DETERMINANTS OF TOTAL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN BOYS AND MEN WITH SPECIAL REFERENCE TO DIET

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

Co-referent: dr.C.E.West, wetenschappelijk hoofdmedewerker aan de Landbouwhogeschool

J.T. KNUIMAN

DETERMINANTS OF TOTAL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN BOYS AND MEN WITH SPECIAL REFERENCE TO DIET

PROEFSCHRIFT
TER VERKRIJGING VAN DE GRAAD VAN
DOCTOR IN DE LANDBOUWWETENSCHAPPEN,
OP GEZAG VAN DE RECTOR MAGNIFICUS,
DR. C.C. OOSTERLEE,
HOOGLERAAR IN DE VEETEELTWETENSCHAP,
IN HET OPENBAAR TE VERDEDIGEN
OP VRIJDAG 20 MEI 1983
DES NAMIDDAGS TE VIER UUR IN DE AULA
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN

ISN = 18113[illegible]-03

Financial support by the Netherlands Heart Foundation for the publication of this thesis is gratefully acknowledged.

STELLINGEN

1. Zowel de concentraties van totaal als van high density lipoprotein (HDL) cholesterol in het serum zijn hoger bij een meer "verwesterde" voeding rijk aan verzadigd vet en arm aan complexe koolhydraten.
   Dit proefschrift.

2. Een lage concentratie van HDL cholesterol in het serum bij een habitueel lage vetopname brengt wellicht geen hoger risico met zich mee op het krijgen van coronaire hartziekten dan een hoge concentratie van HDL cholesterol bij een habitueel hoge vetopname.
   Dit proefschrift.

3. Relaties tussen parameters van de voedselopname en de concentraties van totaal en HDL cholesterol in het serum zijn aantoonbaar binnen populaties.
   Dit proefschrift.

4. De afwezigheid van een negatieve relatie tussen de gemiddelde HDL cholesterol niveaus in het serum en de sterftecijfers van coronaire hartziekten van populaties is niet onverenigbaar met de beschermende functie die het HDL cholesterol wordt toebedacht.
   Dit proefschrift.

5. De thans gebruikte aanbevolen hoeveelheden zijn ongeschikt voor de evaluatie van de voedselopname van individuen en ook voor die van groepen.

6. Niets lijkt zoveel op een opvallend interessante ontdekking als een resultaat bereikt met een onnauwkeurige methode.

7. Het preventieve effect van een verlaging van het cholesterolgehalte in het serum bereikt door wijzigingen in de voeding dient niet zonder meer gelijkgesteld te worden aan het effect van een zelfde verlaging bereikt door medicijnen.

8. Eet wat u wordt voorgezet en geneest de zieken die er zijn. Want wat uw mond binnengaat zal u niet onrein maken, maar wat uw mond uitgaat dat maakt u onrein.

   Jezus Christus in het Thomas evangelie, logion 14.
   Uitgeverij Karnak, Amsterdam, 1980.

9. Het onderzoek naar biochemische indicatoren van de voedselopname dient sterk te worden gestimuleerd.

10. Teneinde een gelijke verdeling van de lasten en lusten bij het krijgen van een kind te bevorderen, zou het aanbeveling verdienen ook de in het arbeidsproces participerende vader een aantal weken met gedeeltelijk "geboorteverlof" te zenden.

Proefschrift J.T.Knuiman
Determinants of total and high density lipoprotein cholesterol in boys and men with special reference to diet
Wageningen, 20 mei 1983

CONTENTS

PREFACE

In this thesis several international studies on the determinants of total and high density lipoprotein (HDL) cholesterol are described. Special attention was given to the standardization of measurements and the estimation of food intake. The project was carried out in the Department of Human Nutrition of the Agricultural University in Wageningen, the Netherlands in cooperation with institutes in several countries. The project was financially supported by the Netherlands Heart Foundation. I am very grateful to the very many people who gave advice, support and assistance in carrying out the various studies. The people involved are too numerous to mention them all by name but I would like to thank them sincerely.

I would like to thank Prof. J.G.A.J.Hautvast for his confidence in me to design, to carry out and to coordinate the various studies. I am grateful for the fruitful discussions we have had throughout the project. I would also like to thank my co-referent, Dr C.E.West, for his assistance with all aspects of the project, especially for his help in preparing the working plans for the various studies and for critically reviewing the manuscripts. I am very grateful to Susanne Westenbrink and Lidwien van der Heyden for their invaluable assistance with the food consumption study and their cheerful support throughout the project. I am also very grateful to Jan Burema for his assistance with the statistical analysis.

I am indebted to the many people who helped with the international study described in Chapter 5 and many of whom also assisted with the studies described in Chapters 2 and 3. I would particularly like to mention: Assoc. Prof. L.Räsänen, Dr J.Viikari and Ms L.Virkkunen, M.Sc. from Finland; Ms P.Lokko, M.Sc. and Prof. J.O.M.Pobee from Ghana; Prof. A.Ferro-Luzzi, Dr A.M.Ferrini, Dr C. Scaccini and Ms S.Sette from Italy; Ms G.M.Villavieja, M.Sc. and Dr.J.Bulatao-Jayme from the Philippines.

In addition, I would like to thank the following people who were also involved in carrying out the two studies described in Chapters 2 and 3. Dr G.P. Ravelli, Dr J.Pierre and Dr P.J.Lamotte from Ivory Coast; Dr J.B.Fashakin, Dr C.O.Adesanya and Dr F.I.Anjorin from Nigeria; Dr A.Hilvers, Prof. I.Oemrawsingh and Ir M.Asmoredjo-Kirchmann from Suriname; Prof. R.M.Lauer and Dr M.F.Sowers from the U.S.A.; Dr M.Ilyas from Pakistan; Dr P.Dijkhuizen (NUFFIC-ICFSN project Los Banos) from the Philippines; Dr K.Widhalm from Austria; Dr K.Ibsen, Dr H. Meinertz and Dr O.Faergeman from Denmark; Prof. P.Puska from Finland; Prof.

C.Aravanis from Greece; Dr I.Kamarás from Hungary; Prof. O.Conor Ward from Ireland; Dr S.L.Rywik from Poland; Dr A.M.C.Nunes, Dr F.de Padua and Dr J.A.Gil Forte from Portugal; Dr I.Balaguer-Vintro from Spain; and Dr L.I.Hardell from Sweden.

Throughout the project I had stimulating and critical discussions with the following people: Dr M.B.Katan and Ms W.A.van Staveren, M.Sc. in the Department of Human Nutrition and also with Prof. R.J.J.Hermus, Dr J.Vandenbroucke, Dr D.Kromhout, Dr F.van der Haar, Dr A.Cramwinckel, Ms W.Boeijen, Drs M.J.Jansen, Dr E.Dekker, Prof. F.ten Hoor and Prof. H.K.A.Visser. I am also grateful to Ms J.Marr from the Department of Clinical Epidemiology, Royal Free Hospital, London, England and to Dr T.Strasser from the Cardiovascular Disease Unit, World Health Organization, Geneva, Switzerland for their advice in the planning of the various studies.

Many people gave practical advice and assistance with the various studies and I would especially like to mention: Mr M.Callebert, Mr H.Overweg, Ms M. Riemens-Dijksma and Dr J.van Haaren who helped us in making contact with the men who participated in the study described in Chapter 4; Drs G.Makkink and her colleagues for their assistance in the various studies with the schoolboys; and Drs A.L.M.Verbeek for his advice and carrying out the clinical examinations of the macrobiotics in Belgium.

There are many people in the Department of Human Nutrition for whose assistance in carrying out the project I am particularly grateful: Helmi van der Wiel-Wetzels for the measurement of skinfolds; Ans Soffers, Joke Barendse-van Leeuwen and Cock Germing-Nouwen for technical assistance; Annemieke Jaartsveld and Christel Sijstermans-Mertens for assistance in collecting the food intake data; Janna de Boer for the computer analysis of the data described in Chapter 5; Hannie van Oosten-Van der Goes, Ben Scholte and Jaap Booy for their assistance with data processing; Truus Kosmeyer-Schuil, Jannie Bos, Elly Koot-Gronsveld, Lizelotte van der Snoek and Peter van den Bovenkamp for the analysis of food samples; Henny Verwey and Frans Schouten for the analysis of total and HDL cholesterol; Riekie van der Molen-Janssen and Marianne Reinink for typing articles included in the thesis. I am grateful to Mr P.Middelburg and Ms N.Th.Broekmeyer-Rustige for assistance with administration and financial matters.

Finally, I would like to thank my wife, Willy, for the patience and understanding and also for typing the thesis.

Wageningen, February 1983 Jan Knuiman

1 INTRODUCTION*

Coronary heart disease and its determinants

Mortality from coronary heart disease is high in western countries especially when compared with less developed countries. In the Netherlands since the Second World War, there has been a trend towards a higher incidence at a younger age (11) and this premature morbidity and mortality from coronary heart disease has an enormous social and economic impact. Therefore, a tremendous amount of work has been done throughout the world to identify factors causing the disease.

The disease appears to be multifactorial and a number of factors related to the individual and his environment would appear to be associated with the high mortality from coronary heart disease. Nutritional factors are likely to play an important role (12). Much of the evidence indicates that nutritional factors operate through the action of the lipids and lipoproteins in the serum. For example, high correlations have been found between the proportion of energy in the diet derived from saturated fat, the concentration of cholesterol in the serum, the mortality from coronary heart disease and the extent of atherosclerotic plaques in the coronary artery between populations (13 - 16). There is a relationship, both between and within populations, of the concentration of serum cholesterol with the risk of coronary heart disease (14, 17). Animals receiving hypercholesterolaemic diets are more prone to coronary heart disease than animals on normocholesterolaemic diets (18, 19). Children genetically predisposed to having elevated serum cholesterol concentrations, particularly those homozygous for type II hyperlipoproteinaemia (familial hyperlipoproteinaemia), often die from coronary heart disease before reaching the age of 20 (20). Long term intervention studies have shown that it is possible to reduce the incidence of coronary heart disease when the dietary intake of saturated fat is decreased and that of linoleic acid is increased (21, 22). Analysis of the components of the atherosclerotic plaque and studies on the origin of material in the plaque are consistent with the concept that serum lipoproteins have a role in the aetiology of coronary heart disease (23).

*Largely based on reference 1. In addition aspects of the work related to that described here have also been published (2 - 10).

The concept of serum lipoproteins

Since the earlier descriptions of the relationship between the concentration of cholesterol in serum and the incidence of coronary heart disease, the picture has been refined. Cholesterol is transported in serum together with other lipids bound to proteins as serum lipoproteins. These lipoproteins can be separated by ultracentrifugation in a density gradient on the basis of their density into a number of classes: the very low density lipoproteins (VLDL), the low density lipoproteins (LDL) and the high density lipoproteins (HDL).

Epidemiological studies indicate that LDL particles appear to be damaging to the arteries while HDL particles apparently are beneficial (24 - 27) and laboratory studies support these findings. For example Steinberg et al (28) have shown that the binding of LDL to the surface of aortic smooth muscle cells is reduced by HDL and Miller et al (29) have reported similar observations with fibroblasts. It is also interesting to mention the study of Zilversmit et al (30) in which it was shown that mink fed a commercial ration moderately high in cholesterol or a semipurified diet have serum cholesterol concentrations similar to those found in humans. However, even after two years on such diets, the aortas and coronary arteries of the mink were found to be free of fatty streaks and atherosclerotic plaques. This may be due to the fact that in mink, unlike in humans, 80 percent of the cholesterol in the serum is carried in the HDL subfraction.

Coronary heart disease and children

Despite the enormous body of knowledge that has accumulated on the relationship between nutrition and coronary heart disease, the results of nutritional intervention studies aimed at reducing the incidence of coronary heart disease (21, 22) have been somewhat disappointing. It is possible that intervention earlier in life would be more effective. Studies in non-human primates have shown that experimentally induced fatty streaks and fibrous plaques can be made to regress by feeding diets low in cholesterol and saturated fat (31). However, only the simplest fatty streaks appear to be truly reversible. It may well be, as suggested by Pearson et al (32), that two populations of fatty streaks exist: one destined, perhaps by its location or risk factors, to develop into fibrous plaques; and a second which remains as fatty streaks and never goes on to become fibrous plaques.

Fibrous plaques can already be found in the coronary arteries of persons

in their early twenties (33, 34) and even in children below 15 years of age (35). The concept that atherosclerosis possibly begins in childhood probably dates from the paper of Holman (36) in 1961 in which he drew attention to the pathology of atherosclerosis in children. As a result of this early work, many studies on the risk factors for coronary heart disease in children have already been carried out or have commenced.

Objectives for research in infants and children

According to the World Health Organization studies in children should try to provide answers to the following questions (37):

- what is the magnitude of the risk indicators of coronary heart disease in infants and children living in different biological, social and cultural environments;
- how does the magnitude of the risk indicators in the infants and young children relate to the magnitude of the risk indicators and to the disease itself in the parents and grandparents;
- at what time and under what circumstances does an increased level of the risk indicators appear and what is the cause of this increase;
- what can be done to prevent an increased level of risk indicators from developing and, more importantly, to prevent the onset of severe coronary heart disease and its complications.

Results of studies carried out in children

Many studies in infants and children have been carried out in the last two decades, especially in the more affluent countries in the developed world (38 - 50). In the Netherlands too, a number of studies was undertaken (40, 41). From 1974 to 1976 Van der Haar and Kromhout (41) carried out a regional comparative study in children aged 6 - 10 years in three towns. The mean serum cholesterol concentration was found to be about 4.5 mmol/l and the mean HDL cholesterol concentration was about 1.4 mmol/l. A need was felt to compare the results of this study with those of children in other countries. However, relatively little was known about the concentration of total and HDL cholesterol in the serum of children living in countries with low mortality of coronary heart disease. In addition, it was also difficult to compare published data on serum total and HDL cholesterol concentrations, because of variations not due to biological factors of the actual concentrations (51). Variability may

arise from differences in the methods used or by the use of similar methods not only by different people but also by the same people under different conditions or at a different time. Variability may also arise from differences in the methods of blood sampling, the storage of serum, etc.

The studies described in this thesis

The first study described in this thesis (Chapter 2) was designed to obtain reliable data on the concentrations of total and HDL cholesterol in the serum of 7 and 8 year-old boys from sixteen countries with different rates of mortality from coronary heart disease. A standardized protocol was used for the collection of samples with the analyses being carried out in a single laboratory.

Surprisingly, the results of this study showed that the mean concentrations of HDL cholesterol appeared to increase linearly with that of total cholesterol ($r = 0.90$, $n = 26$ regions). Even for the 15 European regions, the correlation coefficient was almost identical ($r = 0.89$). This result suggested that the mean concentration of HDL cholesterol would also be positively related to the mortality of coronary heart disease as the mean concentration of total cholesterol is highly correlated with the mortality rate of coronary heart disease between populations and provided that our findings in boys could be extrapolated to adults. This result also suggested that a western type of diet with a relatively high contribution of animal products and therefore rich in saturated fat and relatively poor in complex carbohydrates, is responsible for the higher serum total and HDL cholesterol concentrations in the boys of the more developed countries.

