (N=16)
Age (years)	24.3 ± 7.6	22.9 ± 3.7	25.9 ± 6.7	24.4 ± 6.7
Height (cm)	182.9 ± 5.3	169.4 ± 5.3	184.8 ± 5.5	171.1 ± 7.4
Weight (kg)	73.1 ± 7.0	61.6 ± 5.7	75.1 ± 7.8	62.3 ± 7.2
Body mass index (kg/m^2)	21.9 ± 2.3	21.5 ± 2.1	22.0 ± 2.1	21.2 ± 1.4

* Values are means ± SD.

the subjects and during the control period. However, the dietary fat composition was different. The energy intake from saturated fat intake increased from a group average of 14.6 percent on the habitual diets to 19.3 percent during the control period, while the intake from polyunsaturates decreased on average by 1.7 percent. This resulted in a decrease of the ratio of polyunsaturated to saturated fatty acids from 0.45 to 0.21. After the switch-over to the two unsaturated-fat diets total fat, alcohol, cholesterol and dietary fiber intake did not change. The energy intake from saturated fat decreased by 6.5 percent on both test diets. This decrease was compensated for by an increased intake of monounsaturated (3.5 percent) and polyunsaturated fatty acids (3.0 percent) on the monounsaturated-fat diet and by polyunsaturates alone on the polyunsaturated-fat diet. The intake of sodium, potassium, calcium, and magnesium was constant throughout the study

Dietary adherence

Weight changes did not exceed 2.5 kg over the eight weeks of the experiment, except for one man on the monounsaturated-fat diet who lost 3.2 kg; there was an increase of on average (mean ± SD) 0.2 ± 1.3 kg in the monounsaturated-fat group and of 0.2 ± 1.0 kg in the polyunsaturated-fat group. Changes in weight were not correlated with changes in blood pressure.

Table 2. Mean daily intake of energy and composition of the diets.*

	Type of diet			
	Habitual* (N=58)	Control+ (N=58)	Mono-unsaturated-fat+ (N=29)	Poly-unsaturated-fat+ (N=29)
Energy				
MJ/day	10.1 ± 2.5	11.7	11.8	11.9
kcal/day	2414 ± 598	2796	2820	2844
Protein (% of energy)	14.3 ± 1.8	13.1	13.4	13.1
Fat (% of energy)	35.8 ± 5.6	36.7	37.4	37.6
Saturated fatty acids	14.6 ± 2.9	19.3	12.9	12.6
Monounsaturated fatty acids	12.4 ± 2.2	11.5	15.1	10.8
Polyunsaturated fatty acids	6.3 ± 1.9	4.6	7.9	12.7
Linoleic acid	5.5 ± 1.8	4.1	7.7	12.6
P/S ratio¥	0.45 ± 0.2	0.21	0.61	1.00
Carbohydrates (% of energy)	48.6 ± 6.0	49.1	47.8	48.5
Alcohol (% of energy)	1.7 ± 2.1	1.2	1.5	1.0
Cholesterol (mg/MJ)§	31.4 ± 12.6	33.4	35.8	35.3
Dietary fiber (g/MJ)§	3.1 ± 0.9	4.0	4.1	4.1
Sodium (mmol/MJ)§	12.3 ± 2.3	13.3	12.9	12.5
Potassium (mmol/MJ)§	9.0 ± 1.9	10.3	10.0	10.2
Calcium (mmol/MJ)§	3.6 ± 0.9	3.0	3.1	2.9
Magnesium (mmol/MJ)§¶		1.5	1.5	1.5

* Values were calculated from three-day weighed inventories before the experiment began, and they are means ± SD.

\+ Based on chemical analysis of duplicate diets. The standard deviations for energy intake between subjects during the study were 2.7 MJ for the control diet, 2.8 MJ for the monounsaturated-fat diet, and 2.3 MJ for the polyunsaturated-fat diet. The variation between subjects in the composition of the study diets was negligible; therefore, no standard deviations are given for the nutrients.

¥ Ratio of polyunsaturated to saturated fatty acids.

§ To convert values for the intake of cholesterol to milligrams, dietary fiber to grams, and sodium, potassium, calcium and magnesium to mmol per 1,000 kcal, multiply by 4.184.

¶ No figures for magnesium were available in the food composition table used. Thus, values on the study diets are without the minor contribution of magnesium from free-choice items.

Coffee consumption was not different between the two diet groups and amounted to on average 2.6 cups per day on both the control diet and on the test diet for the monounsaturated-fat group; for the polyunsaturated-fat group coffee consumption was on average 2.4 cups per day on the control diet and 2.6 cups on the test diet.

Dietary adherence was confirmed by the ratio of oleic to linoleic acid of serum cholesteryl esters, which decreased from 0.33 ± 0.04 to 0.29 ± 0.05 in the monounsaturated-fat group, and from 0.33 ± 0.04 to 0.21 ± 0.03 in the polyunsaturated-fat group ($P<0.0001$ for the difference in changes between the two diet groups).

Serum cholesterol levels of the 56 subjects who were also measured 11 weeks after the experiment were 4.83 ± 0.69 mmol/L before the experiment and 4.89 ± 0.78 mmol/L 11 weeks after the experiment (change, 0.06 ± 0.51 mmol/L; not significant). This suggests that subjects had returned to their habitual dietary fat intake.

Blood pressure

The blood pressure values obtained during the study are shown in Figure 1. Systolic blood pressure was the same in the two diet groups and remained virtually unchanged during the course of the experiment. Due to higher innate levels diastolic pressure in the polyunsaturated-fat group was slightly higher at the end of the control period and it remained so during the test period. Mean blood pressures for both diet groups are shown in Table 3 for men and women separately. Changes in systolic blood pressure from the saturated to the unsaturated-fat diets were slight; they varied from -2.4 ± 10.2 mm Hg for men on the polyunsaturated-fat diet to 2.0 ± 7.6 mm Hg for women on the polyunsaturated-fat diet. The change in diastolic blood pressure of -2.9 mm Hg for women on the monounsaturated-fat diet was significantly different from that of 2.4 mm Hg for women on the polyunsaturated-fat diet (95 percent confidence interval for the difference in changes between the diet groups, -10.5 to -0.1 mm Hg; $P=0.047$). This effect, however, was not observed in men.

When post-experimental blood pressure levels were compared with those obtained at the end of the experiment, no difference in changes between the two diets groups was observed.

Excluding the persons who had fallen ill during the experiment from the analysis ($N=53$) did not change the results. The change in systolic blood

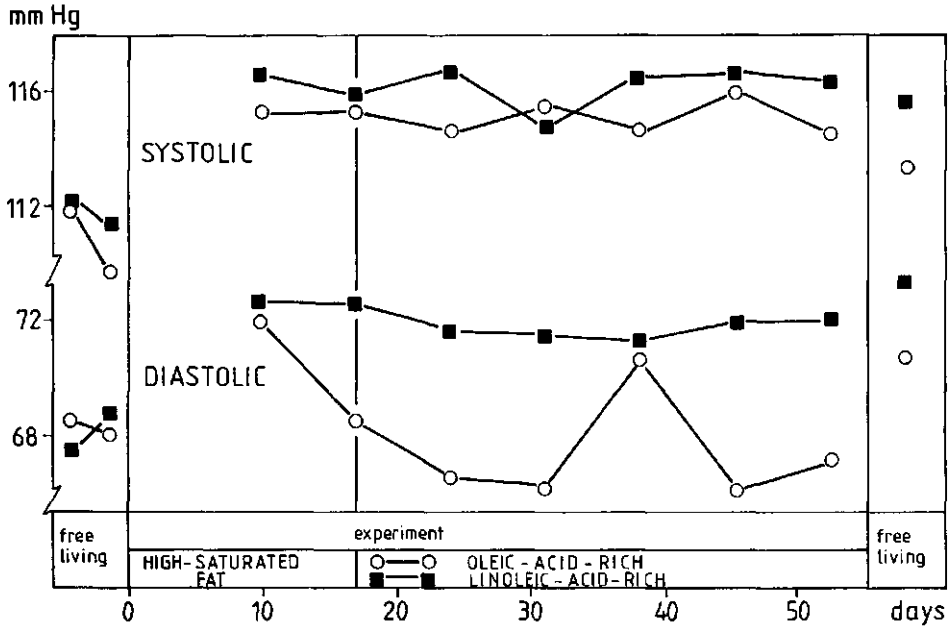

Figure 1. Mean systolic and diastolic blood pressure before, during, and after the experiment.

Subjects were volunteers from the general population who were consuming a diet high in saturated fat (week 3) followed by test diets high in either oleic acid (N=29) or in linoleic acid (N=29).

pressure for men on the monounsaturated-fat diet (N=12) was now 1.6 ± 10.2 mm Hg and in diastolic blood pressure 0.7 ± 7.9 mm Hg. These changes were not significantly different from those of -1.0 ± 9.1 mm Hg and -4.7 ± 9.5 mm Hg for men on the polyunsaturated-fat diet (N=11). For women on the monounsaturated-fat diet (N=14), the change in systolic blood pressure was -1.1 ± 7.6 mm Hg as compared with 2.0 ± 7.6 mm Hg for women on the polyunsaturated-fat diet (N=16). The change in diastolic blood pressure of -3.1 ± 5.6 mm Hg for women on the monounsaturated-fat diet was still greater than that of 2.4 ± 8.3 for women on the polyunsaturated-fat diet (95 percent confidence interval, -10.9 to -0.1 mm Hg; P=0.045).