The second study (Chapter 3) was carried out in adult men from thirteen countries with different rates of mortality from coronary heart disease to examine whether the findings in the boys can actually be extrapolated to adults. The study was carried out in a similar way as the first study.

The third and fourth studies (Chapter 4 and 5, respectively) were carried out to examine whether a diet relatively rich in saturated fat and relatively poor in complex carbohydrates is associated with relatively high concentrations of both total and HDL cholesterol. The third study (Chapter 4) was carried out in macrobiotic, vegetarian and non-vegetarian men and boys living in the Netherlands and the Flemish part of Belgium. As the study was carried out in such a relatively small geographical area, the influence of confounding factors, which may result from a between country comparison, was

reduced. The fourth study (Chapter 5) was carried out in boys living in Finland, the Netherlands, Italy, the Philippines and Ghana. The relationships between dietary variables and the concentrations of total and HDL cholesterol were examined by comparing the mean values for the groups from the different countries and by looking for associations between dietary variables and the concentrations of total and HDL cholesterol within the groups from the various countries.

Finally, a general discussion of the studies is presented in Chapter 6.

REFERENCES

1. Knuiman JT, West CE, Hautvast JGAJ. Infant and child nutrition: the effects on serum lipids and the consequences in later life. Biblthca Nutr Dieta 31, 131 (1982).
2. Knuiman JT, West CE, Hermus RJJ, Hautvast JGAJ. Is serum cholesterol outmoded? Lancet ii, 1183 (1979).
3. Knuiman JT, Hermus RJJ, Hautvast JGAJ. Serum total and HDL cholesterol concentrations in rural and urban boys from 16 countries. Atherosclerosis 36, 529 (1980).
4. Knuiman JT, West CE. HDL cholesterol in men from 13 countries. Lancet ii, 367 (1981).
5. Knuiman JT, West CE, Hautvast JGAJ. Role of diet to HDL cholesterol and coronary heart disease: serum total and high density lipoprotein cholesterol in boys and men from developing and developed countries. Am Heart J 103, 447 (1982).
6. Knuiman JT, West CE, Burema J. Serum total and high density lipoprotein cholesterol concentrations and body mass index in adult men from 13 countries. Am J Epid 116, 631 (1982).
7. Knuiman JT, West CE. The concentration of cholesterol in serum and in various serum lipoproteins in macrobiotic, vegetarian and non-vegetarian men and boys. Atherosclerosis 43, 71 (1982).
8. Knuiman JT, Van der Berg H, Verbeek ALM. Enige klinische, klinisch-chemische en antropometrische bevindingen in een vooronderzoek bij zich macrobiotisch voedende mannen en jongens. Voeding 43, 250 (1982).
9. Knuiman JT, West CE. Differences in HDL cholesterol between populations: no paradox? Lancet i, 296 (1983).
10. Knuiman JT, Westenbrink S, Van der Heyden L, West CE, Burema J, De Boer J, Hautvast JGAJ, Räsänen L, Virkkunen L, Viikari J, Lokko P, Pobee JOM, Ferro-Luzzi A, Ferrini AM, Scaccini C, Sette S, Villavieja GM, Bulatao-Jayme J. Determinants of total and high density lipoprotein cholesterol in boys from Finland, the Netherlands, Italy, the Philippines and Ghana with special reference to diet. Submitted.
11. Van Schaik TFSM. Nota voor de commissie meervoudig onverzadigde vetzuren. Voeding 35, 17 (1974).
12. Epstein FH. Nutrition, atherosclerosis and coronary heart disease. Evidence

from epidemiological observations. Atheroscler Rev 5, 149 (1979).

13. Stamler J, Stamler R, Shekelle RB. Regional differences in prevalence, incidence and mortality from atherosclerotic coronary disease. In: Ischaemic heart disease. eds. De Haas JH, Hemker HC, Snellen HA. Leiden University Press, Leiden (1970).

14. Keys A. Coronary heart disease in seven countries. American Heart Association Monograph No 29. Am Heart Association, New York (1970).

15. Strong JP, Eggen DA. Risk factors and atherosclerotic lesions. In: Atherosclerosis, Proceedings 2nd International Symposium. ed. Jones RJ. Springer, Berlin (1970).

16. Scrimshaw NS, Guzman MA. Diet and atherosclerosis. Lab Invest 18, 623 (1968).

17. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins and the risk of CHD. The Framingham Study. Ann Intern Med 74, 1 (1971).

18. Anitschkow N. Experimental arteriosclerosis in animals, a survey of the problem. In: Arteriosclerosis. ed. Cowdry EV. McMillan, New York (1933).

19. Hermus RJJ. Experimental atherosclerosis in rabbits on diets with milk fat and different proteins. PhD thesis. Centre for Agricultural Publishing and Documentation. PUDOC, Wageningen, 1975.

20. Kwiterovich PO, Levy RI, Frederickson DS. Neonatal diagnosis of familial type II hyperlipoproteinaemia. Lancet i, 118 (1973).

21. Miettinen M, Turpeinen O, Karvonen MJ, Elosuo F, Paavilainen E. Effect of cholesterol-lowering diet on mortality from coronary heart disease and other causes. Lancet ii, 835 (1972).

22. Dayton S, Pearce ML, Gofman H, Harnish A, Shickman M, Winfield M, Zager A, Dixon W. Controlled trial of a diet high in unsaturated fat for prevention of atherosclerotic complications. Lancet ii, 1060 (1968).

23. Smith EB, Slater RS. Relationships between low-density lipoprotein in aortic intima and serum lipid levels. Lancet i, 463 (1972).

24. Rhoads GG, Gulbrandsen CL, Kagan A. Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. N Engl J Med 294, 293 (1976).

25. Gofman JW, Hanig M, Jones HB, Lauffer MA, Lawry EY, Lewis LA. Mann GV, Moore FE, Olmsted F, Yeager JF, Andrus EC, Barach JH, Beams JW, Fertig JW, Page JH, Shannon JH, Stare FJ, White PD. Evaluation of serum lipoproteins and cholesterol measurements as predictors of clinical complications of atherosclerosis. Circulation 14, 691 (1956).

26. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease - The Framingham Study. Am J Med 62, 707 (1977).

27. Miller NE, Førde OH, Thelle DS, Mjøs OD. The Tromsø heart study. High density lipoprotein and coronary heart disease - a prospective case-control study. Lancet i, 965 (1977).

28. Steinberg D, Carew TE, Weinstein DB, Koshinsky T. Binding, uptake and catabolism of low density (LDL) and high density (HDL) lipoproteins by cultured smooth muscle cells. In: Lipoprotein metabolism. ed. Greten H. Springer, Heidelberg (1976).

29. Miller NE, Weinstein DB, Carew TE, Koshinsky T, Steinberg D. Interaction between high density and low density lipoproteins during uptake and degradation by cultured human fibroblasts. J Clin Invest 60, 78 (1977).

30. Zilversmit DB, Clarkson TB, Hughes LB. High plasma cholesterol in mink (Mustela vison) without atherosclerosis. Atherosclerosis 26, 97 (1977).

31. Armstrong ML. Regression of atherosclerosis. Atheroscler Rev 1, 137 (1976).

32. Pearson TA, Dillman JM, Solez K, Heptinstall RH. Evidence for two populations of fatty streaks with different roles in the atherogenic process. Lancet ii, 496 (1980).

33. Enos WJ, Beijer JC, Holmes FH. Pathogenesis of coronary disease in American soldiers killed in Korea. JAMA 158, 912 (1955).

34. McNamara J, Malot MA, Stremple JF, Cutting RT. Coronary artery disease in combat casualties in Vietnam. JAMA 216, 1185 (1971).

35. Vihert AM. Atherosclerosis of the aorta in five towns. Bull Wld Hlth Org 53, 501 (1976).

36. Holman RL. Atherosclerosis - a pediatric nutrition problem ? Am J Clin Nutr 9, 565 (1961).

37. WHO/ISFC Meeting on precursors of atherosclerosis in children. CVD/78.1. WHO, Geneva (1977).

38. Darmady JM, Fosbrooke AS, Lloyd JK. Prospective study of serum cholesterol levels during the first years of life. Br Med J ii, 685 (1972).

39. Nestel PJ, Poyser A, Boulton TJC. Changes in cholesterol metabolism in response to dietary cholesterol and fat. Am J Clin Nutr 32, 2177 (1977).

40. Uppal SC. Coronary heart disease. Risk pattern in Dutch youth. A pilot study in Westland schoolchildren. PhD thesis. Leiden (1974).

41. Van der Haar F, Kromhout D. Food intake, nutritional anthropometry and blood chemical parameters in 3 selected Dutch schoolchildren populations. PhD thesis. Med Landbouwhogeschool, Wageningen (1978).

42. Frerichs RR, Srinavasan SR, Webber LS, Berenson GS. Serum cholesterol and triglyceride levels in 3,446 children from a biracial community - The Bogalusa Heart Study. Circulation 54, 302 (1976).

43. Golubjatnikov R, Paskey T, Inhorn SL. Serum cholesterol levels of Mexican and Wisconsin schoolchildren. Am J Epid 96, 30 (1972).

44. Johnson BC, Epstein FH, Kjelsberg MO. Distributions and familial studies of blood pressure and serum cholesterol levels in a total community Tecumseh Michigan. J Chron Dis 18, 147 (1965).

45. Lauer RM, Connor WE, Leaverton PE, Reiter MA, Clarke WR. Coronary heart disease risk factors in schoolchildren - The Muscatine Study. J Pediat 86, 697 (1975).

46. Wilmore JH, McNamara JJ. Prevalence of coronary heart disease risk factors in boys, 8 to 12 years of age. J Pediat 84, 527 (1974).

47. Morrison JA, De Groot I, Edwards BK, Kelly KA, Mellies MJ, Khury P, Glueck CJ. Lipids and lipoproteins in 927 schoolchildren, aged 6 to 17 years. Pediatrics 62, 990 (1978).

48. Dyerberg J, Hjörne N. Plasma lipid and lipoprotein levels in childhood and

adolescence. Scand J Clin Lab Invest 31, 473 (1973).

49. Räsänen L, Wilska M, Kantero RL, Näntö V, Ahlström A, Hallmann N. Nutrition survey of Finnish rural children. IV. Serum cholesterol values in relation to dietary variables. Am J Clin Nutr 31, 1050 (1978).

50. Walker ARP, Walker BF. High density lipoprotein cholesterol in African children and adults in a population free of coronary heart disease. Br Med J ii, 1336 (1978).

51. Whitehead TP, Brownings DM, Gregory A. A comparative survey of the results of analyses of blood serum in clinical chemistry laboratories in the U.K. J Clin Path 26, 435 (1973).

2 SERUM TOTAL AND HIGH DENSITY LIPOPROTEIN (HDL) CHOLESTEROL CONCENTRATIONS IN RURAL AND URBAN BOYS FROM 16 COUNTRIES

J.T. KNUIMAN, R.J.J. HERMUS * and J.G.A.J. HAUTVAST

Department of Human Nutrition, Agricultural University, Wageningen (The Netherlands)

(Received 2 November, 1979)
(Revised, received 15 February, 1980)
(Accepted 18 February, 1980)

Summary

Serum total and HDL cholesterol concentrations were measured in schoolboy populations from 16 countries. Total and HDL cholesterol concentrations were determined in one laboratory to reduce methodological variability as much as possible. A standardized protocol was used for the drawing of blood, preparation, storage and transport of serum.

Mean serum total cholesterol concentrations were relatively low in the less developed countries and relatively high in the more developed ones. Mean serum HDL cholesterol concentrations showed a distribution similar to that of mean total cholesterol: relatively low levels in the less developed countries and relatively high levels in the more developed ones.

Statistically significant relationships were found between serum total and HDL cholesterol between individuals, between populations and within populations.

The relationship between HDL cholesterol concentrations and diet is discussed.

Key words: *School boys (international comparison) — Serum HDL cholesterol — Serum total cholesterol*

Supported by The Netherlands Heart Foundation, Grant No. 78.064.

* Present address: Central Institute for Nutrition and Food Research, CIVO-TNO, Zeist, The Netherlands.

Address for correspondence and reprints: J.T. Knuiman, M.Sc., Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands.

Introduction

Relatively little is known about the serum concentrations of total and high density lipoprotein (HDL) cholesterol of children living in different countries of the world with differing rates of mortality from coronary heart disease (CHD). Information on serum concentrations of total and HDL cholesterol would be particularly interesting as it is possible that atherogenesis begins in childhood [1—3] and because of the postulated protective effect of high concentrations of HDL cholesterol [4,5].

It is difficult to compare the known data on serum total and HDL cholesterol concentrations because of the variations, not due to biological factors, in the results obtained. Apparent concentrations may vary up to 40% by the use of different methods or even by the use of the same technique by people in different laboratories [6]. Variability possibly also results from differences in the methods of blood sampling, storage of serum, etc.

This study was designed to obtain reliable data on a limited number of 7- and 8-year-old boys in countries with different rates of CHD mortality, by using a standardized protocol for the collection of samples and by carrying out the analyses in a single laboratory using standardized methods. It is hoped that a more comprehensive study will be carried out in the future, using a larger number of samples from a wider range of people.

The results obtained indicate that the serum total cholesterol concentration is lower in the developing countries studied in Africa and Asia, and higher in the developed countries of Europe and in the U.S.A. The serum HDL cholesterol concentrations were generally correlated with the total cholesterol concentrations.

Methods

Samples were collected from 16 countries in various regions of the world selected on the basis of having different patterns of diet [7] and different rates of mortality from coronary heart disease (1974/1975 data for men aged 55—64 years were provided by Dr. T. Strasser, Cardiovascular Diseases Unit, WHO, Geneva). In each country, co-operation was sought from a well known scientist or institute to collect samples from 35 boys in five schools in both an urban and a rural environment. The investigators involved and the location of study areas are shown in Table 1. Samples were collected from boys aged 7 and 8 years after obtaining the necessary parental consent as required locally. The boys were chosen at random and samples were taken from those found to be healthy by visual inspection.

Blood was drawn [8] without the addition of EDTA as only the serum was required. The serum was separated from the blood cells and stored at −20° C until shipment to The Netherlands in the frozen state by air express. All samples arrived at the laboratory in the frozen state and were then stored at −20° C until analysis which was carried out within 2.5 months of blood sampling. Storage for periods up to 6 months at −20° C has been shown not to affect measurement of HDL and total cholesterol concentration (M.B. Katan et al., unpublished observations) Miller et al. [4] have previously reported that

TABLE 1

List of participating investigators and location of study areas

Country	Region	Investigators	Study areas	No. of schools sampled	Time of data collection
Africa					
Ghana (GH)	R	J.O.M. Pobee	Surroundings of Accra	7	Mar. 1979
Ghana	U	J.O.M. Pobee	Accra	7	Feb. 1979
Ivory Coast (IC)	R	G.P. Ravelli	Adiopodoume	1	Oct. 1978
Ivory Coast	U	P.J. Lamotte	Bouake	5	Dec. 1978
Nigeria (NI)	R	J.B. Fashakin	Surroundings of Île-Ife	3	Oct. 1978
Nigeria	U	J.B. Fashakin	Île-Ife	2	Oct. 1978
America					
U.S.A. (US)	R	R.M. Lauer, M.F. Sowers	Muscatine, Iowa	4	Feb. 1979
Asia					
Pakistan (PA)	R	M. Ilyas	Surroundings of Peshawar	5	Nov. 1978
Pakistan	U	M. Ilyas	Peshawar	5	Nov. 1978
Philippines (PH)	R	P. Dijkhuizen	Suburbs of San Pablo	1	Dec. 1978
Philippines	U	P. Dijkhuizen	San Pablo	5	Dec. 1978
Europe					
Austria (AU)	U	K. Widbalm	Vienna	5	Dec. 1978
Denmark (DK)	U	K. Ibsen, H. Meinertz, O. Faergeman	Copenhagen	5	Mar. 1979
Finland (FI)	R	J. Viikari	Surroundings of Turku, Helsinki, Tampere, Kuopio, Oulu	8	Nov., Dec. 1978
Finland	U	J. Viikari	Turku, Helsinki, Oulu, Tampere, Kuopio	8	Nov., Dec. 1978
Greece (GR)	R	C. Aravanis	Zelion, Exarxos	2	Nov. 1978
Hungary (HU)	U	I. Kamaras	Budapest	5	May 1979
Ireland (IR)	R	O.C. Ward	Area 25 miles from Dublin	5	Nov. 1978
Ireland	U	O.C. Ward	Dublin	5	Nov. 1978
Netherlands (NE)	R	J.T. Knuiman	Surroundings of Ede	5	Nov. 1978
Netherlands	U	J.T. Knuiman	Wageningen	5	Nov. 1978
Poland (PL)	U	S.L. Rywik	Warsaw	5	Nov. 1978
Portugal (PR)	R	A.M.C. Nunes	Fundao area	5	Nov. 1978
Portugal	U	F. de Padua, J.A.G. Forte	Lisbon	5	Nov. 1978
Sweden (SW)	R	L.I. Hardell	Surroundings of Uppsala	6	Oct.—Dec. 1978
Sweden	U	L.I. Hardell	Uppsala	6	Oct.—Dec. 1978

R = rural; U = urban.

storage of serum for 2 months at −20° C has no effect on the measurement of HDL cholesterol. To ensure uniformity in the method of blood sampling and transport, all the necessary materials were supplied from the laboratory in Wageningen.

Serum total cholesterol was measured by the direct method of Huang et al. [9,10] and calibrated against the method of Abell et al. [11]. The precision of the method varied between 0.7 and 2.7% (coefficient of variation). The accuracy defined as the difference between the concentration of total cholesterol in standard sera as measured in our laboratory and compared with the values as provided by the Center for Disease Control, Atlanta, GA, U.S.A. was between 0 and 2.5%. HDL cholesterol determinations were preceded by precipitation of LDL and VLDL by heparin—$MnCl_2$ [10,12]. The precision of the HDL cholesterol determinations varied between 1.7 and 2.5% and the accuracy between 0.3 and 1.8%. Since 1977 the Lipid Laboratory of the Department of Human Nutrition has been certified by the Center of Disease Control as meeting the criteria for precision and accuracy as specified for standardization by the WHO Collaborating Center for Reference and Research in Blood Lipids [13].

The HDL cholesterol/total cholesterol ratio was calculated for each child and the means and SEM of the total cholesterol and HDL cholesterol concentration and the ratio calculated for each urban and rural group. The relationships between the HDL and total cholesterol concentrations were examined by calculating the correlation co-efficients both within and between the population groups studied. In addition the total cholesterol and HDL cholesterol concentrations were correlated with the availability of animal products expressed as percentage of the total energy supply per capita [7] of the various countries.

Results

The mean serum concentrations of total cholesterol and HDL cholesterol in all subjects are presented in Table 2. The mean concentrations of total cholesterol in the three West African countries (Ghana, Ivory Coast and Nigeria) were low (2.64—3.53 mmol/l), the lowest being observed in the rural Nigerian boys. The values from Pakistan (3.31 and 3.76 mmol/l) were also quite low. The serum total cholesterol concentrations in the rural groups were significantly lower (Student t-test, $P < 0.05$) than those in the corresponding urban groups in Ghana, Nigeria and Pakistan. Intermediate values were observed in the Philippines, Greece, Portugal and Hungary (3.78—4.17 mmol/l). Higher serum total cholesterol concentrations were found in the boys in the remaining European countries and the United States (4.27—5.16 mmol/l). In this group of countries, a comparison of the values from countries where data from both rural and urban environments were available (Ireland, the Netherlands, Sweden and Finland) showed that the mean serum total cholesterol concentration in boys from the rural areas (4.69 ± 0.069 mmol/l, n = 147) was significantly higher ($P < 0.05$) than the value found in boys from the urban areas (4.50 ± 0.056 mmol/l, n = 145).

HDL cholesterol concentrations also varied over a wide range. Generally the mean serum HDL cholesterol concentrations showed a distribution similar to

TABLE 2

TOTAL AND HDL CHOLESTEROL CONCENTRATIONS IN SERUM OF BOYS AGED 7 AND 8 YEARS IN RURAL AND URBAN AREAS OF 16 COUNTRIES

The correlation coefficient of total and HDL cholesterol between individuals in all countries was 0.60 ($P < 0.001$, n = 901) and the mean correlation co-efficient between total and HDL cholesterol in each population group ($\bar{r}$) was 0.35 ($P < 0.001$, n = 26)

Country	Area	Sample size	Total cholesterol (mmol/l) [a]	HDL cholesterol (mmol/l) [a]	HDL/total cholesterol [a]	Correlation coefficient [b]
Africa						
Ghana	R	35	3.13 ± 0.13	0.92 ± 0.051	0.30 ± 0.015	0.60 **
Ghana	U	35	3.53 ± 0.12	1.06 ± 0.037	0.30 ± 0.010	0.38 *
Ivory Coast	R	31	3.26 ± 0.13	1.04 ± 0.050	0.32 ± 0.014	0.58 **
Ivory Coast	U	35	3.14 ± 0.11	0.93 ± 0.035	0.30 ± 0.010	0.47 **
Nigeria	R	36	2.64 ± 0.10	0.81 ± 0.042	0.32 ± 0.017	0.41 *
Nigeria	U	36	3.37 ± 0.12	1.21 ± 0.060	0.36 ± 0.017	0.58 **
America						
U.S.A.	R	34	4.35 ± 0.12	1.37 ± 0.046	0.32 ± 0.010	0.34 *
Asia						
Pakistan	R	34	3.31 ± 0.11	0.78 ± 0.027	0.24 ± 0.009	0.34 *
Pakistan	U	36	3.76 ± 0.11	0.92 ± 0.033	0.25 ± 0.012	0.11
Philippines	R	34	4.17 ± 0.10	1.11 ± 0.039	0.27 ± 0.010	0.33
Philippines	U	27	4.09 ± 0.14	1.06 ± 0.054	0.27 ± 0.013	0.47 *
Europe						
Austria	U	30	4.45 ± 0.11	1.38 ± 0.058	0.32 ± 0.016	−0.12
Denmark	U	36	4.49 ± 0.13	1.51 ± 0.053	0.34 ± 0.013	0.34 *
Finland	R	35	5.16 ± 0.15	1.68 ± 0.056	0.33 ± 0.012	0.31
Finland	U	35	4.82 ± 0.13	1.72 ± 0.057	0.36 ± 0.012	0.48 **
Greece	R	38	3.78 ± 0.12	1.21 ± 0.037	0.33 ± 0.011	0.31
Hungary	U	38	4.11 ± 0.14	1.46 ± 0.045	0.36 ± 0.015	−0.05
Ireland	R	35	4.51 ± 0.14	1.49 ± 0.051	0.34 ± 0.015	0.14
Ireland	U	36	4.43 ± 0.11	1.40 ± 0.048	0.32 ± 0.010	0.40 *
Netherlands	R	41	4.56 ± 0.12	1.46 ± 0.044	0.33 ± 0.009	0.47 **
Netherlands	U	39	4.27 ± 0.10	1.33 ± 0.053	0.31 ± 0.013	0.49 **
Poland	U	33	4.56 ± 0.10	1.65 ± 0.059	0.37 ± 0.012	0.35 *
Portugal	R	33	3.76 ± 0.09	1.20 ± 0.045	0.32 ± 0.016	0.38 *
Portugal	U	35	3.94 ± 0.12	1.31 ± 0.049	0.34 ± 0.010	0.51 **
Sweden	R	36	4.60 ± 0.13	1.54 ± 0.053	0.34 ± 0.013	0.33 *
Sweden	U	35	4.49 ± 0.10	1.49 ± 0.052	0.34 ± 0.013	0.10

[a] Values expressed as mean ± SEM.

[b] Correlation coefficient between total and HDL cholesterol within groups.

Significance of the correlation coefficient: * $P < 0.05$; ** $P < 0.01$.

that observed for the total cholesterol concentrations; relatively low values (0.78—1.31 mmol/l) in the developing and the less developed countries (in West Africa, Asia, Greece and Portugal), and higher values (1.33—1.72 mmol/l) in the developed countries (U.S.A. and the remaining European countries). As with serum total cholesterol, in Ghana, Nigeria and Pakistan, the means of the serum HDL cholesterol concentrations were significantly lower ($P < 0.05$) in the boys from the rural areas (0.84 ± 0.025 mmol/l, n = 107) compared with those from the urban areas (1.05 ± 0.028 mmol/l, n = 107). The serum HDL cholesterol concentrations in rural boys from the developed countries tended to be higher than those from the urban areas but the difference was not significant.

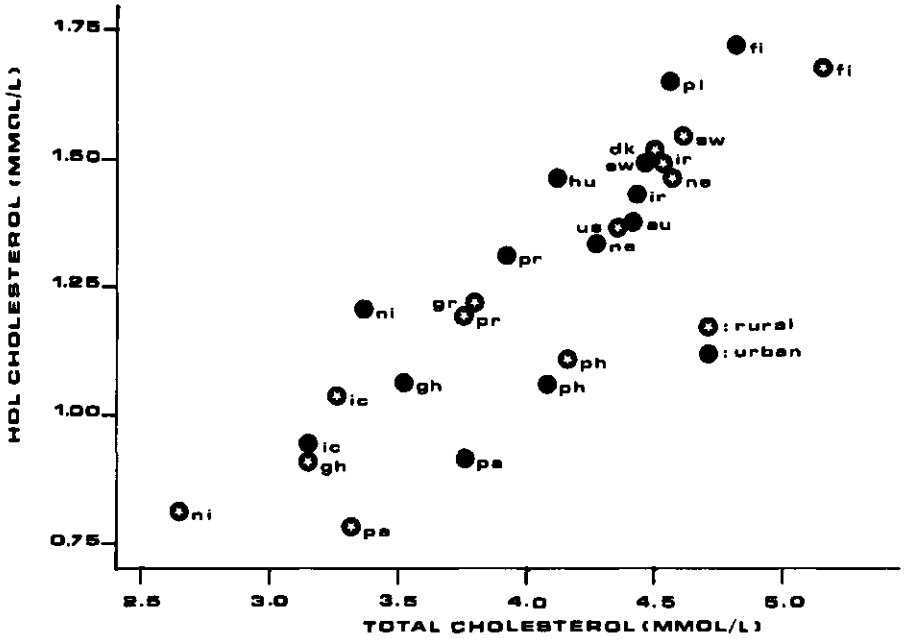

Fig. 1. Relationship between HDL cholesterol and total cholesterol between populations ($r = 0.90$, $P < 0.001$, $n = 26$). For abbreviations see Table 1.

From the total cholesterol concentrations and the HDL cholesterol concentrations for each boy studied, correlation co-efficients can be calculated and these are also presented in Table 2. In most cases (18 out of 26), the values are positive and significant, the mean value (r) being 0.35 ($P < 0.001$). When the correlation co-efficient is calculated for all individuals in the study grouped into rural and urban areas of the various countries, it is very high ($r = 0.90$, $P < 0.001$; Fig. 1) although the correlation co-efficient falls to 0.60 ($P < 0.001$) when all individuals are compared disregarding their location.

The serum HDL cholesterol/total cholesterol ratio in the Asian boys (0.24—0.27) was found to be much lower than in all the other groups studied (0.30—0.37).

It was possible to compare the mean serum total cholesterol and HDL cholesterol concentrations of the pooled urban and rural populations with the availability of animal products in 1973/1974 per capita in all the participating countries and expressed as percentage of the total energy supply. The correlation co-efficient using the total cholesterol concentrations was 0.91 and using the HDL cholesterol concentrations was 0.90 ($P < 0.05$ for both values).

Discussion

This study clearly shows that there are large variations in mean total and HDL cholesterol concentrations in serum between populations of 7- and 8-year-old boys. The differences observed are real because extensive internal and external quality control of the measurements were employed. The data

obtained are of a preliminary nature because of the small number of boys studied in each population. In a larger study, more attention would need to be paid to the selection of individuals within each population.

The serum total and HDL cholesterol concentrations reported here (Table 2) are basically similar to those reported by other workers. For example, for total cholesterol, workers in the U.S.A. found concentrations of 4.09—4.82 mmol/l [14—19]; in Denmark, 5.06 ± 0.73 mmol/l (mean ± SD) [20]; in Finland, 6.09 ± 1.01 mmol/l; and in the Netherlands, 4.53 ± 0.70 mmol/l [12]. For HDL cholesterol, concentrations of 1.45—1.61 mmol/l have been reported for American children [19] and 1.36 mmol/l for Dutch children [12]. However, values for HDL cholesterol in black South African children have been reported to be as high as 1.66 mmol/l [22], while comparable values in this study (from West Africa) are 0.81—1.21 mmol/l. The HDL cholesterol/total cholesterol ratio for the South African children was 0.55 compared with 0.30—0.36 for the West African boys presented here. At this stage it is not possible to attribute these differences to biological or to methodological factors and so further work is required in this area.

Lower total and HDL cholesterol levels in the rural regions of some developing countries (Ghana, Nigeria and Pakistan) possibly reflect a lower total food consumption in the rural boys from the countries concerned, or a lower intake of a specific food component such as saturated fats. It is interesting that in some European countries (Finland, Ireland, The Netherlands and Sweden), the reverse could be observed: lower total and HDL cholesterol levels in the urban regions. The differences between urban and rural regions possibly result from differences in socio-economic conditions leading to differences in food consumption.