Table 3. Effects of the monounsaturated-fat and polyunsaturated-fat diet on blood pressure.*

	Men		Women		All	
	Mono-unsaturated-fat diet (N=14)	Poly-unsaturated-fat diet (N=13)	Mono-unsaturated-fat diet (N=15)	Poly-unsaturated-fat diet (N=16)	Mono-unsaturated-fat diet (N=29)	Poly unsaturated-fat diet (N=29)
Systolic						
Pre-experimental	119.4 ± 10.4	118.8 ± 10.4	102.5 ± 7.1	106.2 ± 11.2	110.6 ± 12.2	111.6 ± 12.4
Experimental						
Control period	123.0 ± 8.3	122.4 ± 15.1	108.4 ± 9.5	111.7 ± 11.1	115.5 ± 11.5	116.5 ± 13.9
Test period	122.6 ± 10.7	120.0 ± 9.7	107.2 ± 9.2	113.7 ± 10.9	114.6 ± 12.5	116.5 ± 10.7
Change	-0.4 ± 11.0	-2.4 ± 10.2	-1.2 ± 7.3	2.0 ± 7.6	-0.8 ± 9.1	0.0 ± 9.0
Post-experimental+	120.6 ± 7.7	121.3 ± 8.8	107.1 ± 10.3	112.8 ± 9.1	113.4 ± 11.4	116.4 ± 9.8
Diastolic						
Pre-experimental	69.6 ± 8.3	65.9 ± 7.8	67.3 ± 6.7	69.8 ± 7.6	68.4 ± 7.4	68.1 ± 7.8
Experimental						
Control period	68.8 ± 7.4	69.1 ± 10.9	65.5 ± 6.8	74.4 ± 8.6	67.1 ± 9.6	72.7 ± 9.0
Test period	68.8 ± 12.0	69.1 ± 10.9	65.5 ± 6.8	74.4 ± 8.6	67.1 ± 9.6	72.0 ± 9.8
Change	0.1 ± 7.5	-4.4 ± 9.0	-2.9 ± 5.5¥	2.4 ± 8.4	-1.5 ± 6.6	-0.7 ± 9.2
Post-experimental+	73.5 ± 9.0	72.4 ± 9.7	68.7 ± 7.2	74.2 ± 9.0	70.9 ± 8.3	73.4 ± 9.2

* Values are expressed in mm Hg and are means ± SD.
\+ One man missing from each group
¥ Denotes a significant difference in changes between diet groups ($P<0.05$).

DISCUSSION

In this study with healthy, normotensive subjects we could not demonstrate a favorable effect on blood pressure of linoleic acid, the major essential fatty acid of the (n-6) family, relative to oleic acid when total fat intake was 37 percent of energy. The effect on diastolic pressure for women was even more favorable on the monounsaturated-fat diet than on the polyunsaturated-fat diet. This finding, however, may be due to chance, as the direction of the change was opposite to that expected and the effect was not observed in men. The hypothesis was that blood pressure levels, for the two sexes combined, would not be affected by linoleic acid relative to oleic acid. This hypothesis could not be rejected. With 26 subjects per diet group the statistical power for detecting a true difference of 4 mm Hg between both diets in our study was 52 percent. To increase the power to 80 percent the true difference between both diets should have been 6 mm Hg. That a decrease of 7 mm Hg in systolic blood pressure is possible in normotensive subjects by dietary modification has been shown [20].

The intake of other dietary factors which might influence blood pressure such as sodium, potassium, calcium, magnesium, alcohol, and dietary fiber, was kept constant throughout the study. Changes in weight also have not affected blood pressure levels, as they were small and not related to changes in blood pressure. In addition, blood pressure measurements were done automatically to exclude observer bias and participants did not know the possible effects on blood pressure of the two test diets. Thus, results were not confounded by other factors known to affect blood pressure.

Our results are in agreement with the results of Sacks et al [21], who also found no effect on blood pressure in normotensive subjects when exchanging oleic acid for linoleic acid. Margetts et al [5] did not observe a change in blood pressure when they increased the ratio of polyunsaturated to saturated fatty acids in the diet from 0.3 to 1.0 by replacing saturated and monounsaturated by polyunsaturated fatty acids. In another study it was shown that blood pressure was not affected if polyunsaturated fat intake increased from 3 to 19 percent of energy intake at the expense of a mixture of saturated and monounsaturated fats [6]. Energy intake from fat in these studies [5, 6, 21] was about 40 percent; these results suggest that a high-fat, linoleic-acid-rich diet has no hypotensive effect in normotensive subjects. Other studies, however, demonstrated a decrease in blood pressure when linoleic acid intake was increased at constant fat intake. Heagerty et

al [22] found in a double blind cross-over trial that a supplement of 4 g of safflower oil per day decreased supine systolic pressure in normotensive volunteers. A decrease in both systolic and diastolic blood pressure was reported when the ratio of poyunsaturated to saturated fatty acids was increased from 0.3 to 1.0, irrespective of whether fat intake was 25 or 43 percent of energy [23].

A possible explanation for the discrepancy between the present and other studies is that both diets had the same effect on blood pressure. The linoleic acid intake was increased on both diets, and it is possible that the relationship between linoleic acid and blood pressure can only be demonstrated at lower intakes of linoleic acid. However, blood pressure levels did not decrease in either group upon switching from the high-saturated-fat control diet to either unsaturated diet. Although it cannot be ruled out that a downward effect of both unsaturated diets was balanced by an opposite upward drift, this explanation is unlikely: drifts of blood pressure in time are usually downward and not upward in experiments such as ours.

Another explanation for the absence of an effect of linoleate on blood pressure in our study might be attributed to the relatively low initial blood pressure levels of the volunteers. Puska et al [4] indeed found a more pronounced decrease in both systolic and diastolic blood pressure on a low-fat, linoleic-acid-enriched diet in hypertensives than in normotensives. Strazzullo et al [24], however, reported that saturated fat significantly increased systolic pressure in subjects who had a mean systolic pressure that was even lower than that in our sample.

Our results suggest that two diets differing in the level of unsaturated fatty acids but not in total fat do not influence blood pressure in young normotensive men and women. We conclude that at present there is no conclusive evidence that dietary fat is involved in blood pressure regulation in normotensive men and women.

REFERENCES

1. Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361 662 men. Lancet 1986; 2:933-936.
2. Dawber TR. The Framingham Study. Cambridge, Massachusetts and London: Harvard University Press, 1980.
3. Iacono JM, Marshall MW, Dougherty RM, Wheeler MA, Mackin JF, Canary JJ. Reduction in blood pressure associated with high polyunsaturated fat

diets that reduce blood cholesterol in man. Prev Med 1975; 4:426-443.
4. Puska P, Iacono JM, Nissinen A, et al. Controlled, randomised trial of the effect of dietary fat on blood pressure. Lancet 1983; i:1-5.
5. Margetts BM, Beilin LJ, Armstrong BK, et al. Blood pressure and dietary polyunsaturated and saturated fats: a controlled trial. Clin Sci 1985; 69:165-175.
6. Brussaard JH, van Raaij JMA, Stasse-Wolthuis M, Katan MB, Hautvast JGAJ. Blood pressure and diet in normotensive volunteers: absence of an effect of dietary fiber, protein, or fat. Am J Clin Nutr 1981; 34:2023-2029.
7. Oster P, Arab L, Schellenberg B, Heuck CC, Mordasini R, Schlierf G. Blood pressure and adipose tissue linoleic acid. Res Exp Med (Berl) 1979; 175:287-291.
8. Berry EM, Hirsch J. Does dietary linolenic acid influence blood pressure. Am J Clin Nutr 1986; 44:336-340.
9. Mensink RP, Katan MB. Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. N Engl J Med 1989; 321:436-441.
10. Commissie UCV. UCV Tabel. The Hague: Voorlichtingsbureau voor de Voeding, 1985.
11. Aravanis C, Mensink RP, Karalias N, Christodoulou B, Kafatos A, Katan MB. Serum lipids, apoproteins and nutrient intake in rural Cretan boys consuming high-olive-oil diets. J Clin Epidemiol, 1988; 41:1117-1123.
12. Osborne DR, Voogt P. The analysis of nutrients in foods. London: Academic Press, 1978.
13. Williams S, ed. Official methods of analysis of the association of official analytical chemists. 14th ed. Arlington, VA: AOAC Inc, 1984.
14. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226:497-509.
15. Metcalfe LD, Schmitz AA, Pelka JR. Rapid preparation of fatty acid esters from lipids for gas chromotographic analysis. Anal Chem 1966; 38:514-515.
16. Prosky L, Asp NG, Furda J, de Vries JW, Schweizer TF, Harland BF. Determination of total dietary fiber in foods, food products and total diets: interlaboratory study. J Assoc Off Anal Chem 1984; 67:1044-1053.
17. Van de Bovenkamp P, Katan MB. Cholesterol content of chicken skin. J Food Sci 1981; 46:291.
18. Analytical methods for atomic absorption spectrophotometry. Norwalk: Perkin- Elmer Cooperation, 1976.
19. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames: Iowa State University Press, 1980.
20. Iacono JM, Puska P, Dougherty RM, et al. Effect of dietary fat on blood pressure in a rural Finnish population. Am J Clin Nutr 1983; 38:860-869.
21. Sacks FM, Stampfer MJ, Munoz A, McManus K, Canessa M, Kass EH. Effect of linoleic and oleic acids on blood pressure, blood viscosity, and erythrocyte cation transport. J Am Coll Nutr 1987; 6:179-185.
22. Iacono JM, Judd JT, Marshall MW, et al. The role of dietary essential fatty acids and prostaglandins in reducing blood pressure. Prog Lipid Res 1981; 20:349-364.
23. Heagerty AM, Ollerenshaw JD, Robertson DI, Bing RF, Swales JD. Influence of dietary linoleic acid on leucocyte sodium transport and blood pressure. Br Med J 1986; 293:295-297.
24. Strazzullo P, Ferro-Luzzi A, Siani A, et al. Changing the Mediterranean diet: effects on blood pressure. J Hypertension 1986; 4:407-412.