It is interesting to note the relatively low HDL cholesterol/total cholesterol ratio in the Asian populations studied. The relationship of this finding to the risk of CHD in later life is not yet understood.

In this study higher levels of serum total cholesterol in the various groups were generally accompanied by higher HDL cholesterol concentrations (Fig. 1, Table 2). The correlation co-efficient between the parameters of the various populations ($r = 0.90$) was much higher than within the populations ($r = 0.35$). The inter-individual population correlation co-efficient (disregarding groups) was between these 2 values ($r = 0.60$). Inter-individual variations and the relatively narrow range of values for both HDL and total cholesterol within populations may be responsible for these differences in correlation co-efficients.

Recently, Robinson et al. [23] reported relatively low HDL cholesterol concentrations in Masai people. These low concentrations of HDL cholesterol were associated with relatively low concentrations of total cholesterol compared with a European control group although the HDL cholesterol/total cholesterol ratio was the same in both groups (0.23). In the study reported here, similar HDL cholesterol/total cholesterol ratios were found in boys from West Africa and from Europe.

It is tempting to suggest that a more Western type of diet characterized by a relatively high fat intake with a large contribution from animal products is responsible for the higher serum total and HDL cholesterol concentrations in the

developed countries. This is borne out by the correlations between the per capita availability of animal products expressed as a percentage of total energy and the total and HDL cholesterol concentrations. Specific characteristics of the food components such as the polyunsaturated fat/saturated fat ratio (P/S ratio) and the cholesterol content may be important in this regard. Dietary intervention studies could possibly throw more light on the behavior of total and HDL cholesterol in relation to diet than can inter-country studies. One such study carried out in 19—23-year-old healthy volunteers showed that a limitation of the total fat intake from 35 energy % to 17 energy % over a period of 6 weeks resulted in both a decrease in total cholesterol (from 5.2 to 4.7 mmol/l, $P < 0.001$) and in HDL cholesterol (from 1.50 to 1.29 mmol/l, $P < 0.001$), while in the control group (35 energy % of fat), no significant changes were observed [24]. In another study, Shepherd et al. [25] examined the effect of two diets equal in total fat, protein, carbohydrate and cholesterol content but differing in the P/S ratio of the dietary fat. The polyunsaturated fat diet (P/S ratio = 4.0) reduced the plasma total cholesterol by 24% ($P < 0.01$) and the HDL cholesterol by 33% ($P < 0.01$) compared with the saturated fat diet (P/S ratio = 0.25). Brunner et al. [26] studied the effect of a high-caloric, high-fat diet and that of a low-caloric, low-fat diet in healthy subjects. The high-caloric, high-fat diet resulted in an increase of both serum total and HDL cholesterol compared with the low-caloric, low-fat diet. The HDL cholesterol/total cholesterol ratio displayed only very small changes during this experiment that lasted for 12 months. A study of Burslem et al. [27] showed that a diet rich in total fat and cholesterol and with a low P/S ratio was associated with both higher total and HDL cholesterol concentrations compared with a diet low in total fat with a high P/S ratio consumed by vegetarians.

Thus, it would appear that when the total cholesterol concentration is high as a result of the diet consumed, the HDL cholesterol concentration also tends to be high. This effect of diet on HDL cholesterol is probably more clear between populations than within populations because the differences in diets are probably greater between populations than within populations. Other possible determinants of the HDL cholesterol concentration e.g. the amount of alcohol consumed and physical activity may further obscure the effect of diet on HDL cholesterol within populations.

HDL is possibly involved in the transport of cholesterol out of the walls of the arteries [28]. This suggests that HDL may counterbalance the effect of a diet enhancing the risk from coronary heart disease. Therefore it may well be that, within a population, those people with a higher HDL cholesterol concentration are more able to cope with the effects of a risk-enhancing diet.

Acknowledgements

We would like to thank all those who contributed to the execution of this study, expecially the co-operative investigators abroad (Table 1).

We would also like to thank Dr.T.Strasser, Cardiovascular Disease Unit, WHO, Geneva and Dr. E. Dekker, Netherlands Heart Foundation, The Hague for advice during the preliminary stages of the study, Dr. C.E. West of this Department for assistance in writing the paper and H. Verwey and J. Barendse for technical assistance in the analysis.

References

1 Hautvast, J.G.A.J. and Valkenburg, H.A. (Eds.), Proceedings of the Workshop on Atherosclerosis and the Child, Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands, 1977.

2 Study of atherosclerosis precursors in children, Report of a WHO consultation on prevention of adult cardiovascular diseases in childhood, CVD/74.1 Geneva, WHO, 4—6 February, 1974.

3 WHO/ISFC meeting on precursors of atherosclerosis in children, CVD/78.1, Geneva, WHO, 12—14 October, 1977.

4 Miller, N.E., Førde, O.H., Thelle, D.S. and Mjøs, O.D., The Tromsø Heart Study — High density lipoprotein and coronary heart disease: a prospective case-control study Lancet, i (1977) 965.

5 Gordon, T., Castelli, W.P., Hjortland, M.C., Kannel, W.B. and Dawber, T.R., High density lipoprotein as a protective factor against coronary disease — The Framingham Study, Amer. J. Med., 62 (1977) 707.

6 Whitehead, T.P., Brownings, D.M. and Gregory, A., A comparative survey of the results of analyses of blood serum in clinical chemistry laboratories in the U.K., J. Clin. Path., 26 (1973) 435.

7 Provisional Food Balance Sheets, Rome, FAO, 1977.

8 Manual of Laboratory Operations, Lipid Research Clinics Program, Vol. 1 (Lipid and Lipoprotein Analyses) (DHEW Publ. No. (NIH) 75), National Institute of Health, Bethesda, MD, 1974, p. 628.

9 Huang, T.C., Chen, C.P., Wefler, V. and Raferty, A., A stable reagent for the Liebermann—Burchard reaction, Anal. Chem., 33 (1961) 1045.

10 Van der Haar, F., Van Gent, C.M., Schouten, F.M. and Van der Voort, H.A., Methods for the estimation of high density cholesterol, comparison between two laboratories, Clin. Chim. Acta, 88 (1978) 469.

11 Abell, A.A., Levy, B.B., Broady, B.B. and Kendall, F.E., A simplified method for the estimation of total cholesterol in serum and a demonstration of its specificity, J. Biol. Chem., 195 (1952) 357.

12 Van der Haar, F. and Kromhout, D., Food intake, nutritional anthropometry and blood chemical parameters in 3 selected Dutch schoolchildren populations, Meded. Landbouwhogeschool, Wageningen, The Netherlands, 78-9 (1978).

13 Schouten, F.J.M., Van der Haar, F., Hermus, R.J.J., Katan, M.B. and Kromhout, D., Kwaliteitsbewaking van serum cholesterol bepalingen, Voeding, 40 (1979) 93.

14 Frerichs, R.R., Srinavasan, S.R., Webber, L.S. and Berenson, G.S., Serum cholesterol and triglyceride levels in 3,446 children from a biracial community — The Bogalusa Heart Study, Circulation, 54 (1976) 302.

15 Golubjatnikov, R., Paskey, T. and Inhorn, S.L., Serum cholesterol levels of Mexican and Wisconsin schoolchildren, Amer. J. Epid., 96 (1972) 36.

16 Johnson, B.C., Epstein, F.H. and Kjelsberg, M.O., Distributions and familial studies of blood pressure and serum cholesterol levels in a total community Tecumseh Michigan, J. Chron. Dis., 18 (1965) 147.

17 Lauer, R.M., Connor, W.E., Leaverton, P.E., Reiter, M.A. and Clarke, W.R., Coronary heart disease risk factors in schoolchildren — The Muscatine Study, J. Ped., 86 (1975) 697.

18 Wilmore, J.H. and McNamara, J.J., Prevalence of coronary heart disease risk factors in boys, 8 to 12 years of age, J. Ped., 84 (1974) 527.

19 Morrison, J.A., De Groot, I., Edwards, B.K. et al., Lipids and lipoproteins in 927 schoolchildren, aged 6 to 17 years, Pediatrics, 62 (1978) 990.

20 Dyerberg, J. and Hjörne, N., Plasma lipid and lipoprotein levels in childhood and adolescence, Scand. J. Clin. Lab. Invest., 31 (1973) 473.

21 Räsänen, L., Wilska, M., Kantero, R.L., Näntö, V., Ahlström, A. and Hallmann, N., Nutrition survey of Finnish rural children, Part 4 (Serum cholesterol values in relation to dietary variables), Amer. J. Clin. Nutr., 31 (1978) 1050.

22 Walker, A.R.P. and Walker, B.F., High-density-lipoprotein cholesterol in African children and adults in a population free of coronary heart disease, Brit. Med. J., 2 (1978) 1336.

23 Robinson, D. and Williams, P., High density lipoprotein cholesterol in the Maasai of East Africa — A cautionary note, Brit. Med. J., 1 (1979) 1249.

24 Deurenberg, P., De invloed van een vetbeperkte, koolhydraatverrijkte voeding en van een vezelverrijkte voeding op de lipidespiegel in het serum bij vrouwelijke studenten van een diëtistenopleiding, Voeding, 39 (1978) 222.

25 Shepherd, J., Packard, C.J., Patsch, J.R., Gotto, A.M. and Taunton, O.D., Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apoprotein in A-1, J. Clin. Invest, 61 (1978) 1582.

26 Brunner, D., Weissbort, J., Fischer, M. et al., Serum lipid response to a high caloric, high fat diet in agricultural workers during 12 months, Amer. J. Clin. Nutr., 32 (1979) 1342.

27 Burslem, J., Schonfeld, G., Howald, M.A., Weidman, S.W. and Miller, J.P., Plasma apoprotein and lipoprotein lipid levels in vegetarians, Metabolism, 27 (1978) 711.

28 Miller, N.E., The evidence for the antiatherogenicity of high density lipoprotein in man, Lipids, 13 (1978) 914.

3 SERUM TOTAL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL CONCENTRATIONS AND BODY MASS INDEX IN ADULT MEN FROM 13 COUNTRIES

JAN T. KNUIMAN, CLIVE E. WEST AND JAN BUREMA

Knuiman, J. T. (Dept. of Human Nutrition, Agricultural U., The Netherlands), C. E. West and J. Burema. Serum total and high density lipoprotein cholesterol concentrations and body mass index in adult men from 13 countries. *Am J Epidemiol* 1982;116:631-42.

In 1980, serum total and high density lipoprotein (HDL) cholesterol concentrations and body mass index (weight/height^2) were measured in groups of adult men from 13 countries. A standardized protocol was used for the drawing of blood and the preparation, storage, and transport of serum. Total and HDL cholesterol concentrations were determined in one laboratory to reduce methodological variability as much as possible. Mean serum total cholesterol concentrations were low in the groups from Africa (3.0–4.3 mmol/liter; 1 mmol/liter = 38.7 mg/100 ml), intermediate in the groups from Pakistan, the Philippines, Surinam, Hungary, Poland, and the Mediterranean countries (4.4–5.5 mmol/liter), and high in those from the Netherlands and Finland (5.6–6.4 mmol/liter). Mean serum HDL cholesterol concentrations tended to be lower in the men from Africa, Asia, and Surinam (0.7–1.3 mmol/liter) than in those from Europe (1.1–1.5 mmol/liter), the highest values for both total and HDL cholesterol being found in the men from eastern Finland. The ratio of HDL cholesterol/total cholesterol varied from 0.15 to 0.32 and was on average slightly higher in the groups from Africa (0.26–0.32) than it was in the groups from Europe (0.20–0.28) and from Asia and Surinam (0.15–0.22). The body mass index was positively related to the concentration of total cholesterol and negatively related to the concentration of HDL cholesterol and the HDL cholesterol/total cholesterol ratio. The relationships between the concentration of HDL cholesterol and mortality from coronary heart disease within and between populations are discussed.

body weight; cholesterol; lipoproteins, HDL

It has been found that the concentration of high density lipoprotein (HDL) cholesterol and the ratio of HDL cholesterol to total cholesterol are negatively related to the risk of coronary heart disease within a number of populations (1–6). However, this inverse association between the concentration of HDL cholesterol and the risk of coronary heart disease has not been found in comparisons between countries. For instance, Keys (7) did not find important differences between HDL cholesterol in southern Japan where coronary heart disease was rela-

Received for publication March 2, 1982, and in final form April 16, 1982.

Abbreviation: HDL, high density lipoprotein.

[1] Department of Human Nutrition, Agricultural University, De Dreijen 12, 6703 BC Wageningen, The Netherlands. (Reprint requests to Dr. Knuiman.)

This work was supported by the Netherlands Heart Foundation.

The authors thank all those who contributed to the execution of the study, especially the cooperative investigators abroad (see table 1). They also thank A. E. M. F. Soffers, J. C. M. Barendsevan Leeuwen, G. Germing-Nouwen, and H. J. Verwey for technical assistance, J. de Boer for assistance in the statistical analysis, and Dr. M. B. Katan and Professor J. G. A. J. Hautvast for discussion.

tively rare and in Finland where coronary heart disease was quite common. Other authors have reported extremely different HDL cholesterol concentrations for populations relatively free of coronary heart disease. A high value (2.1 mmol/liter; 1 mmol/liter = 38.7 mg/100 ml) has been found for Nigerians on "their typical Nigerian" diet (8) and a very low value (0.7 mmol/liter) has been reported for Tarahumara Indians (9) on diets low in saturated fat (2 per cent of calories) and cholesterol (71 mg/day). It is possible that these extreme findings are partly the result of differences in the methods used in estimating the concentrations of HDL cholesterol (10–12).

Recently, we have carried out several studies (13–15) to obtain comparable data on the concentrations of total and HDL cholesterol for samples from countries with different rates of mortality from coronary heart disease by using standardized protocols for the collection of samples and by carrying out the analyses in a single laboratory using standardized methods and employing rigid external and internal quality control of the measurements.

Our first study (14) in boys aged seven and eight years in both urban and rural regions from 16 countries demonstrated clearly that marked differences exist in the serum concentrations of total and HDL cholesterol between groups of boys from different countries. The highest concentrations for both total and HDL cholesterol were found in the serum of Finnish boys and the lowest concentrations in the serum of boys from Africa (Ghana, Ivory Coast, and Nigeria) and from Pakistan. Surprisingly, the mean concentrations of HDL cholesterol appeared to increase linearly with that of total cholesterol ($r = 0.90$, $n = 26$ regions). Even for the 15 European regions (from Austria, Denmark, Finland, Greece, Hungary, Ireland, the Netherlands, Poland, Portugal, and Sweden), the correlation coefficient was almost identical ($r = 0.89$). Since the mean concentration of total cholesterol is highly correlated with the mortality rate of coronary heart disease between populations (16), this would indicate that the mean concentration of HDL cholesterol would also be positively related to the mortality of coronary heart disease between populations provided that our findings in boys can be extrapolated to adults.

To examine whether our findings in boys can actually be extrapolated to adults, we have carried out a study in groups of adult men from 13 countries with different rates of mortality from coronary heart disease. Therefore, the purpose of this study was to examine the relationship between mean total and HDL cholesterol concentrations over a wide range of mean total cholesterol levels. Our findings are compared with those in boys from 16 countries (13, 14). Furthermore, it is discussed why the mean concentration of HDL cholesterol is not negatively related to mortality from coronary heart disease between populations, while there is a negative relationship between the concentration of HDL cholesterol and mortality from coronary heart disease within populations. Since differences in body mass index between populations play an important role in this regard, as will be argued under Discussion, height and weight were also measured.

Methods

Population samples

In 1980, samples were collected from 13 countries in various regions of the world on the basis of having different patterns of diet (17) and different rates of mortality from coronary heart disease (1974/1975 data for men aged 55–64 years from Europe and the Philippines were kindly provided by Dr. T. Strasser, Cardiovascular Disease Unit, World Health Organization, Geneva). In each country, cooperation was sought from a well known scientist or institute to collect samples from 40 men

aged 33–38 years and also from 40 men aged 43–48 years. Since we were particularly interested in the relationship between mean total and HDL cholesterol concentrations over a wide range of values for total cholesterol concentrations rather than in representative data for the participating countries, we preferred to draw relatively small samples in a relatively large number of countries, rather than draw bigger samples from a smaller number of countries. Since we wanted to obtain results from men to compare with those from boys (13), we would have liked, in principle, to collect data from adult men of all ages. However, because our resources were limited and because the relationship between mean total and HDL cholesterol was probably considered to be age-dependent, we decided to examine two narrow age categories in this study.

Strict random samples were not required from the cooperating investigators, since this was considered unnecessary in achieving the objectives of the study. However, care was taken to ensure that we would obtain samples with low mean concentrations of total cholesterol from the developing countries. Therefore, the elite groups in the developing countries were excluded from the study. The diets of these groups and their concentrations of total cholesterol tend to be entirely different from the nonelite groups (18, 19). The men selected were generally laborers and were recruited from several locations in each country, such as factories, farms, workshops, transport and railway companies (see table 1). Only men found to be apparently healthy as determined by visual inspection were enrolled in the study.

Anthropometry, blood sampling, shipment and storage of samples, and estimation of total and HDL cholesterol

Height and weight were measured as described previously (13) and blood was drawn (20) in a nonfasting condition for the estimation of total and HDL cholesterol in whole serum (13). Previous studies (21, 22) have shown that there are no definable differences in the concentrations of total and HDL cholesterol with time of day and time since last meal. A nonfasting condition was preferred to a fasting condition for logistic reasons. The serum was stored at −20 C until shipment to the Netherlands in the frozen state by air express which was carried out within four weeks after blood sampling. After arrival, the samples were stored at −80 C until analysis which was carried out within three months. To ensure uniformity in the method of blood sampling and transport, all necessary materials were supplied from the laboratory in Wageningen.

The concentration of total cholesterol in serum was measured as described previously (13). Reproducibility for blind control sera provided by the Centers for Disease Control, Atlanta, GA, was 0.9 per cent and accuracy was 1.4 per cent of the true (target) values. The concentration of HDL cholesterol was measured after precipitation of the low and very low density lipoproteins by heparin-$MnCl_2$ (23, 24). Reproducibility for blind control sera obtained in the Centers for Disease Control Survey of HDL cholesterol measurements (12) was 2.2 per cent and bias with regard to the overall survey mean was on average 0.1 per cent.

From the measurement of height and weight, the body mass index expressed as weight/height^2 (kg/m^2) was calculated. It has been shown in previous studies (25, 26) that the ratio of weight/height^2 is a satisfactory indicator of relative obesity as judged by criteria of correlation with height (lowest correlation is best) and measures of body fatness (highest correlation is best), even for nonwestern populations.

Statistical methods

The relationships of the concentrations of total and HDL cholesterol and the ratio

TABLE 1

Description of the population samples in the 13 countries studied (1980)

Country	Investigator(s)	Location	Composition of sample	Ethnic background/nationality
Africa				
Ghana	J. O. M. Pobee	Accra and its surroundings	Hospital and factory workers	12 ethnic groups but mainly Akan (n = 37), Ewe (n = 14), and Ga (n = 12)
Ivory Coast	G. P. Ravelli, J. Pierre	Abidjan	Laborers	20 ethnic groups but mainly Baoulé (n = 67), Sénoufo (n = 23), and Malinké (n = 12) from the Ivory Coast and Mossi (n = 17) from Upper Volta
Nigeria	C. O. Adesanya, F. I. Anjorin	Zaria	Traders, farmers, laborers	Hausa
America				
Surinam	A. Hilvers, I. Oemrawsingh	Nickerie	Farmers, laborers, drivers	Hindustani
Asia				
Pakistan	M. Ilyas	Peshawar	Flour mill workers, hotel waiters, drivers, clerks, hospital staff	Pathan
Philippines	J. Bulatao-Jayme	Bulacan	Cottage industry workers, traders, drivers	Filipino
Europe				
E. Finland	P. Puska	Joensuu in North Karelia	General sample	Finnish
W. Finland	J. Viikari	Turku	Workers from laboratory, shipyard, and brewery	Finnish
Hungary	I. Kamarás	Budapest	Factory workers	Hungarian
Italy	A. Ferro-Luzzi	Villages south of Naples	Farmers, laborers, clerks	Italian
Netherlands	J. T. Knuiman	Boxmeer, Cuyk	Factory workers	Dutch
Poland	S. Rywik	Warsaw	Factory workers	Slavonic
Portugal	J. M. Pereira Miguel	Lisbon	Sample from population register	Portugese
Spain	I. Balaguer-Vintro	Barcelona	Factory workers	Spanish

of HDL cholesterol/total cholesterol with body mass index were examined by linear regression equations using the *Statistical Package for the Social Sciences* (27). The regression coefficients for the groups from the different countries were estimated from a multiple regression analysis on the pooled data and by using a model allowing for age- and country-specific slopes and intercepts. Body mass index, age category (dummy), a set of country-specific dummies, and a set of interaction terms (country-specific dummies times body mass index) were introduced consecutively into the regression model. The introduction of new variables or set of variables to the model was omitted when this did not result in a significant improvement of the accuracy of estimation of the dependent variable.

Results

The mean values for the anthropometric data and serum concentrations of total and HDL cholesterol are presented in table 2 and figures 1 and 2. The mean values for body mass index were low in the men from Africa (Ghana, Ivory Coast, and Nigeria), the Philippines, and Surinam (20.8–23.5 kg/m^2) compared with those in the European men and the men from Pakistan (24.5–27.5 kg/m^2). Low mean concentrations of total cholesterol were found in the men from Africa (3.0–4.3 mmol/liter), the lowest being observed in the Nigerian men. Intermediate values were found in the men from Surinam, Pakistan, the Philippines, Hungary, Poland, Portugal, Spain, and Italy (4.4–5.5 mmol/liter) and high values were found in the men from eastern and western Finland and the Netherlands (5.6–6.4 mmol/liter), the highest being found in the men from eastern Finland. HDL cholesterol concentrations were on average lower in the men from Africa, Asia, and Surinam (0.7–1.3 mmol/liter) than in the European men (1.1–1.5 mmol/liter), the highest concentrations being found in the men from eastern Finland. The mean concentration of HDL cholesterol was positively correlated with that of total cholesterol (r = 0.64 for the men aged 33–38 years and r = 0.57 for the men aged 43–48 years). The ratio of HDL cholesterol/total cholesterol was higher in the African men (0.26–0.32) than in the men from Asia and Surinam (0.15–0.22) and the men from Europe (0.20–0.28).

The regression coefficients for the relationships of the concentrations of total and HDL cholesterol and the ratio of HDL cholesterol/total cholesterol with body mass index are summarized in table 3. For total cholesterol, there was a positive relationship with body mass index after adjustment had been made for country-specific levels of total cholesterol. Introduction of age- and country-specific slopes into the regression model did not significantly improve the accuracy of estimation of total cholesterol concentration. For HDL cholesterol, there was a negative relationship with body mass index after allowance had been made for country-specific levels of HDL cholesterol. However, the regression coefficient for the Ghanaian men was significantly different from all other slopes. The ratio of HDL cholesterol/total cholesterol was also negatively related to body mass index. Allowing for country-specific regression coefficients did not significantly improve the accuracy of estimation of the HDL cholesterol/total cholesterol ratio.

Allowing for age in the regression models did not result in a significant improvement in the accuracy of estimation in any of the relationships examined.

Discussion

This study clearly shows that there are considerable differences in the mean concentrations in serum of total and HDL cholesterol between groups of adult men from different countries. The differences observed are real because extensive

TABLE 2

*Anthropometric data and the concentration in serum of total and HDL cholesterol in the 13 countries studied (1980)**

Country and age category (years)	Sample size	Weight (kg)	Height (cm)	Body mass index (kg/m^2)	Total cholesterol (mmol/liter)	HDL cholesterol (mmol/liter)	HDL cholesterol/ total cholesterol
Africa							
Ghana							
33–38	40	60 ± 8	167 ± 6	21.5 ± 2.1	4.3 ± 1.0	1.31 ± 0.30	0.32 ± 0.08
43–48	36	61 ± 8	170 ± 7	21.3 ± 2.8	3.9 ± 1.0	1.13 ± 0.42	0.29 ± 0.08
Ivory Coast							
33–38	80	66 ± 7	169 ± 6	23.1 ± 2.3	4.0 ± 0.8	1.14 ± 0.32	0.30 ± 0.09
43–48	80	65 ± 8	167 ± 7	23.5 ± 2.8	4.2 ± 0.8	1.20 ± 0.33	0.29 ± 0.09
Nigeria							
33–38	40	60 ± 6	169 ± 5	20.8 ± 1.8	3.1 ± 0.6	0.81 ± 0.22	0.26 ± 0.07
43–48	40	62 ± 7	169 ± 5	21.9 ± 1.9	3.0 ± 0.6	0.84 ± 0.22	0.29 ± 0.08
America							
Surinam							
33–38	40	67 ± 9	169 ± 6	23.5 ± 3.7	5.3 ± 1.0	1.06 ± 0.28	0.21 ± 0.06
43–48	39	66 ± 10	169 ± 6	23.2 ± 3.0	5.3 ± 1.1	1.08 ± 0.30	0.21 ± 0.08
Asia							
Pakistan							
33–38	39	70 ± 9	166 ± 5	25.5 ± 3.4	4.4 ± 1.0	0.76 ± 0.22	0.18 ± 0.03
43–48	39	75 ± 7	165 ± 5	27.5 ± 2.6	5.1 ± 1.0	0.71 ± 0.19	0.15 ± 0.05
Philippines							
33–38	39	61 ± 9	164 ± 6	22.7 ± 3.2	4.6 ± 1.0	0.98 ± 0.22	0.22 ± 0.07
43–48	40	57 ± 8	162 ± 5	21.8 ± 3.0	5.0 ± 1.1	1.06 ± 0.30	0.22 ± 0.07
Europe							
E. Finland							
33–38	41	78 ± 9	174 ± 6	25.9 ± 2.8	6.3 ± 1.1	1.32 ± 0.42	0.22 ± 0.09
43–48	40	77 ± 12	170 ± 7	26.6 ± 4.0	6.4 ± 1.2	1.50 ± 0.36	0.24 ± 0.07
W. Finland							
33–38	38	78 ± 9	177 ± 6	25.0 ± 2.5	5.9 ± 1.2	1.39 ± 0.36	0.25 ± 0.10
43–48	38	80 ± 9	175 ± 8	26.2 ± 2.5	6.2 ± 1.0	1.38 ± 0.38	0.23 ± 0.07
Hungary							
33–38	33	74 ± 9	177 ± 5	24.6 ± 2.8	5.2 ± 1.0	1.41 ± 0.31	0.28 ± 0.08
43–48	18	75 ± 7	175 ± 8	24.6 ± 2.4	5.3 ± 0.8	1.43 ± 0.26	0.28 ± 0.07

Italy							
33–38	38	74 ± 9	168 ± 6	26.1 ± 2.6	5.1 ± 0.8	1.22 ± 0.38	0.24 ± 0.08
43–48	26	73 ± 11	167 ± 6	26.2 ± 4.2	5.3 ± 1.0	1.15 ± 0.28	0.23 ± 0.08
Netherlands							
33–38	41	77 ± 8	178 ± 5	24.5 ± 2.4	5.6 ± 1.0	1.18 ± 0.27	0.22 ± 0.08
43–48	42	80 ± 9	176 ± 7	25.7 ± 2.6	5.8 ± 1.1	1.10 ± 0.31	0.20 ± 0.07
Poland							
33–38	40	74 ± 9	170 ± 5	25.7 ± 2.6	4.8 ± 0.9	1.27 ± 0.30	0.28 ± 0.09
43–48	40	77 ± 9	170 ± 6	26.6 ± 3.1	5.1 ± 0.9	1.13 ± 0.34	0.23 ± 0.08
Portugal							
33–38	40	68 ± 9	166 ± 7	24.7 ± 2.9	5.4 ± 1.1	1.27 ± 0.40	0.24 ± 0.07
43–48	37	69 ± 11	165 ± 6	25.3 ± 3.6	5.1 ± 0.9	1.41 ± 0.52	0.28 ± 0.09
Spain							
33–38	39	75 ± 11	169 ± 6	26.1 ± 3.7	5.3 ± 1.0	1.19 ± 0.24	0.23 ± 0.08
43–48	39	73 ± 8	167 ± 7	26.1 ± 2.4	5.5 ± 0.9	1.21 ± 0.29	0.23 ± 0.06

* All results are expressed as mean ± SD (standard deviation). 1 mmol/liter = 38.7 mg/dl.

internal and external quality control of the measurements was employed.

Although the aim of the study was not to collect data representative of the different countries, it appears that our findings are basically similar to those reported previously. For instance, total cholesterol concentrations of 6.4–7.0 mmol/liter have been reported from eastern Finland (28). Values reported from the western part of Finland have always been lower than those from the eastern part (29, 30), and this was also the case in this study. Earlier work from this laboratory showed that the concentrations of serum total cholesterol in adult men in the Netherlands was 5.4–6.1 mmol/liter (31) and concentrations of 5.0–5.3 mmol/liter have been reported for Italian men (30). For healthy men from Africa (Ethiopia and Nigeria), values between 2.7 and 5.2 mmol/liter have been reported (8, 18, 32), and for Asian men from the Punjab in India and from Thailand, values between 4.0 and 5.2 mmol/liter (19, 33) have been reported.