CHAPTER 8

Effect of dietary trans fatty acids on blood pressure in normotensive subjects

Ronald P. Mensink, Marian H.J. de Louw, Martijn B. Katan

ABSTRACT

Consumption of essential fatty acids of the (n-6) series may affect blood pressure in man. Trans fatty acids in the diet interfere with the metabolism of essential fatty acids in rats. We therefore measured the effect of dietary trans fatty acids on blood pressure in 25 men and 34 women. Each subject consumed, in random order, three mixed natural diets, each for three weeks. The composition of the three diets was similar, except for 10.4 percent of energy, which was provided by either oleic acid, trans fatty acids, or saturated fatty acids. The intake from polyunsaturated fatty acids was 3.4 percent on the saturated-fat diet and 4.6 percent on the other two diets.

On the oleic-acid diet systolic blood pressure was 113 ± 12.8 mm Hg and diastolic blood pressure 66 ± 8.3 mm Hg, on the trans-fatty-acid diet 112 ± 12.2 mm Hg and 67 ± 8.1 mm Hg, and on the saturated-fat diet 112 ± 12.6 mm Hg and 67 ± 8.1 mm Hg. No significant diet effects were observed.

We conclude that neither trans nor saturated fatty acids influence blood pressure levels in normotensive subjects relative to oleic acid.

INTRODUCTION

Trans fatty acids can be formed during hydrogenation or hardening of oils rich in unsaturated fatty acids. Hydrogenation is a widely used process to make fats which can be used for production of certain types of margarines and shortenings [1]. In addition, trans fatty acids are also present in small amounts in fat from ruminants [2]. High intakes of trans fatty acids have been claimed to interfere with the metabolism of polyunsaturated fatty acids in rat [3]. In man, there is some evidence that the intake of these essential fatty acids of the (n-6) and (n-3) series influences blood pressure levels [4, 5], possibly through their effects on prostaglandin formation [6]. It is doubtful whether an interaction between trans fatty

acids and polyusaturated fatty acids also exist in man. However, our controlled trial on the effects of trans fatty acids on serum lipids and lipoproteins [7] gave us the opportunity to examine the effects of trans fatty acids on blood pressure. Trans fatty acids in the diet were replaced by either oleic acid, a monounsaturated fatty acids with the cis configuration, or by saturated fatty acids. Thus, this study also allowed us to examine whether saturated fatty acids affect blood pressure relative to oleic acid. The intake of other nutrients and minerals was similar on all three diets.

METHODS

Subjects

Subjects were 34 female and 25 male volunteers, who participated in an experiment to study the effects of trans fatty acids on serum lipids [7]. Most of them were students at the Agricultural University. All subjects were healthy, as indicated by a medical questionnaire and by the absence of protein and glucose in the urine. One woman used a beta-adrenergic blocking agent throughout the experiment, without any change in dosage. Eight women used oral contraceptives. Two of the women and none of the men were smokers. Baseline characteristics are shown in Table 1.

The protocol and aim of the study were fully explained, and subjects gave their informed consent. Prior approval for the study was obtained from the ethics committee of the Department of Human Nutrition.

Design and diets

All subjects consumed three different diets during three consecutive periods. One diet was high in oleic acid, another high in trans isomers of oleic acid, and the third diet was high in saturated fatty acids. Before the study, subjects were categorized according to sex, and females also according to use of oral contraceptives. From each category about one sixth of the subjects were randomly allocated to one of the six possible diet sequences. Each dietary period lasted three weeks. All subjects were studied simultaneously for 63 days from 26 September to 28 November 1988.

Diets used during the study consisted of conventional solid foods, and

menus were changed daily in a cycle of 21 days. The composition of the three diets was similar, except for 10.4 percent of total energy, which was provided by either oleic acid, trans fatty acids, or saturated fatty acids (Table 2). Special fats and margarines were prepared by the Unilever Research Laboratory, Vlaardingen, The Netherlands. The intakes of protein, fat, polyunsaturated fatty acids, carbohydrates, fiber, sodium, potassium, calcium, and magnesium were similar on the three diets.

Diets were formulated at twenty-eight levels of energy intake, ranging from 5.5 to 20.0 MJ per day. The amount of food necessary to meet each individual's energy requirement was weighed out. Body weights were checked by us twice weekly, and energy intake was adjusted when necessary. All food was provided, except for free-choice items, free of fat and cholesterol. Free-choice items were listed and accorded points corresponding to their energy value, with one point equalling 41.8 kJ (10 kcal). Each subject was required to consume a constant number of points per day, which varied with total energy intake and corresponded with 7 to 11 percent of the total energy intake. Typical choices were an apple (6 points), orange juice (6 points) or a glass of beer (9 points). In our experience, the free-choice system helps subjects to reconcile the rigid requirements of participation in a trial with their social life and personal preferences. The importance of not changing one's selection of free-choice items between periods was repeatedly explained and stressed. Subjects recorded in diaries their free-choice items, the amount of coffee used, and any deviations from the protocol.

Subjects were asked not to change their smoking habits, use of oral contraceptive, or their physical activity pattern during the study.

For each of the three diets, duplicate portions for one imaginary participant with an energy intake of 10 MJ (2390 kcal) per day were collected daily and stored at -20 °C for later analysis.

Other details have been published [7].

Measurements

Baseline data were obtained in August and/or September 1988. Standing height was measured without shoes, and weight without shoes or heavy clothing was recorded to the nearest 0.1 kg. During this period subjects were also asked to weigh and record their food intake on three separate days, two working days and one weekend day. Foods were coded and the

composition of the diets calculated using the 1985 edition of the Netherlands Nutrient Data Bank [8]. From our previous studies it was known that such records tend to underestimate actual energy needs by some 10 to 15 percent. The calculated energy content was therefore increased by 10 percent and this value was used as starting level of energy intake at the start of the experiment.

Blood pressure was measured during the morning hours with an automatic sphygmomanometer with recorder (Takeda Medical UA-751, Adquipment Medical BV, Rotterdam, The Netherlands) on one occasion before and once a week during the experiment. The cuff used was 14 cm wide and had a greatest lenght of 40 cm. Systolic pressure was recorded at Korotkoff phase I and diastolic pressure at Korotkoff phase V. Subjects were asked not to perform physical activity or eat or smoke for one hour prior to the blood pressure measurements. Four measurements at each session were made in a quiet room after approximately 5 min of rest on the left arm with the subject sitting upright. Subjects wore loosely fitting sleeves. During the measurement they rested their left forearm on a table. One trained investigator (MdL), who was blind to each subject's diet, performed all the measurements, using the same sphygmomanometer throughout. All measurements on a particular subject were generally made at the same time of the day to eliminate effects of diurnal variations in blood pressure. Subjects were never told their blood pressure readings, while the experiment lasted.

The fatty-acid composition of erythrocyte membranes on the last day of each dietary period was determined to estimate dietary adherence.

The duplicate portions of each diet period were mixed thoroughly, and then freeze-dried. The ash content and the moisture level [9] were determined and then the material was stored at -20 °C. Aliquots were analysed for protein [10], total fat [11], the proportions of individual fatty acids [12], dietary fiber [13], and cholesterol [14]. Available carbohydrate was calculated by difference. Sodium, potassium, calcium and magnesium were determined by atomic absorption spectrophotometry [15] in the freeze-dried material after it had been wet ashed and neutralized. The mean composition of the diets was calculated from to the duplicate portion analysis plus the calculated contribution of the free-choice items.

Statistical methods

The first blood pressure measurement of each session was discarded and

the remaining three measurements were averaged per subject. Body weights were averaged per week. To compare the effects of the three different diets, an analysis of variance was carried out using the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) [16]. The design of the study made it possible to eliminate variation due to time effects or residual effects of the previous diet [17]. All P-values are two-tailed.

RESULTS

Subjects

The volunteers were between 19 and 57 years of age. The body mass indexes ranged between 18.4 and 25.4 kg/m^2 for men and between 17.4 and 29.7 kg/m^2 for women. Systolic blood pressure, as measured four weeks before the study, ranged from 90 to 156 mm Hg and diastolic blood pressure from 47 to 90 mm Hg (Table 1).

Table 1. Baseline characteristics of the subjects.