For HDL cholesterol, concentrations of 1.2–1.5 mmol/liter have been found in both black and white Americans (34, 35), while concentrations of 1.0–1.1 mmol/liter have been reported for Finns, Lapps, and Norsemen from Scandinavia (36). Lewis et al. (37) found little or no differences in the mean HDL cholesterol concentrations (1.35–1.42 mmol/liter) in adult men from London, Naples, Uppsala, and Geneva. In adult men from the Netherlands, values of 1.2–1.3 mmol/liter have been measured (31). Therefore, the HDL cholesterol concentrations found by us correspond reasonably well with previously reported findings for the more "westernized" populations. However, HDL cholesterol concentrations for non-elite groups from Asia, Africa, and Surinam measured by us (0.7–1.3 mmol/liter) are more in line with the findings of Connor et al. (9) for Tarahumara Indians (0.7 mmol/liter) and those of Robinson

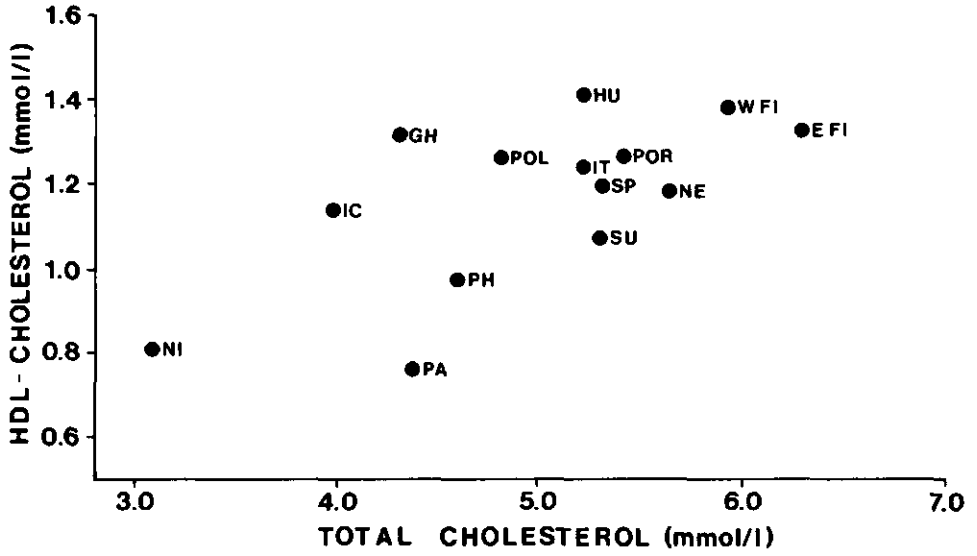

FIGURE 1. Relationship between the mean concentrations of total and high density lipoprotein cholesterol for groups of men aged 33–38 years from 13 countries (1980). EFI, eastern Finland; GH, Ghana; HU, Hungary; IC, Ivory Coast; IT, Italy; NE, The Netherlands; NI, Nigeria; PA, Pakistan; PH, The Philippines; POL, Poland; POR, Portugal; SP, Spain; SU, Surinam; WFI, western Finland.

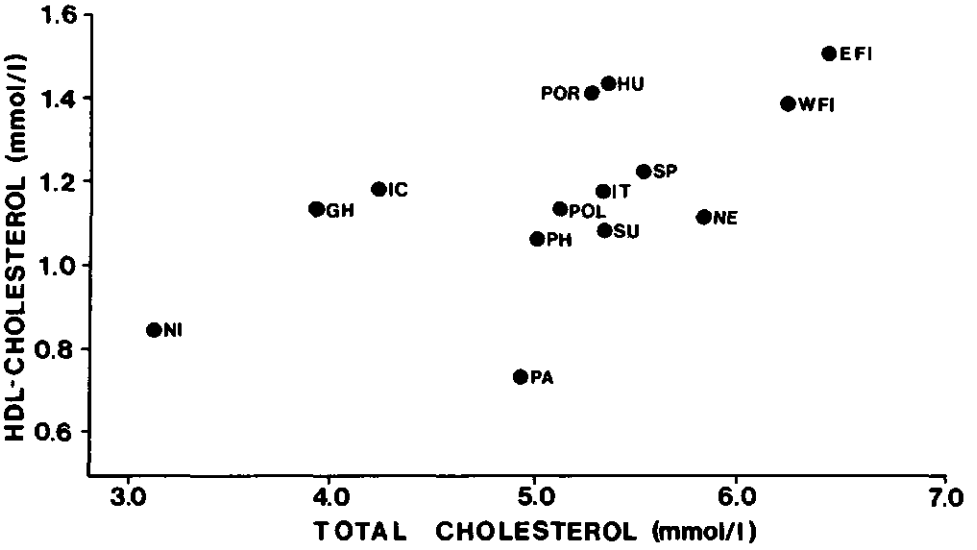

FIGURE 2. Relationship between the mean concentrations of total and high density lipoprotein cholesterol for groups of men aged 43–48 years from 13 countries (1980). EFI, eastern Finland; GH, Ghana; HU, Hungary; IC, Ivory Coast; IT, Italy; NE, The Netherlands; NI, Nigeria; PA, Pakistan; PH, The Philippines; POL, Poland; POR, Portugal; SP, Spain; SU, Surinam; WFI, western Finland.

TABLE 3

Regression coefficients for estimating the concentration of total and HDL cholesterol and the HDL cholesterol/total cholesterol ratio with body mass index for the pooled data of the men from 13 countries*

Parameter	Regression coefficient	Standard error
Total cholesterol	0.0719	0.0100
HDL cholesterol		
Ghana	0.0281	0.0154
Other countries	−0.0220	0.0034
HDL cholesterol/total cholesterol	−0.0071	0.0008

* After adjustment for differences in level between countries.

and Williams (38) for Masai men (1.05 mmol/liter) than with those of Ononogbu (8) for Nigerians (2.1 mmol/liter). In this study, we found lower, although sometimes only slightly lower, concentrations of total and HDL cholesterol in the men from Africa, Asia, and Surinam compared with those from Europe. Therefore, it is not very likely that a negative relationship exists between the mean concentration of HDL cholesterol and mortality from coronary heart disease of different countries, nor is it very likely that there is a negative relationship between the mean ratio of HDL cholesterol/total cholesterol and mortality from coronary heart disease of different countries. The ratios for the groups from Asia and Surinam (Hindustani of Asian descent) were on average lower than for the other groups. In our study in boys (13), the ratios for the Asian groups were also lower (0.24−0.27) than for the other groups (0.30−0.37). The reason for this is not clear.

When correlated with mortality from coronary heart disease (1974/1975 data for men aged 55−64 years for the European countries and the Philippines), it appeared that the correlation was highest for total cholesterol (r = 0.86, n = 8 regions). For HDL cholesterol and the ratio of HDL cholesterol/total cholesterol, the correlations were r = 0.57 and r = 0.25, respectively. Therefore, our data suggest that the mean concentration of total cholesterol is the best indicator of coronary heart disease mortality of countries and that the concentration of HDL cholesterol in populations with lower mortality from coronary heart disease is lower or, at most, equal to that in populations with higher mortality from coronary heart disease.

When compared with our findings in boys from 16 countries (13, 14), the tendency for a concomitant increase of mean HDL cholesterol and total cholesterol between groups from different countries was less clear (see figures 1 and 2). Especially within Europe, there seems to be no association at all. On the basis of the results of our study in boys in which there appeared to be a linear increase in HDL cholesterol with total cholesterol (r = 0.90, n = 26 regions), it was proposed that the concentration of both total and HDL cholesterol increases under the influence of a "westernized" diet rich in animal products and therefore rich in saturated fat and cholesterol. Supporting evidence for this hypothesis has been collected from studies in Tarahumara Indians (9) and in the Masai (38) and also from studies in vegans and macrobiotics (39, 40). In these epidemiologic studies, lower concentrations of HDL cholesterol were associated with lower concentrations of total cholesterol. In experimental studies (41−48), it has been found that on dietary intervention the effects on total and HDL cholesterol were parallel. That the tendency for a concomitant increase of mean total and HDL cholesterol in adults was less clear

than it was in boys (13) is probably explained by the fact that in adults other factors like alcohol consumption (49), smoking (50), obesity (51–55), drug use (56), and physical activity (57–59) play a bigger role in affecting the concentration of HDL cholesterol than they do in boys. Furthermore, the effect of a higher degree of obesity on HDL cholesterol concentration is opposite that of a diet rich in fat and cholesterol, while the effects of a diet rich in fat and cholesterol and of a higher degree of obesity on total cholesterol concentration are parallel. For this reason, the mean HDL cholesterol concentrations in the European countries are probably somewhat lower than could be expected on the basis of the mean total cholesterol concentrations. The regression coefficient for HDL cholesterol on body mass index was equal to -0.022 (mmol liter^{-1}kg^{-1}m^2) which corresponds well with those of Glueck et al. (55) for males aged 20–44 years (-6.33 mg/100 ml/1000 kg/cm^2 = -0.016 mmol liter^{-1}kg^{-1}m^2) and for males aged 45–64 years (-8.46 mg/100 ml/1000 kg/cm^2 = -0.022 mmol liter^{-1}kg^{-1}m^2).

We would like to suggest that the influence of nonnutritional factors, relative to nutritional factors on HDL cholesterol concentration, will determine whether this association between HDL cholesterol concentration and mortality from coronary heart disease will be negative, absent, or positive. Within populations on relatively homogeneous diets, nonnutritional determinants are probably dominant, resulting in a negative association between HDL cholesterol concentration and mortality from coronary heart disease, while between populations, nutritional factors are probably dominant, resulting in a lack of association or even a positive relationship between mean HDL cholesterol and mortality from coronary heart disease.

We would also like to suggest that high concentrations of HDL cholesterol associated with a high intake of animal fat merely reflect a higher capacity to handle large amounts of dietary fat. Thus, people in an affluent population with high HDL cholesterol concentrations would have a relatively low risk of coronary heart disease compared with people with a low HDL cholesterol concentration. Such people with a low HDL cholesterol concentration, probably because of nonnutritional factors, might have a relatively low capacity to handle large amounts of dietary fat, and this would be associated with a relatively high risk of coronary heart disease. Alternatively, people with low intakes of animal fat, such as those in the developing countries, have low levels of HDL cholesterol, but this would not be associated with an enhanced risk of coronary heart disease.

In conclusion, we suggest that in the absence of considerable differences in diet, e.g., within countries, the observed negative association between HDL cholesterol and mortality from coronary heart disease is due to nonnutritional factors, such as physical activity, smoking, alcohol consumption, use of drugs, and the nonnutritional determinants of obesity. On the other hand, when differences in nutritional factors play an important role and the individual influences of the nonnutritional factors are averaged out by taking means for each country, a positive relationship between HDL cholesterol and mortality from coronary heart disease results.

References

1. Miller NE, Førde OH, Thelle DS, et al. The Tromsø Heart Study—High density lipoprotein and coronary heart disease: a prospective case-control study. Lancet 1977;1:965-7.
2. Gordon T, Castelli WP, Hjortland MC, et al. High density lipoprotein as a protective factor against coronary heart disease: The Framingham Study. Am J Med 1977;62:707-13.
3. Castelli WP, Doyle JT, Gordon T, et al. HDL cholesterol and other lipids in coronary heart disease. The Cooperative Lipoprotein Phenotyping Study. Circulation 1977;55:767-72.
4. Jensen G, Schnor P, Faergeman O, et al. HDL cholesterol and ischaemic cardiovascular dis-

ease in the Copenhagen city heart study. Dan Med Bull 1980;27:139-42.
5. Rhoads GG, Gulbrandsen CL, Kagan A. Serum lipoproteins and coronary heart disease in a population study of Hawaii Japanese men. N Engl J Med 1976;294:293-8.
6. Gordon T, Castelli WP, Hjortland MC, et al. Predicting coronary heart disease in middle aged and older persons. JAMA 1977;238:497-9.
7. Keys A. Alpha lipoprotein (HDL) cholesterol in the serum and the risk of coronary heart disease and death. Lancet 1980;2:603-9.
8. Ononogbu IC. Comparison of high density lipoprotein and serum cholesterol levels in a European and African community. Atherosclerosis 1979;34:49-52.
9. Connor WE, Cerqueira MT, Connor RW, et al. The plasma lipids, lipoproteins and diet of the Tarahumara Indians of Mexico. Am J Clin Nutr 1978;31:1131-42.
10. Castelli WP, Cooper GR, Doyle JT, et al. Distribution of triglyceride and total, LDL and HDL cholesterol in several populations: a cooperative lipoprotein phenotyping study. J Chronic Dis 1977;30:147-69.
11. Whitehead TP, Brownings DM, Gregory A. A comparative survey of the results of analysis of blood serum in clinical chemistry laboratories in the U.K. J Clin Pathol 1973;26:435-45.
12. Hainline A, Cooper GR, Olansky AS, et al. CDC survey of high density lipoprotein cholesterol measurement: a report. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA, 1980.
13. Knuiman JT, West CE, Hermus RJJ, et al. Is serum cholesterol outmoded? Lancet 1979;2: 1183-4.
14. Knuiman JT, Hermus RJJ, Hautvast JGAJ. Serum total and high density lipoprotein (HDL) cholesterol concentrations in rural and urban boys from 16 countries. Atherosclerosis 1980;36:529-37.
15. Knuiman JT, West CE. The concentration of cholesterol in serum and in various serum lipoproteins in macrobiotic, vegetarian and non-vegetarian men and boys. Atherosclerosis 1982;43:71-82.
16. Keys A. Coronary heart disease in seven countries. Circulation 1970;41 and 42(suppl 1):1-211.
17. Provisional Food Balance Sheets, Rome, Food and Agricultural Organization, 1977.
18. Ostwald R, Gebre-Medhin M. Westernization of diet and serum lipids in Ethiopians. Am J Clin Nutr 1978;31:1028-40.
19. Pongpaew P, Saovakontha S, Schelp F, et al. Serum lipid pattern in urban and rural Thai population. J Nutr Sci Vitaminol (Tokyo) 1978;24:289-96.
20. Manual of Laboratory Operations, Lipid Research Clinics Program, Vol 1, Lipid and Lipoprotein Analysis. (DHEW Publication no. (NIH) 75), National Institutes of Health, Bethesda, MD, 1974.
21. Mayer KH, Stamler J, Dyer AR, et al. Epidemiologic findings on the relationship of time of day and time since last meal to five clinical variables: serum cholesterol, hematocrit, systolic and diastolic blood pressure, and heart rate. Prev Med 1978;7:22-7.
22. Henderson LO, Saritelli AL, LaGarde E, et al. Minimal within-day variation of high density lipoprotein cholesterol and apoprotein A-1 levels in normal subjects. J Lipid Res 1980;21:953-55.
23. Burstein M, Samaille J. Sur un dosage rapide du cholesterol lié au α- et β-lipoproteins du serum. Clin Chim Acta 1960;5:609-17.
24. van der Haar F, van Gent CM, Schouten FM, et al. Methods for the estimation of high density cholesterol, comparison between two laboratories. Clin Chim Acta 1978;88:469-81.
25. Keys A, Fidanza F, Karvonen MJ, et al. Indices of relative weight and obesity. J Chronic Dis 1972;25:329-43.
26. Evans JG, Prior IAM. Indices of obesity derived from height and weight in two Polynesian populations. Br J Prev Soc Med 1969;23:56-9.
27. Kim J, Kohout FJ. Special topics in general linear models. In: Nie NH, Hadlai Hull C, Jenkins JG, et al., eds. Statistical package for the social sciences (SPSS). 2nd ed. New York: McGraw-Hill Book Company, 1975;368-97.
28. Puska P, Tuomilehto J, Salonen J, et al. Changes in coronary risk factors during comprehensive five-year community programme to control cardiovascular diseases (North Karelia project). Br Med J 1979;2:1173-8.
29. Keys A, Karvonen MJ, Fidanza F. Serum cholesterol studies in Finland. Lancet 1958;2:175-8.
30. Keys A. Seven countries: a multivariate analysis of death and coronary heart disease. Cambridge, MA: Harvard University Press, 1980;121-35.
31. van der Haar F, Kromhout D. Food intake, nutritional anthropometry and blood chemical parameters in 3 selected Dutch schoolchildren populations. Med Landbouwhogeschool, Wageningen, The Netherlands. 1978;78-9:119-22.
32. Onitiri AC, Sander M, Boyo AE. Serum lipids and lipoproteins in healthy Africans. Clin Chim Acta 1977;81:57-61.
33. Werner GT, Sareen DK. Serum cholesterol levels in the populations of Punjab in North West India. Am J Clin Nutr 1978;31:1479-83.
34. Tyroler HA, Glueck CJ, Christensen B, et al. Plasma high density lipoprotein cholesterol comparisons in black and white populations. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62(suppl IV):99-107.
35. Rifkind BM, Tamir I, Heiss G, et al. Distributions of high density and other lipoproteins in selected LRC prevalence study populations: a brief survey. Lipids 1979;14:105-12.
36. Førde OH, Thelle DS, Miller NE, et al. The Tromsø Heart Study. Acta Med Scand 1978; 203:21-6.
37. Lewis B, Chait A, Sigurdson G, et al. Serum lipoproteins in four European communities: a