	Men (N=25)		Women (N=34)	
	Mean ± SD	Range	Mean ± SD	Range
Age (years)	24.7 ± 8.3	19 - 52	25.6 ± 9.9	19 - 57
Height (cm)	185 ± 6.2	175 - 200	170 ± 6.8	157 - 187
Weight (kg)	75 ± 6.2	65 - 87	64 ± 8.3	54 - 90
Body mass index (kg/m^2)	22.0 ± 1.9	18.4 - 25.4	22.0 ± 2.5	17.4 - 29.7
Blood pressure (mm Hg)				
- diastolic	74 ± 7.9	58 - 90	70 ± 9.6	47 - 88
- systolic	129 ± 12.2	115 - 157	109 ± 10.4	90 - 129

Body weights were on average 69.1 ± 9.3 kg on the oleic acid diet, 69.3 ± 9.4 kg on the trans-fatty-acid diet, and 69.0 ± 9.4 kg on the saturated-fat diet. Over the nine weeks of the study average body weight decreased by 0.3 ± 1.2 kg (range, -3.1 to 2.4 kg).

Table 2. Mean daily intake of energy and composition of the diets.*

	Type of diet			
	Habitual*	Oleic-acid+	Trans-fatty-acid+	Saturated-fat+
Energy				
MJ/day	10.3 ± 3.4	11.6	11.5	11.4
kcal/day	2464 ± 813	2780	2751	2734
Protein (% of energy)	14.3 ± 1.9	13.1	13.3	14.0
Fat (% of energy)	34.7 ± 5.6	39.6	40.2	38.8
Saturated fatty acids	13.9 ± 2.7	9.5	10.0	19.4
Monounsaturated fatty acids	12.3 ± 2.3	24.1	24.2	14.7
Cis-C18:1		23.0	12.6	12.8
Trans-C18:1		0.0	10.9	1.8
Polyunsaturated fatty acids	6.4 ± 2.3	4.6	4.6	3.4
Linoleic acid	5.4 ± 2.2	4.0	4.2	3.9
P/S ratio¥	0.48 ± 0.25	0.48	0.46	0.18
Carbohydrates (% of energy)	49.7 ± 6.5	46.3	45.6	46.1
Mono- and disaccharides	24.7 ± 5.6	26.2	25.3	24.0
Polysaccharides	24.9 ± 4.7	19.9	20.2	22.0
Alcohol (% of energy)	1.6 ± 2.1	1.1	0.9	1.3
Dietary fiber (g/MJ)§	3.2 ± 0.8	4.1	4.1	4.3
Cholesterol (mg/MJ)§		33.4	35.8	35.3
Sodium (mmol/MJ)§	11.5 ± 2.3	11.9	12.1	11.8
Potassium (mmol/MJ)§	10.1 ± 2.0	11.4	11.0	11.1
Calcium (mmol/MJ)§	3.2 ± 0.9	3.0	3.3	3.7
Magnesium (mmol/MJ)§¶		1.5	1.6	1.6

* Values were calculated from three-day weighed inventories before the experiment began, and they are means ± SD. No values for trans fatty acids are available in the food composition table used.

\+ Based on chemical analysis of duplicate diets. Each value represents the mean of three different periods, during which each diet was consumed by a different third of the subjects. Differences in composition of the study diets between periods and between subjects were negligible; therefore, no standard deviations are given for the nutrients.

¥ Ratio of polyunsaturated to saturated fatty acids.

§ To convert values for the intake of cholesterol to milligrams, dietary fiber to grams, and sodium, potassium, calcium and magnesium to mmol per 1,000 kcal, multiply by 4.184.

¶ No figures for magnesium were available in the food composition table used. Thus, values on the study diets are without the minor contribution of magnesium from free-choice items.

Nutrient intake

The mean daily intake of energy and the composition of the diets before and during the study are shown in Table 2. Compared with the diet high in oleic acid the intake from saturated fatty acids did not change on the trans-fatty-acid diet, but the intake increased from 9.5 to 19.4 percent of energy on the saturated-fat diet. The percent of total energy from polyunsaturated fatty acids was 4.6 on the oleic-acid and trans-fatty-acid diet and 3.4 on the saturated-fat diet. The ratio of polyunsaturated to saturated fatty acids was 0.48 on the oleic-acid diet, 0.46 on the trans-fatty-acid diet, and 0.18 on the saturated-fat diet. The consumption of alcohol on the three test diets was similar. The intake of dietary fiber, sodium, potassium, calcium, and magnesium was similar on all three diets. Coffee intake averaged 2.2 cups per day during consumption of the oleic-acid and saturated-fat diet, and 2.3 cups per day during consumption of the trans-fatty-acid diet.

Dietary adherence was confirmed by the fatty-acid composition of erythrocyte membranes. The ratio of cis-C18:1 to trans-C18:1 was 17.1 ± 6.3 on the oleic acid diet, 4.6 ± 1.2 on the trans-fatty-acid diet, and 14.5 ± 4.9 on the saturated-fat diet.

Inspection of the diaries did not reveal any deviations from the study protocol.

Blood pressure

Time courses of blood pressure values are shown in Figure 1. In the third week on the oleic-acid diet, the mean systolic blood pressure was 113 ± 12.8 mm Hg and the mean diastolic blood pressure was 66 ± 8.3 mm Hg. On the trans-fatty-acid diet these values were 113 ± 13.3 (95 percent confidence interval for difference with the oleic-acid diet, -2.9 to 1.5 mm Hg) and 67 ± 8.1 mm Hg (95 percent confidence interval, -0.9 to 2.7 mm Hg), and on the diet high in saturated fat 112 ± 12.6 (95 percent confidence interval, -2.9 to 1.5 mm Hg) and 67 ± 8.3 mm Hg (95 percent confidence interval, -1.0 to 2.5 mm Hg) (Table 3). The blood pressure values on the three test diets were not significantly different from each other. In the thirty subjects with the highest diastolic blood pressures at the start of the study, levels were 78 ± 5.4 mm Hg on their habitual diet, 70 ± 5.4 mm Hg on the oleic-acid diet, 72 ± 6.6 mm Hg on the trans-fatty-acid diet, and 71 ± 8.0 mm Hg on the

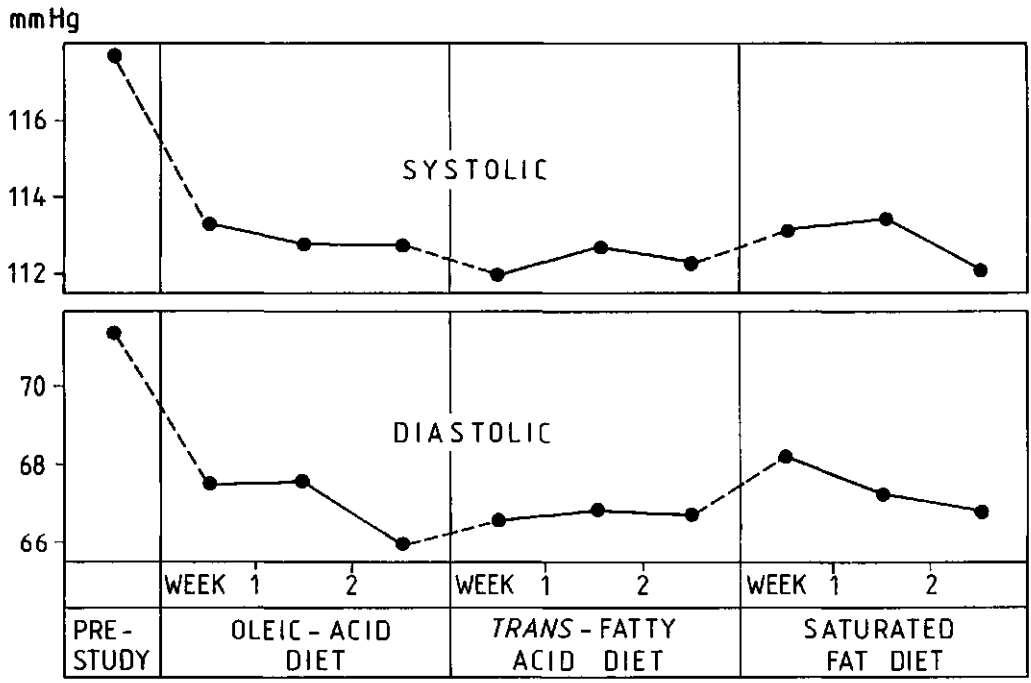

Figure 1. Mean systolic and diastolic blood pressure before and during the experiment.

During the study 24 men and 35 women consumed each diet in random order for three weeks.

saturated-fat diet. The values on the three test diets were not significantly different from each other. The mean systolic blood pressure of the thirty subjects with the highest systolic blood pressure levels was 129 ± 10.8 mm Hg on their habitual diet, 120 ± 12.1 mm Hg on the oleic-acid diet, 121 ± 11.6 mm Hg on the trans-fatty-acid diet, and 119 ± 10.4 mm Hg on the saturated-fat diet. Again, no significant differences were found between the three test diets.