quantitative comparison. Eur J Clin Invest 1978;8:165-73.
38. Robinson D, Williams P. High density lipoprotein cholesterol in the Masai of East Africa: a cautionary note. Br Med J 1979;1:1249.
39. Sacks FM, Castelli WP, Donner A, et al. Plasma lipids and lipoproteins in vegetarians and controls. N Engl J Med 1975;292:1148-51.
40. Burslem J, Schonfeld G, Howald MA, et al. Plasma apoprotein and lipoprotein lipid levels in vegetarians. Metabolism 1978;27:711-19.
41. Brussaard JH, Dallinga-Thie G, Groot PHE, et al. Effects of amount and type of dietary fat on serum lipids, lipoproteins and apoproteins in man. Atherosclerosis 1980;36:515-27.
42. Ernst M, Bowen P, Fischer M, et al. Changes in plasma lipids and lipoproteins after a modified fat diet. Lancet 1980;2:111-12.
43. Brunner F, Weissbort J, Fischer M, et al. Serum lipid response to a high caloric, high fat diet in agricultural workers during 12 months. Am J Clin Nutr 1979;32:1342-9.
44. Shepherd J, Packard CJ, Patsch JR, et al. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apoprotein A-1. J Clin Invest 1978;61:1582-92.
45. Deurenberg P. De invloed van een vetbeperkte, koolhydraatverrijkte voeding en van een vezelverrijkte voeding op de lipidespiegel in het serum bij vrouwelijke studenten van een diëtistenopleiding. Voeding (Dutch Journal of Nutrition) 1978;39:222-6.
46. Schonfeld G, Weidman SW, Witztum JL, et al. Alterations in levels and interrelations of plasma apolipoproteins induced by diet. Metabolism 1976;25:261-75.
47. Schaefer EJ, Levy RI, Ernst ND, et al. 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;34:1758-63.
48. Tan MH, Dickinson MA, Albers JJ, et al. The effect of a high cholesterol and saturated fat diet on serum high density lipoprotein cholesterol, apoprotein A-1, and apoprotein E levels in normolipidemic humans. Am J Clin Nutr 1980; 33:2559-65.
49. Ernst M, Fischer M, Smith W, et al. The association of plasma high density lipoprotein cholesterol with dietary intake and alcohol consumption. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62(suppl IV):41-52.
50. Criqui MH, Wallace RB, Heiss G, et al. Cigarette smoking and plasma high density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 1980; 62(suppl IV):70-6.
51. Albrink MJ, Krauss RM, Lindgren FT, et al. Intercorrelations among plasma high density lipoprotein, obesity and triglycerides in a normal population. Lipids 1980;15:668-76.
52. Avogaro P, Cazzolato G, Bittolo Bon G, et al. HDL cholesterol, apolipoproteins A1 and B: age and index body weight. Atherosclerosis 1978;31:85-91.
53. Berchtold P, Berger M, Jörgens, V, et al. Cardiovascular risk factors and HDL cholesterol levels in obesity. Int J Obes 1981;5:1-10.
54. Contaldo F, Strazullo P, Postiglione A, et al. Plasma high density lipoprotein in severe obesity after stable weight loss. Atherosclerosis 1980;37:163-7.
55. Glueck CJ, Taylor HL, Jacobs D, et al. Plasma high density lipoprotein cholesterol: associations with measurements of body mass. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62(suppl IV):62-9.
56. Wallace RB, Hunninghake DB, Reiland S, et al. Alterations of plasma high density lipoprotein cholesterol levels associated with consumption of selected mediations. The Lipid Research Clinics Program Prevalence Study. Circulation 1980;62(suppl IV):77-82.
57. Enger SC, Herbjørnsen K, Eriksen J, et al. High density lipoproteins (HDL) and physical activity: the influence of physical exercise, age and smoking on HDL cholesterol and the HDL/ total cholesterol ratio. Scand J Clin Lab Invest 1977;37:251-5.
58. Hartung GH, Foreyt JP, Mitchelli RE, et al. Relation of diet to high density lipoprotein cholesterol in middle-aged marathon runners, joggers, and inactive men. N Engl J Med 1980;302: 357-61.
59. Haskell WL, Taylor HL, Wood PD, et al. Strenuous physical activity, treadmill exercise test performance and plasma high density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 1980; 62(suppl IV):53-61.

4 THE CONCENTRATION OF CHOLESTEROL IN SERUM AND IN VARIOUS SERUM LIPOPROTEINS IN MACROBIOTIC, VEGETARIAN AND NON-VEGETARIAN MEN AND BOYS

J.T. Knuiman and C.E. West

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

(Received 21 July, 1981)
(Revised, received 5 November, 1981)
(Accepted 9 November, 1981)

Summary

The concentrations of total and high density lipoprotein (HDL)-cholesterol and the ratio of HDL-cholesterol to total cholesterol have been examined in groups of non-vegetarian, semi-lactovegetarian, lactovegetarian and macrobiotic men aged 30–39 years and boys aged 6–11 years.

In the men, the concentration of total cholesterol ranged from 3.8 mmol/l in the macrobiotics to 5.5 mmol/l in the non-vegetarians, while the concentration of HDL-cholesterol varied between 1.2 mmol/l and 1.4 mmol/l. The ratio of HDL-cholesterol/total cholesterol varied from 0.23 in the non-vegetarian men to 0.31 in the macrobiotics and it was negatively related to the body mass index (ratio of weight to height^2).

In the boys the concentration of total cholesterol ranged from 3.4 mmol/l in the macrobiotics to 4.3 mmol/l in the semi-lactovegetarians, while the concentration of HDL-cholesterol varied between 1.2 mmol/l to 1.4 mmol/l. The ratio of HDL-cholesterol/total cholesterol was similar in the four groups (0.33–0.35). The concentration of cholesterol in various lipoprotein fractions separated by ultracentrifugation was also estimated in subsamples of the populations.

The variation between groups in the concentration of HDL-cholesterol appeared to be largely due to variations in the concentration of cholesterol in the HDL_2 fraction ($1.063 < \rho_{20} < 1.125$).

Supported by the Netherlands Heart Foundation.
Address correspondence and reprints to J.T. Knuiman.

0021-9150/82/0000–0000/$02.75

Key words: *HDL cholesterol – HDL subfractions – Lipoprotein cholesterol – Macrobiotics – Total cholesterol – Vegetarians*

Introduction

It has been found that the concentration of total cholesterol is positively related to the risk of coronary heart disease (CHD) [1–3], and that of HDL-cholesterol negative to the risk of CHD [4–7]. Furthermore it has been suggested [8] that the ratio HDL-cholesterol/total cholesterol actually provides a better indicator of the contribution of serum lipids to the risk of coronary heart disease than that of the concentration of either total or HDL-cholesterol. Therefore it is worthwhile to seek possible determinants of this ratio.

In our study of 7- and 8-year-old boys in both rural and urban areas of 16 countries [9,10] we found that the mean of the HDL-cholesterol/total cholesterol ratio varied within only narrow limits: 0.24–0.27 in Asia (Pakistan and the Philippines), 0.30–0.36 in Africa (Ghana, Ivory Coast, Nigeria) and 0.30–0.37 in the U.S.A. and Europe (Austria, Denmark, Finland, Greece, Hungary, Ireland, the Netherlands, Poland, Portugal and Sweden).

On the basis of these findings the hypothesis was formulated that the concentration of both total and HDL-cholesterol increase under the influence of a more 'Westernized' diet, which has a large proportion of food of animal origin. As a result, the ratio of HDL-cholesterol to total cholesterol would be relatively independent of the composition of the diet consumed. However, this hypothesis is based on our findings in children and therefore needs to be tested in adults. For this reason a study in adult men from 13 countries is now in progress.

It was also considered worthwhile to test the same hypothesis in both young boys and adult men with extremely different diets, but living in one region, to eliminate or to diminish the influence of confounding factors affecting the results of a between-country comparison. Thus, the study described in this paper was carried out in adult men aged 30–39 years and in boys aged 6–11 years living in the Netherlands or Belgium and consuming diets differing in the extreme, especially with respect to the contribution to the diet of products of animal origin. Since it has been suggested [11] that the beneficial effect of HDL can be ascribed to a particular subfraction of HDL, the lipoprotein patterns of the boys and men were also studied.

Methods

The men, who were aged between 30 and 39 years and the boys, who were aged between 6 and 11 years, volunteered to participate in the study. They were selected on the basis of having different dietary patterns: macrobiotic, lactovegetarians, semi-lactovegetarians and non-vegetarians. The macrobiotics lived throughout the Flemish part of Belgium and in the Netherlands and were mainly recruited through

Lima N.V. (St. Martens-Latem, Belgium), a manufacturer of macrobiotic products. Macrobiotics are defined as people who adhere to the principles of the macrobiotic system which implies that they almost entirely avoid the use of animal products such as meat, milk and eggs, but consume diets based on whole grains, beans and vegetables supplemented with seaweed and fermented soy products. It is a diet low in fat and rich in carbohydrates. The lactovegetarians and semi-lactovegetarians who lived throughout the Netherlands, were recruited through the Netherlands Vegetarian Society and through advertisements lodged in papers and in shops for biologically grown products. Lactovegetarians are defined as persons who regularly eat both milk and egg products but never, or only seldomly, eat meat or fish products, while semi-lactovegetarians eat meat or fish products less than once a week. The non-vegetarians were recruited from three factories in different areas of the Netherlands.

The classification of people into the various groups defined above was not done on the basis of an extensive dietary survey but on the usual frequency of consumption of products derived from milk, eggs, meat and fish. The frequencies were recorded as the number of times per day or per week that products from milk, eggs, meat and fish were eaten or by indicating that these products were eaten less than once a week or never. Questionnaires recording the frequencies were completed at home by all participants. The time that the members of the three vegetarian groups were on their respective dietary regimen is given in Table 1.

Standing height was measured with a Microtoise (Stanley, Mabo, France) to the nearest 0.1 cm. On examination the person stood erect, without shoes, his back against the wall, chin parallel to the floor, heels together and his feet forming an angle of 45°. Body weight was measured to the nearest 0.1 kg with an electronic weighing scale (Seca, Model 770 alpha, Federal Republic of Germany) which was calibrated regularly during use. The person being weighed stood without shoes, heavy garments and heavy objects in the centre of the platform while being weighed. From the results from each person, the body mass index (weight/height^2, expressed as kg/m^2) was calculated.

Physical activity and the use of alcohol and tobacco or drugs, all of which may influence the HDL-cholesterol/total cholesterol ratio, were not measured.

Blood was taken from each person in a non-fasting condition as described previously [10] for the estimation of total cholesterol and HDL-cholesterol (by the heparin–$MnCl_2$ precipitation method) in whole serum and for the estimation of cholesterol in lipoprotein fractions separated by density gradient ultracentrifugation. The serum was separated from the blood cells by centrifugation. Separation of lipoproteins was carried out on unfrozen serum within 3 days and the serum for the other estimations was stored at −80°C until analysis, which was carried out within one month of blood sampling. The concentration of total cholesterol in serum was measured as described previously [10]. Reproducibility for blind control sera provided by the Centre for Disease Control, Atlanta, GA, U.S.A., was ±0.9% and accuracy was within 1.4% of the true (target) values. The concentration of cholesterol in HDL was measured after the precipitation of the low and very low density lipoproteins by heparin–$MnCl_2$ [12,13]. Reproducibility for blind control sera obtained in the Centre for Disease Control Survey of HDL-cholesterol Measure-

TABLE 1

ANTHROPOMETRIC DATA AND THE CONCENTRATION IN SERUM OF TOTAL AND HDL-CHOLESTEROL OF THE PARTICIPANTS

Results are expressed as mean ± SD.

Group	Sample size	Age (yr)	Time on diet (yr)		Height (cm)
			Median	Range	
Non-vegetarian					
men	52	34.6 ± 2.6	–	–	178 ± 6
boys	54	9.6 ± 1.6	–	–	141 ± 11
Semi-lactovegetarian					
men	43	34.2 ± 2.6	3.5	0.5 – 9.0	180 ± 6
boys	15	9.1 ± 1.6	4.0	1.0 – 9.0	136 ± 11
Lactovegetarian					
men	56	33.7 ± 3.0	5.5	0.8 – 38.0	180 ± 6
boys	15	7.9 ± 1.6	4.0	0.8 – 11.0	132 ± 12 *
Macrobiotic					
men	33	32.5 ± 4.0	4.0	1.0 – 9.0	176 ± 6 *
boys	6	9.7 ± 2.8	4.0	1.0 – 7.0	135 ± 14
All vegetarians pooled					
men	132	33.6 ± 3.1	4.5	0.5 – 38.0	179 ± 6
boys	36	8.7 ± 1.8	4.0	0.8 – 11.0	134 ± 14 **

Statistical comparison by Student's t-test with non-vegetarians: * $P < 0.05$; ** $P < 0.01$.

ments [14] was ±2.2% and bias with regard to the overall survey mean was on average 0.1%. The HDL-cholesterol/total cholesterol ratio was calculated for each person and the means and SD of this ratio calculated for each group. The relationships between the concentrations of total and HDL-cholesterol and the HDL-cholesterol/total cholesterol ratio and the body mass index were examined by calculating regression lines.