Table 3. Systolic and diastolic blood pressure during consumption of diets high in oleic acid, in trans monounsaturated fatty acids, or in saturated fatty acids.*

	Oleic-acid diet	Trans-fatty-acid diet	Saturated-fat diet
Systolic blood (mm Hg)			
Men	121 ± 11.8	122 ± 12.2	122 ± 8.7
Women	106 ± 9.6	105 ± 9.3	105 ± 10.2
All	113 ± 12.8	112 ± 8.1	112 ± 12.6
Diastolic (mm Hg)			
Men	66 ± 8.3	69 ± 8.5	68 ± 7.6
Women	66 ± 8.5	65 ± 7.7	65 ± 8.3
All	66 ± 8.3	67 ± 8.1	67 ± 8.1

* Values are means ± SD. Each diet was fed for three weeks to each of the 25 men and 34 women, in random order.

DISCUSSION

Animal studies have shown that high levels of trans fatty acids in the diet require an increased intake from the essential fatty acid linoleic acid [3]. In man linoleic acid might lower blood pressure [4]. In theory, diets high in trans fatty acids may thus increase blood pressure. In the present study no effects of trans fatty acids on blood pressure levels in normotensive subjects could be demonstrated relative to cis monounsaturated or saturated fatty acids. Linoleic acid provided 3 to 5 percent of energy intake and total fat 39 to 40 percent in all three diets. The power in this experiment for detecting a true difference of 3 mm Hg between two diets was over the 90 percent. Thus, it is not likely that we have missed a biologically significant change in blood pressure purely by chance. We conclude that trans fatty acids have no effects on blood pressure at the levels of linoleic acid intake that are common in most populations.

Our study also showed that saturated fatty acids have the same effect on blood pressure as cis monounsaturated fatty acids. Few studies have examined the effect of an increased intake of saturated fatty acids on blood pressure at a nearly constant intake of polyunsaturated fatty acids. Two groups of

investigators [18, 19] did not observe an effect on blood pressure when the intake of saturated fat plus monounsaturated fat was decreased and replaced by carbohydrates. In contrast, slight effects on systolic [20] or on systolic and diastolic blood pressure have also been reported [21]. Thus, studies in normotensive subjects have not produced consistent results. It should be noted that in some of these studies saturated fatty acids were replaced by monounsaturated fatty acids [20] but in others by carbohydrates [18, 19, 21]. However, we have earlier shown that monounsaturated fatty acids and carbohydrates have similar effects on blood pressure [22].

It is known that the extent of changes in blood pressure levels caused by diet is related to initial blood pressure values. Puska et al [23] found a greater decrease in both systolic and diastolic blood pressure after dietary fat modification in subjects with mild hypertension than in normotensive subjects. However, it is not likely that the level of blood pressure in our study was too low to allow changes to be seen. Blood pressure levels of the subjects in the two studies where effects of decreasing saturated fat intake were reported [20, 21] were even lower than of our subjects. In addition, we could not demonstrate differences in responses between subjects from the upper and the lower half of the blood pressure distribution.

The experimental diets in our study were consumed for three weeks. It could be argued that this period might have been too short to induce a change in blood pressure. However, other diet studies suggested that blood pressure levels were already stabilized after three weeks [19, 24].

Results in our study were not confounded by other factors which may affect blood pressure: body weights were kept constant, and the intake of dietary fiber, alcohol, sodium, potassium, calcium and magnesium was similar on all three diets. The exchange of trans monounsaturated for either cis monounsaturated or saturated fatty acids was the only dietary intervention. We conclude that at intake levels of linoleic acid higher than 3 percent of energy, trans monounsaturated fatty acids, cis monounsaturated fatty acids and saturated fatty acids have the same effect on blood pressure in normotensive subjects.

REFERENCES

1. Wiedermann LH. Margarine and margarine oil, formulation and control. JAOCS 1978; 55:823-829.
2. Parodi PW. Distribution of isomeric octadecenoic fatty acids in milk fat. J Diary Sci 1976; 59:1870-1873.

3. Hill EG, Johnson SB, Holman RT. Intensification of essential fatty acid deficiency in the rat by dietary trans fatty acids. J Nutr 1979; 109:1759-1766.
4. Stern B, Heyden S, Miller D, Latham G, Klimas A, Pilkington K. Intervention study in high school students with elevated blood pressures. Dietary experiment with polyunsaturated fatty acids. Nutr Metab 1980; 24:137-147.
5. Knapp HR, FitzGerald GA. The antihypertensive effects of fish oil. A controlled study of polyunsaturated fatty acid supplements in essential hypertension. N Engl J Med 1989; 320:1037-1043.
6. Heinemann HO, Lee JB. Prostaglandins and blood pressure control. Am J Med 1976; 61:681-695.
7. Mensink RP, Katan MB. Effect of dietary trans fatty acids on levels of high-density and low-density lipoprotein cholesterol in healthy women and men. This thesis, chapter 5.
8. Commissie UCV. UCV Tabel. The Hague: Voorlichtingsbureau voor de Voeding, 1985.
9. Osborne DR, Voogt P. The analysis of nutrients in foods. London: Academic Press, 1978.
10. Williams S, ed. Official methods of analysis of the association of official analytical chemists. 14th ed. Arlington, VA: AOAC Inc, 1984.
11. Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 1957; 226:497-509.
12. Metcalfe LD, Schmitz AA, Pelka JR. Rapid preparation of fatty acid esters from lipids for gas chromotographic analysis. Anal Chem 1966; 38:514-515.
13. Prosky L, Asp NG, Furda J, de Vries JW, Schweizer TF, Harland BF. Determination of total dietary fiber in foods, food products and total diets: interlaboratory study. J Assoc Off Anal Chem 1984; 67:1044-1053.
14. Van de Bovenkamp P, Katan MB. Cholesterol content of chicken skin. J Food Sci 1981; 46:291.
15. Analytical methods for atomic absorption spectrophotometry. Norwalk: Perkin- Elmer Cooperation, 1976.
16. SAS Institue Inc. SAS User's guide: Statistics Version 5 Edition. Cary, NC: SAS Institue Inc., 1985.
17. Snedecor GW, Cochran WG. Statistical methods. 7th ed. Ames: Iowa State University Press, 1980.
18. Sacks FM, Marais GE, Handysides G, et al. Lack of an effect of dietary saturated fat and cholesterol on blood pressure in normotensives. Hypertension 1984; 6:193-198.
19. Iacono JM, Judd JT, Marshall MW, et al. The role of dietary essential fatty acids and prostaglandins in reducing blood pressure. Prog Lipid Res 1981; 20:349-364.
20. Sacks FM, Donnor A, Castelli WP, et al. Effect of ingestion of meat on plasma cholesterol of vegetarians. JAMA 1981; 246:650-644.
21. Strazzullo P, Ferro-Luzzi A, Siani A, et al. Changing the Mediterranean diet: effects on blood pressure. J Hypertension 1986; 4:407-412.
22. Mensink RP, Janssen MC, Katan MB. Effect on blood pressure of two diets differing in total fat but not in saturated and polyunsaturated fatty acids in healthy volunteers. Am J Clin Nutr 1988; 47:976-980.
23. Puska P, Iacono JM, Nissinen A, et al. Controlled, randomised trial of the effect of dietary fat on blood pressure. Lancet 1983; i:1-5.
24. Iacono JM, Puska P, Dougherty RM, et al. Effect of dietary fat on blood pressure in a rural Finnish population. Am J Clin Nutr 1983;38:860-869.

CHAPTER 9

General discussion

The aim of the studies described in this thesis was to examine the effect of the type and amount of fat in the diet on serum lipids and cholesterol levels in the various lipoproteins, on serum apolipoproteins, and on blood pressure in healthy men and women. Special attention was given to monounsaturated fatty acids.

Execution of the studies

The diets in the studies described in this thesis were well controlled. Factors known to affect serum lipids or blood pressure such as drug treatment and cigarette smoking were absent or did not change during the study, while weight and physical activity were kept as constant as possible. It is therefore highly likely that the effects observed can be attributed to the dietary changes imposed. The monounsaturated fatty acids in the diets consisted largely of oleic acid, polyunsaturated fatty acids consisted of linoleic acid, and the trans fatty acids studied were trans isomers of oleic acid. Complex carbohydrates consisted of a mixture of mono- and disaccharides, polysaccharides, and fiber.

Serum lipids, cholesterol levels in lipoprotein fractions and apolioproteins

The effects of the diets in the three studies on serum lipids, lipoproteins and apolipoproteins are summarized in Table 1.

The results for the serum lipids and lipoproteins suggest that:

- a low-fat diet lowers both HDL and LDL cholesterol, and increases serum triglycerides. In contrast, a high-fat, low-saturated-fat diet lowers predominantly LDL cholesterol;
- polyunsaturated fatty acids have the same effect on HDL cholesterol levels as monounsaturated fatty acids. However, when consumed in amounts above the 8 percent of daily energy intake they are not superior to monounsaturates in lowering the level of LDL cholesterol;

- Trans monounsaturated fatty acids cause an unfavorable distribution of cholesterol over the various lipoproteins;
- Trans-monounsaturated fatty acids and the cholesterol-raising saturated fatty acids raise triglycerides relative to cis unsaturated fatty acids.

Table 1. Summary of the effects of the diets on serum lipids, lipoproteins and apolipoproteins in the three different studies.

Substitution	Cholesterol				Triglycerides	Apolipoprotein		
	Total	LDL	HDL	HDL/LDL		A-I	B	A-I/B
Study I								
Cis monounsaturated fatty acids for carbohydrates	=	=	+ +	+ +	- -	+ +	=	+ +
Study II								
Cis monounsaturated for polyunsaturated fatty acids	=	=	=	=	=	=	=	=
Study III								
Cis for trans monounsaturated fatty acids	-	- -	+ +	+ +	-	+ +	- -	+ +
Cis monounsaturated for saturated fatty acids	- -	- -	=	+ +	-	=	- -	+ +
Trans fatty acids for saturated fatty acids	-	-	- -	-	=	- -	+	- -

The third study showed that trans fatty acids have an HDL cholesterol-lowering effect. This disagrees with the suggestion that all fatty acids raise HDL cholesterol when they replace carbohydrates in the diet [1] In our studies polyunsaturated fatty acids did not decrease HDL cholesterol. Lowering of HDL cholesterol by polyunsaturated fatty acids is frequently seen when intake exceeds 13 percent of energy, but it is rarely seen at lower and more usual intakes [2].

The changes in apolipoprotein A-I were in the same direction as those in HDL cholesterol, and changes in apolipoprotein B were in the same dierection as those in LDL cholesterol. Trans monounsaturated fatty acids, however, increased the level of apolipoprotein B slightly, but decreased that of LDL cholesterol, as relative to saturated fatty acids. These results confirm the

conclusions drawn from the data on serum lipids and lipoproteins.

In summary, our studies show that oils produce more favorable lipoproteins levels than solid fats.

Differences between the sexes

Some studies have suggested that the effect of diet on serum lipids may differ between men and women [3, 4]. Effects of diet on LDL cholesterol levels in our studies were similar for men and women. However, we found that the level of HDL cholesterol decreased and that of triglycerides increased to a greater extent in men than in women when saturated fatty acids were replaced by carbohydrates (Chapter 2). HDL cholesterol and apolipoprotein A-I levels also fell in men but not in women when saturated fatty acids were removed from the diets (Chapter 4). In the third study the effects of dietary fatty acids on serum lipids were not different between the sexes (Chapter 5). By and large these results suggest that present dietary guidelines for optimizing serum lipids levels, which are largely derived from studies on male volunteers, may still be appropriate for women. However, our studies do point out the need for further studies of sex-specific effects of diet on serum lipids.

Blood pressure

In our studies, modification of the amount or type of fat in the diet had no effect on blood pressure in normotensive subjects. Theoretically, we might have missed small differences in changes between diet groups purely by chance. But the differences, if they existed at all, must then be of limited relevance for the formulation of dietary recommendations. Our results are in agreement with those of a number of other investigators [5, 6], although effects of dietary fats on blood pressure in normotensive subjects have indeed be reported [7, 8]. At present, we cannot give an explanation for these discrepant results.

Limitations of our studies

Extrapolation from experiments like ours is limited by a number of considerations. One of these is duration. The results of our studies might help in formulating diets that produce high levels of HDL cholesterol, but

they do not tell whether such an intervention will reduce the risk for coronary heart disease. Long-term intervention studies are needed to evaluate the efficacy of dietary changes for the prevention of coronary heart disease. Another limitation of our studies is the control of energy intake. Weight was strictly kept constant in our studies. However, the spontaneous unrestricted consumption of high-fat diets, which occurs in real life, may increase body weight [9, 10]. Thus, possible beneficial effects of high-fat diets on HDL cholesterol levels might be counteracted by increases in body weight. Finally, our subjects were not a representative sample of the general population. We do not know for certain that our results are valid for other population groups such as subjects who are obese, people with hypertension, or the elderly. These restrictions should be kept in mind when interpreting our results.

Recommendations and conclusions

Health authorities recommend a decrease in total fat intake, in particular in saturated fats, from the current 40 percent of energy intake down to 30 percent or even 20 percent [11, 12]. In order to maintain caloric equilibrium, saturated fatty acids should then be replaced by other macronutrients.

Our findings suggest that replacing saturated fatty acids by unsaturated fatty acids will cause higher HDL cholesterol, but comparable LDL cholesterol levels, as those obtained by replacing saturated fatty acids by complex carbohydrates. However, dietary recommendations are not based solely on the effects of diet on serum lipids. Dietary fatty acids may be involved in the regulation of the blood coagulation system [13], while high-fat diets may increase body weight [10] and are also associated with certain types of cancer [14]. Reducing the fat consumption to 30 percent seems therefore prudent, provided that this reduction is specifically targeted at saturated fatty acids with chain lenghts of 12, 14 or 16 carbon atoms. As far as lipoprotein levels are concerned, our findings do not provide support for an increase in the intake of linoleic acid above 8 percent of energy. Our findings suggest that above that limit, monounsaturated and (n-6)poly-unsaturated fatty acids have the same effect on HDL cholesterol, and are equally effective in reducing the level of LDL cholesterol.

We have found fairly dramatic effects of trans fatty acids on HDL cholesterol levels. Although these results should be confirmed in other

trials, the impact of trans-fatty-acid consumption on lipid levels might not be negligible. It is not appropriate to derive dietary guidelines for the general population on the basis of a single study in a selected group of subjects. However, for the time being, it seems prudent for hypercholesterolemic patients to avoid high intakes of trans fatty acids.

We conclude that oils rich in cis-unsaturated fatty acids, whether they have one double bond (oleic acid) or two double bonds (linoleic acid), might be helpful for the prevention of coronary heart disease. However, the most vital step for optimizing serum lipoprotein levels is to reduce the intake of certain saturated fatty acids and cholesterol. In addition, overweight subjects will also benefit from a reduction in body weight.

REFERENCES

1. Katan MB. Diet and HDL. In: Miller GJ, Miller NE, eds. Metabolic aspects of cardiovascular disease. Vol 3 - Clinical and metabolic aspects of high-density lipoproteins. Oxford: Elsevier 1984; 103-132.
2. Beynen AC, Katan MB. Impact of dietary cholesterol and fatty acids on serum lipids and lipoproteins in man. In: Vergroesen AJ, Crawford MA, eds. The role of fats in human nutrition. London: Academic Press Limited, 1989:237-286.
3. Beveridge JMR, Jagannathan SN, Ford Connel W. The effect of the type and amount of dietary fat on the level of plasma triglycerides in human subjects in the post absorptive state. Can J Biochem 1964; 42:999-1003.
4. Ernst N, Fisher M, Bowen P, Schaefer EJ, Levy RI. Changes in plasma lipids and lipoproteins after a modified fat diet. Lancet 1980; ii:111-113.
5. Sacks FM, Stampfer MJ, Munoz A, McManus K, Canessa M, Kass EH. Effect of linoleic and oleic acids on blood pressure, blood viscosity, and erythrocyte cation transport. J Am Coll Nutr 1987; 6:179-185.
6. Margetts BM, Beilin LJ, Armstrong BK, et al. Blood pressure and dietary polyunsaturated and saturated fats: a controlled trial. Clin Sci 1985; 69:165-175.
7. Iacono JM, Puska P, Dougherty RM, et al. Effect of dietary fat on blood pressure in a rural Finnish population. Am J Clin Nutr 1983; 38:860-869.
8. Puska P, Iacono JM, Nissinen A, et al. Controlled, randomised trial of the effect of dietary fat on blood pressure. Lancet 1983; i:1-5.
9. Aravanis C, Mensink RP, Karalias N, Christodoulou B, Kafatos A, Katan MB. Serum lipids, apoproteins and nutrient intake in rural Cretan boys consuming high-olive-oil diets. J Clin Epidemiol 1988; 41:1117-1123.
10. Lissner L, Levitsky DA, Strupp BJ, Kalkwarf HJ, Roe DA. Dietary fat and the regulation of energy intake in human subjects. Am J Clin Nutr 1987; 46:886-892.
11. Consensus Conference. Lowering blood cholesterol to prevent heart disease. J Am Med Ass 1985; 253:2080-2088.
12. Erkelens DW. Cholesterol consensus in the Netherlands. Eur J Clin Nutr 1989; 43:89-96.

13. Miller GJ, Martin JC, Webster J, et al. Association between dietary fat intake and plasma factor VII coagulant activity - a predictor of cardiovascular mortality. Atherosclerosis 1986; 60:269-277.
14. Rapport opgesteld door de Commissie Voeding en Kanker. Factoren in de voeding en het ontstaan van kanker. Voedingsraad, 1986.

Appendix

Complete fatty-acid composition of the study diets provided, as determined by duplicate portion analyses

Table 1. Fatty-acid composition of the diets used in the "Olive-oil" experiment: see chapter 2, chapter 3 and chapter 4.

Fatty acid	Control diet	Carbohydrate-rich diet	Olive-oil-rich diet
	grams per 100 g of fatty acid		
Saturated			
C8:0	1.2	0.4	0.2
C10:0	2.0	0.4	0.3
C12:0	3.0	1.3	0.9
C14:0	7.8	1.8	1.5
C15:0	0.7	0.3	0.2
C16:0	25.1	14.1	13.8
C17:0	0.7	0.4	0.3
C18:0	10.7	5.6	5.2
C20:0	0.3	1.0	0.4
C22:0	0.2	1.8	0.2
C24:0	0.0	0.9	0.0
Monounsaturated			
C14:1	0.7	0.4	0.0
C16:1	1.8	0.6	1.0
C18:1	27.0	36.2	55.4
C20:1	1.5	3.6	3.2
Polyunsaturated			
C18:2(n-6)	7.7	20.5	10.2
C18:3(n-3)	1.2	0.8	1.5
C18:3(n-6)	0.2	0.2	0.1
C20:2	0.3	0.9	0.4
C20:3	0.0	0.6	0.0
Unknown	7.9	8.2	5.2

Table 2. Fatty-acid composition of the diets used in the "Monopoly" experiment: see chapter 4 and chapter 7.

Fatty acid	Control diet	Mono-unsaturated-fat diet	Poly-unsaturated-fat diet
	grams per 100 g of fatty acid		
Saturated			
C8:0	1.6	0.6	0.6
C10:0	2.2	1.0	0.8
C12:0	3.3	1.9	2.3
C14:0	8.5	3.8	3.4
C15:0	0.9	0.4	0.3
C16:0	24.9	18.1	16.5
C17:0	0.4	0.2	0.1
C18:0	11.0	8.5	9.0
C20:0	0.1	0.4	0.3
C22:0	0.1	0.0	0.4
C24:0	0.0	0.0	0.2
Monounsaturated			
C14:1	0.8	0.3	0.3
C16:1	2.4	1.1	1.0
C18:1	27.7	39.8	27.2
C20:1	0.1	0.3	0.2
C22:1	0.1	0.0	0.2
Polyunsaturated			
C18:2(n-6)	11.0	20.6	34.1
C18:3(n-3)	1.1	1.0	0.5
Unknown	4.0	1.9	2.7

Table 3. Fatty-acid composition of the diets used in the "Cis-Trans" experiment. Each diet was consumed during three different periods: see chapter 5 and chapter 8.

Fatty acid	Oleic-acid diet			Trans-fatty-acid diet			Saturated-fat diet		
	Period			Period			Period		
	I	II	III	I	II	III	I	II	III
	grams per 100 g of fatty acid								
Saturated									
C8:0	0.2	0.3	0.2	0.2	0.2	0.2	1.7	1.4	1.6
C10:0	0.3	0.3	0.3	0.4	0.4	0.3	1.8	1.6	1.6
C12:0	1.1	1.2	1.2	1.4	0.6	0.7	9.1	8.7	8.8
C14:0	1.2	1.2	1.3	1.9	1.5	1.5	7.2	6.7	6.7
C15:0	0.1	0.0	0.0	0.2	0.2	0.2	0.5	0.5	0.4
C16:0	11.6	11.9	11.8	11.3	10.4	10.5	21.4	20.5	20.8
C17:0	0.2	0.0	0.0	0.2	0.0	0.1	0.3	0.2	0.2
C18:0	7.5	7.4	7.6	9.2	9.1	8.7	9.3	9.2	8.7
C20:0	0.5	0.4	0.4	0.4	0.4	0.4	0.3	0.3	0.3
C22:0	0.7	0.6	0.7	0.8	1.0	0.9	0.2	0.3	0.3
C24:0	0.3	0.2	0.3	0.3	0.4	0.3	0.1	0.0	0.2
Monounsaturated									
C14:1	0.0	0.0	0.0	0.1	0.0	0.1	0.4	0.4	0.4
C16:1	0.7	0.6	0.7	0.5	0.4	0.4	0.9	0.8	0.9
Cis-C18:1	61.0	59.5	60.8	33.0	31.6	32.9	33.6	34.1	35.5
Trans-C18:1	0.4	0.0	0.0	26.2	30.1	28.6	1.3	3.1	1.4
C20:1	0.6	0.6	0.5	0.3	0.2	0.3	0.2	0.2	0.2
C22:1	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.2	0.1
C24:1	0.4	0.7	0.1	0.2	0.2	0.0	0.6	0.2	0.2
Polyunsaturated									
C18:2(n-6)	10.0	10.5	10.6	10.5	10.4	11.4	6.9	7.8	8.1
C18:3(n-3)	1.1	1.1	1.1	0.5	0.4	0.6	0.6	0.6	0.7
C18:3(n-6)	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0
C20:2	0.1	0.1	0.0	0.2	0.3	0.0	0.0	0.0	0.1
C20:3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unknown	2.0	3.2	2.2	2.2	2.1	1.7	3.6	3.2	2.9

Summary

Dietary recommendations for reducing the risk of coronary heart disease have been formulated mainly so as to lower the level of total serum cholesterol. The dietary changes proposed involve a reduction in the intake of saturated fatty acids and cholesterol, and for overweight subjects a reduction in energy intake. However, there is uncertainty as to the type of nutrient that should replace the saturated fats in the diet.

The risk of coronary heart disease is positively related to the cholesterol level in the low-density lipoprotein (LDL) fraction, but negatively to that in the high-density lipoprotein (HDL) fraction. The effects of dietary fatty acids and carbohydrates on the concentration of cholesterol in the different lipoproteins have not been well defined. The aim of the research described in this thesis was to examine the effect of monounsaturated fatty acids on HDL and LDL cholesterol levels. Effects on blood pressure, another major determinant for the risk of coronary heart disease, were also studied.

Three strictly controlled dietary studies were carried out with healthy, normolipidemic men and women. Body weights were kept constant throughout the studies. Diets were composed of natural, mixed solid foods, and menus were changed daily. The composition of foods provided was measured by analysis of duplicate portions. Fasting blood samples were analyzed for serum lipids and lipoproteins in a rigidly standardized laboratory.

In the first experiment the effects of monounsaturated fatty acids and carbohydrates on serum lipoproteins were compared. Twenty-four men and 24 women first consumed a control diet high in saturated fatty acids for 17 days. They were then randomly divided over two experimental groups in such a way that both groups contained the same number of males and females. For the next 36 days the energy intake from saturated fatty acids was decreased by 11 percent, and this was compensated for by an increased intake of either monounsaturated fatty acids, in the form of olive oil, or of complex carbohydrates. Serum total cholesterol levels fell by 0.44 mmol/L or 17 mg/dL in the carbohydrate group and by 0.46 mmol/L or 18 mg/dL in the olive oil group. HDL cholesterol levels decreased by 0.19 mmol/L or 7 mg/dL on the high-fiber, high-carbohydrate diet and rose by 0.03 mmol/L or 1 mg/dL the olive oil group ($P<0.001$ for difference in changes between the diet groups).

Triglycerides increased by 0.19 mmol/L or 17 mg/dL on the carbohydrate diet and fell by 0.06 mmol/L or 5 mg/dL on the olive oil diet ($P<0.005$). Changes in both HDL cholesterol and triglycerides were larger in men than in women. We concluded that the olive-oil-rich diet, which combined a high intake of total fat with a low intake of saturated fatty acids, selectively lowered non-HDL cholesterol levels. In contrast, the high-fiber, high-carbohydrate diet lowered both HDL and LDL cholesterol and increased serum trigycerides.

Polyunsaturated fatty acids are thought to lower serum total cholesterol more effectively than do monounsaturated fatty acids. In the second study with 27 men and 31 women, 6.5 percent of total energy from saturated fatty acids was replaced by either a mixture of monounsaturated fatty acids and (n-6)polyunsaturated fatty acids ("monounsaturated-fat" diet) or by (n-6)polyunsaturated fatty acids alone ("polyunsaturated-fat" diet). The difference in intake from polyunsaturated fatty acids between the two diet groups amounted to 4.8 percent. The serum LDL cholesterol level decreased by 17.9 percent on the monounsaturated-fat diet and by 12.9 percent on the polyunsaturated-fat diet ($P<0.05$). The changes produced by the two test diets in HDL cholesterol and triglycerides were similar. However, on both diets HDL cholesterol decreased in men when compared with women. We concluded that polyunsaturated fatty acids, when consumed in amounts above 8 percent of energy, have largely the same effects on serum lipoprotein levels as monounsaturated fatty acids.

Monounsaturated fatty acids as found in natural oils and fats have mainly the cis configuration. Fatty acids with the trans configuration are found in large amounts in hydrogenated oils, but their effects on serum lipoproteins in man are unknown. In our third study 34 women and 25 men consumed three diets for three weeks, in random order. The nutrient composition of the diets was identical except for 10 percent of energy intake, which was provided as either oleic acid - a monounsaturated fatty acid with the cis configuration -, trans isomers of oleic acid, or saturated fatty acids, mainly laurate and palmitate. On the trans-fatty-acid diet subjects' high density lipoprotein (HDL) cholesterol levels fell by 0.17 mmol/L or 6.6 mg/dL compared with values on the diet high in oleic acid ($P<0.017$ for difference with the oleic-acid diet). HDL cholesterol on the saturated-fat diet was the same as on the oleic-acid diet. Low-density lipoprotein cholesterol increased by 0.37 mmol/L or 14.3 mg/dL on the trans-fatty-acid diet ($P<0.017$) and by 0.47 mmol/L or 18.2 mg/dL on the saturated-fat diet ($P<0.017$). The level of triglycerides was 0.13 mmol/L or 11.5 mg/dL higher

on both the trans-fatty-acid diet and the saturated-fat diet than on the oleic-acid diet ($P<0.017$). The differences in effects between the diets were similar in men and in women. These results indicate that the serum lipoprotein profile on the trans-fatty-acid diet was even more unfavorable than that on the saturated-fat diet.

No specific effects on blood pressure were detected in any of the three experiments.

We conclude that replacements of fats high in saturated or trans fatty acids by oils high in oleic acid or linoleic acid might be helpful for the prevention of coronary heart disease, as far as lipoprotein levels concerned. Weight gain, however, might be an unwanted complication of high-oil diets.

Samenvatting

Voedingsrichtlijnen ter voorkoming van coronaire hartziekten zijn er met name op gericht om het serumcholesterolgehalte te verlagen. Hiertoe wordt een daling in de consumptie van verzadigde vetzuren en cholesterol aanbevolen, en een stijging in die van meervoudig onverzadigde vetzuren en complexe koolhydraten. Tevens is behandeling van overgewicht gewenst. Over de inneming van enkelvoudig onverzadigde vetzuren worden nauwelijks concrete uitspraken gedaan. Het is echter gebleken dat, behalve het totale serumcholesterolgehalte, de verdeling van het cholesterol over de verschillende lipoproteinen een belangrijke rol speelt bij het ontstaan van coronaire hartziekten. Een hoog cholesterolgehalte in de lage dichtheidslipoproteinen (LDL) veroorzaakt een verhoogd risico voor coronaire hartziekten, terwijl een hoge concentratie cholesterol in de hoge dichtheidslipoproteinen (HDL) juist een beschermende werking lijkt te hebben, of althans een lager risico voorspelt. Er is weinig bekend over de effecten van de verschillende typen vetzuren en koolhydraten op het cholesterolgehalte in deze afzonderlijke lipoproteinen. In de studies die in dit proefschrift staan beschreven onderzochten wij de effecten van enkelvoudig onverzadigde vetzuren op het HDL- en LDL-cholesterolgehalte bij de mens. Tevens zijn de effecten van deze vetzuren op de bloeddruk onderzocht. Een hoge bloeddruk gaat samen met een verhoogd risico voor hart- en vaatziekten.

Drie gecontroleerde voedingsexperimenten zijn uitgevoerd met gezonde mannen en vrouwen. De samenstelling van de voedingen die aan de deelnemers werden verstrekt werd bepaald door analyses van dubbele porties. Tijdens de proef werd bloed afgenomen, nadat de deelnemers gedurende minstens negen uur geen energie-bevattende voedingsmiddelen hadden gebruikt. De deelnemers behielden een zo constant mogelijk gewicht tijdens de studies.

In de eerste studie werden de effecten van enkelvoudig onverzadigde vetzuren op het totaal en HDL-cholesterolgehalte vergeleken met die van koolhydraten. De eerste zeventien dagen van het onderzoek kregen 24 mannen en 24 vrouwen een voeding rijk aan verzadigde vetzuren. Hierna werden zij aselect ingedeeld in twee groepen; het aantal mannen en vrouwen was in beide groepen gelijk. Eén groep kreeg de volgende 36 dagen een olijfolierijke voeding, terwijl de andere groep een koolhydraatrijke voeding kreeg. Hiertoe werden 11 energieprocenten verzadigd vet vervangen door in de ene groep

enkelvoudig onverzadigde vetzuren en in de andere groep koolhydraten. Het serumcholesterolgehalte daalde 0,44 mmol/L op de koolhydraatrijke voeding en 0,46 mmol/L op de olijfolierijke voeding. Het HDL-cholesterolgehalte daalde 0,19 mmol/L op de koolhydraatrijke voeding en steeg 0,03 mmol/L op de olijfolierijke voeding ($P<0,001$ voor het verschil in effect tussen beide voedingen). Het triglyceridengehalte steeg 0,19 mmol/L bij de deelnemers in de koolhydraatgroep en daalde 0,06 mmol/L in de olijfoliegroep ($P<0,005$). De veranderingen in zowel het HDL-cholesterol als in de triglyceriden waren groter voor mannen dan voor vrouwen. Uit deze resultaten blijkt dat de olijfolierijke voeding, waarin een hoge vetgehalte gecombineerd werd met een laag gehalte aan verzadigd vet, het HDL-cholesterolgehalte niet verlaagde: de daling in serumcholesterol vond voornamelijk plaats in het LDL-cholesterol. De koolhydraatrijke voeding verlaagde echter zowel het LDL-cholesterol als het "beschermende" HDL-cholesterol.

Aan meervoudig onverzadigde vetzuren, met name aan linolzuur, wordt een cholesterolverlagende werking toegeschreven ten opzichte van enkelvoudig onverzadigde vetzuren. In de tweede studie kregen 27 mannen en 31 vrouwen eerst een voeding rijk aan verzadigde vetzuren. Hierna werden 6,5 energieprocenten verzadigd vet vervangen door hetzij enkelvoudig plus meervoudig onverzadigde vetzuren (oliezuur en linolzuur: "Mono-voeding") hetzij door alleen meervoudig onverzadigde vetzuren (linolzuur: "Poly-voeding"). Het verschil in de opneming van meervoudig onverzadigde vetzuren bedroeg 4,8 energieprocenten. De opneming van meervoudig onverzadigd vet in de Poly-groep was 12.7 energieprocenten. Het LDL-cholesterolgehalte daalde 17.9 procent in de Mono-groep en 12.9 in de Poly-groep ($P<0,05$). Beide voeding hadden eenzelfde effect op het HDL-cholesterolgehalte en ook op het triglyceridengehalte. Het HDL-cholesterol echter daalde op beide voedingen bij mannen in vergelijking met vrouwen. Deze resultaten tonen aan dat het vervangen van verzadigde vetzuren door enkelvoudig onverzadigde vetzuren eenzelfde gunstig effect op de serumlipiden kunnen hebben als vervanging door meervoudig onverzadigde vetzuren indien de opneming van linolzuur bij aanvang al meer dan 8 energieprocenten bedraagt.

Tijdens het harden van plantaardige oliën wordt een gedeelte van de cis-dubbele bindingen in onverzadigde vetzuren omgezet in trans-dubbele bindingen. De effecten van transvetzuren op het HDL- en het LDL-cholesterolgehalte bij mensen zijn niet eerder bestudeerd. In onze derde studie kregen 25 mannen en 34 vrouwen drie verschillende voedingen. De samenstelling van de voedingen was gelijk, behalve dat 10 energieprocenten geleverd werd door

hetzij oliezuur, een enkelvoudig onverzadigde vetzuur met één cis-dubbele binding, hetzij door trans-isomeren van oliezuur, hetzij door verzadigde vetzuren, met name laurinezuur en palmitinezuur. Iedere deelnemer kreeg in willekeurige volgorde elk van de drie voedingen gedurende drie weken verstrekt. In vergelijking met de oliezuurrijke voeding was het LDL-cholesterolgehalte 0,37 mmol/L hoger op de trans-vetzuurrijke voeding ($P<0,017$ voor het verschil met de oliezuurrijke voeding) en 0,47 mmol/L hoger op de verzadigd-vetzuurrijke voeding ($P<0,017$). Het HDL-cholesterolgehalte was gelijk op de oliezuurrijke en de verzadigd-vetzuurrijke voeding, maar was 0,17 mmol/L lager op de trans-vetzuurrijke voeding ($P<0,017$). Het triglyceridengehalte was 0,13 mmol/L ($P<0,017$) hoger op de trans-vetzuurrijke en de verzadigd-vetzuurrijke voeding dan op de oliezuurrijke voeding. Deze resultaten laten zien dat transvetzuren een ongunstig effect hebben op het HDL- en LDL-cholesterolgehalte.

De verstrekte voedingen hadden in geen van de studies effect op de bloeddruk.

Uit deze studies blijkt dat het vervangen van verzadigde vetzuren en trans-onverzadigde vetzuren door enkelvoudig onverzadigde vetzuren met een cis-dubbele binding van nut kan zijn bij de preventie van coronaire hartziekten. Echter, men moet erop bedacht zijn dat dergelijke olierijke voedingen gepaard kunnen gaan met een toeneming in het lichaamsgewicht, hetgeen de gunstige effecten op de lipoproteinen weer teniet kan doen.

Curriculum Vitae

Ronald Peter Mensink werd geboren op 30 april 1960 te Velp. In 1978 behaalde hij het gymnasium-ß diploma aan Het Rhedens Lyceum te Rozendaal. In datzelfde jaar begon hij zijn studie aan de toenmalige Landbouwhogeschool te Wageningen. Vanaf juli 1984 tot en met januari 1985 werd een stageperiode doorgebracht bij Dr. Trevor C. Beard "at the Wooden Valley Hospital, Canberra, Australia". In juli 1985 studeerde hij af in de afstudeerrichting "Humane Voeding" met Humane Voeding als verzwaard hoofdvak, Wiskundige Statistiek als bijvak, en Gezondsheidsleer als extra bijvak. Vanaf september 1985 was hij in dienst bij de Vakgroep Humane Voeding, eerst als Tewerkgesteld Erkend Gewetensbezwaarde, later als onderzoeksassistent in het kader van het "Dr. E. Dekker-programma" van de Nederlandse Hartstichting. Gedurende deze periode werd het in dit proefschrift beschreven onderzoek verricht.