The separation of lipoproteins in serum by ultracentrifugation was carried out as described by Terpstra et al. [15] in subsamples randomly chosen from all groups. The concentration of cholesterol in the lipoprotein fractions was estimated by the catalase method of Röschlau et al. [16].

As macrobiotic diets are sometimes regarded as being nutritionally deficient, additional blood was taken from the macrobiotics to carry out a number of analyses to assess their nutritional status. There was no evidence either from clinical examination or from the analysis of haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin or mean corpuscular haemoglobin concentration of nutritional anaemias or other nutritional deficiencies.

Weight (kg)	Body mass index (kg/m^2)	Total cholesterol (mmol/l)	HDL-cholesterol (mmol/l)	HDL-cholesterol/ total cholesterol
77 ± 8	24.4 ± 2.3	5.5 ± 1.0	1.2 ± 0.3	0.23 ± 0.07
32 ± 7	16.0 ± 1.7	4.2 ± 0.6	1.4 ± 0.3	0.34 ± 0.06
72 ± 9 **	22.2 ± 2.3 **	4.9 ± 1.0 *	1.3 ± 0.3 *	0.28 ± 0.08 **
31 ± 6	16.4 ± 1.3	4.3 ± 0.9	1.4 ± 0.2	0.33 ± 0.08
69 ± 7 **	21.4 ± 1.7 **	4.7 ± 0.9 **	1.4 ± 0.3 *	0.30 ± 0.08 **
27 ± 6	15.5 ± 0.8	4.0 ± 0.6	1.4 ± 0.4	0.34 ± 0.08
65 ± 8 **	20.9 ± 2.1 **	3.8 ± 0.8 **	1.2 ± 0.3	0.31 ± 0.08 **
29 ± 8	15.3 ± 1.6	3.4 ± 0.6 **	1.2 ± 0.3	0.35 ± 0.06
69 ± 8 **	21.5 ± 2.1 **	4.5 ± 0.9 **	1.3 ± 0.3	0.29 ± 0.08 **
29 ± 6 *	15.9 ± 1.2	4.0 ± 0.8	1.3 ± 0.4	0.34 ± 0.08

Results

Data on the men in each of the four groups are presented in Tables 1 and 2. Although the differences between the mean heights of the men, which ranged from 176 to 180 cm, were small, there were large differences in the weight and body mass index as shown in Table 1. The weights and body mass indices for the semi-lactovegetarian, lactovegetarian and macrobiotic men were on average lower than for the non-vegetarian men; there being a gradation to both lower weights and indices from the non-vegetarian through the semi-lactovegetarian and lactovegetarian men to the macrobiotic men. The same trend is also seen in the concentration of total cholesterol in serum and the difference between the vegetarian men and non-vegetarian men was significant. However, this trend is not repeated for the concentration of HDL-cholesterol; the concentration for the macobiotic men was similar to that of the non-vegetarian men, while the concentrations in the semi-lactovegetarian and lactovegetarian men were slightly higher.

The ratio of HDL-cholesterol to total cholesterol is significantly lower in the

TABLE 2

RELATIONSHIPS BETWEEN CHOLESTEROL PARAMETERS AND BODY MASS INDEX FOR THE MEN

Group	n	Total cholesterol	SE_b	HDL-cholesterol	SE_b	HDL-cholesterol/ total cholesterol	SE_b
		Regression equation		Regression equation		Regression equation	
Non-vegetarians	52	y=0.176 **x+1.19	0.057	y=−0.027x+1.84	0.015	y=−0.0119 *x+0.518	0.0041
Semi-lactovegetarians	43	y=0.208 **x+0.29	0.063	y=−0.035x+2.08	0.021	y=−0.0174 *x+0.665	0.0049
Lactovegetarians	56	y=0.066x+3.23	0.067	y=−0.026x+1.91	0.025	y=−0.0083x+0.474	0.0060
Macrobiotics	33	y=0.020x+3.40	0.067	y=−0.033x+1.85	0.023	y=−0.0108x+0.539	0.0069

y=bx+c where y=concentration of total or HDL-cholesterol, or the ratio and x=body mass index. Significance of b (slope) from 0: * $P<0.05$, ** $P<0.01$.

non-vegetarian men than in the vegetarian men. As the vegetarian men were on average lighter than the non-vegetarian men, as reflected in the body mass index, it is interesting to relate the concentration of total and HDL-cholesterol and the HDL-cholesterol/total cholesterol ratio to the body mass index (Table 2).

There was a positive relationship between the concentration of total cholesterol and the body mass index and it was significant for the non-vegetarian and semi-lactovegetarian men. A negative relationship was found between the concentration of HDL-cholesterol and the body mass index; however, in no case did it reach statistical significance. A negative relationship was found between the ratio of HDL-cholesterol/total cholesterol and the body mass index and it was significant for the non-vegetarian and semi-lactovegetarian men. Data on the boys in each of the four groups are also presented in Table 1. The heights and weights of the vegetarian (semi-lactovegetarian, vegetarian and macrobiotic) boys were on average lower than that of the non-vegetarian boys but much of this difference can probably be attributed to the higher average age of the non-vegetarian boys. There were only slight differences in body mass index between the groups of boys (range: 15.3–16.4). For the concentration of total cholesterol and HDL-cholesterol and the ratio of HDL-cholesterol/total cholesterol the differences between the groups of boys were also small and not significant except for the difference in the concentration of total cholesterol between the macrobiotic and non-vegetarian boys. There was no significant relationship between the body mass index and the concentration of total cholesterol, HDL-cholesterol or the ratio of HDL-cholesterol/total cholesterol within the groups of boys.

Some of the results of the ultracentrifugation of the prestained serum are shown in Fig. 1. Visible bands were observed in the following density ranges: VLDL, $\rho_{20} < 1.006$ g/ml; LDL, $1.03 < \rho_{20} < 1.05$ g/ml; two HDL bands with $1.075 < \rho_{20} < 1.10$ g/ml and $1.10 < \rho_{20} < 1.13$ g/ml. Sometimes an additional band was seen with $1.06 < \rho_{20} < 1.075$ g/ml. This latter band is probably an Lp(a) band though this was not checked by electrophoresis [15]. The density limit of 1.10 corresponds with the

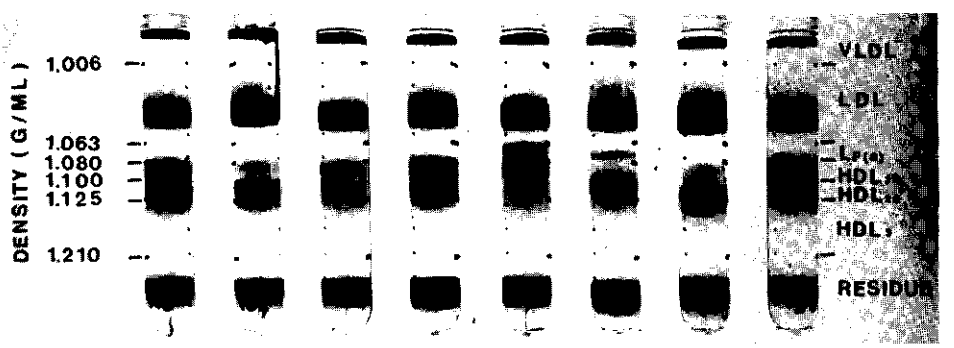

Fig. 1. Photographs of lipoproteins separated by continuous gradient ultracentrifugation after pre-staining of the lipoproteins with Sudan Black.

The separation between HDL_{2a} and HDL_{2b} can be clearly seen and in samples 5 and 6, the presence of material which is probably Lp(a) can also be seen.

TABLE 3

CONCENTRATIONS OF CHOLESTEROL IN THE VARIOUS LIPOPROTEIN FRACTIONS FOR ALL GROUPS (mmol/l)·
Results are expressed as mean ± SD.

Group	Sample size	VLDL [a]	LDL	HDL_{2b}	HDL_{2a}
Non-vegetarians					
men	14	0.70 ± 0.48	2.97 ± 0.71	0.35 ± 0.15	0.32 ± 0.06
boys	15	0.26 ± 0.09	2.43 ± 0.54	0.64 ± 0.26	0.50 ± 0.10
Semi-lactovegetarians					
men	14	0.60 ± 0.34	2.95 ± 0.48	0.57 ± 0.20 *	0.40 ± 0.13 *
boys	10	0.36 ± 0.12 *	2.50 ± 0.61	0.58 ± 0.20	0.42 ± 0.05 *
Lactovegetarians					
men	17	0.45 ± 0.20	3.00 ± 0.70	0.53 ± 0.17 *	0.42 ± 0.09 *
boys	14	0.38 ± 0.16 *	2.30 ± 0.47	0.63 ± 0.33	0.42 ± 0.12
Macrobiotics					
men	13	0.20 ± 0.22 *	2.07 ± 0.95	0.43 ± 0.20	0.31 ± 0.10
boys	6	–	–	–	–

[a] Density ranges (g/ml) for the different lipoproteins: VLDL, $\rho_{20} < 1.006$; LDL, $1.006 < \rho_{20} < 1.063$; HDL_{2b}, $1.063 < \rho_{20} < 1.100$; HDL_{2a}, $1.100 < \rho_{20} < 1.125$; HDL_3, $1.125 < \rho_{20} < 1.210$; Bottom fraction, $\rho_{20} > 1.210$.

boundary between HDL_{2b} ($1.063 < \rho_{20} < 1.100$) and HDL_{2a} ($1.100 < \rho_{20} < 1.125$) and the band with a density $1.100 < \rho_{20} < 1.13$ corresponds to HDL_{2a} and HDL_3.

The concentration of cholesterol in the various lipoprotein fractions was much less in the groups of boys examined than in the various groups of adult men (Table 3). In the men, the concentration of cholesterol in the VLDL fractions was lower in the vegetarians than in the non-vegetarians, while in the LDL-fraction, the cholesterol concentration was similar in all groups, except in the macrobiotics which had significantly lower levels than the non-vegetarians. The concentration of HDL-cholesterol in the men was higher in the semi- and lactovegetarians than the non-vegetarians while the macrobiotics had similar levels to the non-vegetarians. Most of the variation in the concentration of cholesterol in the HDL fraction of both the men and the boys could be accounted for by differences in the HDL_2 fractions, in which the concentration of cholesterol varied over a wide range (HDL_{2b}, $1.063 < \rho_{20} < 1.100$; 0.35–0.64 mmol/l serum and HDL_{2a}, $1.100 < \rho_{20} < 1.125$; 0.32–0.50 mmol/l serum) while there was only slight variation (0.35–0.45 mmol/l serum) between the groups in the cholesterol concentration in the HDL_3 fraction ($1.125 < \rho_{20} < 1.210$).

In this study, the concentration of HDL-cholesterol was measured after precipitation of the VLDL and LDL and also following ultracentrifugation of serum. The results obtained by the two methods generally show very close agreement (Table 3).

HDL_3	Bottom fraction	HDL		Total [b]	Recovery by UC
		by UC	by precipit.		
0.39 ± 0.06	0.14 ± 0.04	1.06 ± 0.24	1.19 ± 0.33	5.01 ± 0.90	97 ± 4
0.43 ± 0.08	0.15 ± 0.03	1.57 ± 0.34	1.40 ± 0.31	4.04 ± 0.55	109 ± 9
0.41 ± 0.07	0.17 ± 0.08	1.38 ± 0.34 *	1.34 ± 0.32	5.02 ± 0.50	101 ± 5
0.39 ± 0.08	0.14 ± 0.08	1.38 ± 0.24	1.37 ± 0.20	4.35 ± 0.81	101 ± 5
0.45 ± 0.09 *	0.19 ± 0.09 *	1.40 ± 0.23 *	1.37 ± 0.21	4.94 ± 0.74	102 ± 6
0.40 ± 0.13	0.14 ± 0.08	1.42 ± 0.36	1.38 ± 0.35	4.11 ± 0.60	103 ± 8
0.42 ± 0.13	0.11 ± 0.04	1.16 ± 0.30	1.21 ± 0.19	3.86 ± 0.99	91 ± 10
–	–	–	1.19 ± 0.33	3.35 ± 0.56	

[b] Values slightly different from those in Table 2 as only a sub-sample was taken from each group. Statistical comparison by Student's *t*-test with non-vegetarians: * $P < 0.05$

Discussion

In this study we have found higher mean concentrations of serum total cholesterol in non-vegetarian men (5.5 mmol/l) compared with three groups of vegetarian men (3.8–4.9 mmol/l). These results agree with the findings reported by other workers [17,18]. Variation in the mean concentration of serum total cholesterol was much smaller in boys than in men, although the concentrations in the macrobiotic boys were comparable to those that we found earlier in boys from Africa and Pakistan [10]. The higher concentrations of total cholesterol found in the non-vegetarian men possibly result from a higher degree of body fatness and also possibly from a higher intake of saturated fat due to the higher proportion of products of animal origin in their diets [19,20].

A negative relationship between the concentration of HDL-cholesterol and body mass index has been reported earlier by other workers [21,22]. We also found a similar relationship in the men in our study, although it was not statistically significant, probably because of the low number of men in each group studied. The negative relationship between the concentration of HDL-cholesterol and body mass index would explain why the semi-lactovegetarian and lactovegetarian men, being lighter, have higher HDL-cholesterol concentrations than the non-vegetarian men. However, the concentration of HDL-cholesterol in the macrobiotic men was lower, being similar to that of the non-vegetarian men. Sacks et al. [23] found relatively low

HDL-cholesterol concentrations in macrobiotics (1.09 mmol/l) compared with non-vegetarians (1.27 mmol/l) and Burslem et al. [18] reported a value of 1.11 mmol/l for vegetarians compared with 1.24 mmol/l in controls.

The results of studies of Ernst et al. [24] and Schonfeld et al. [25] indicate that increased amounts of carbohydrates in the diet reduce the level of HDL-cholesterol in serum. Such evidence suggests that low concentrations of HDL-cholesterol could be the result of diets low in fat and high in carbohydrates. Since the concentration of total cholesterol is also low in vegetarian diets, especially those of macrobiotics, this would result in a ratio that is not vastly different to that of people on non-vegetarian diets. Epidemiological studies in groups using extremely different diets, including those in the Tarahumara Indians from Mexico [26] and the Masai [27] and in our study of young boys from 16 countries [9,10], are in agreement with this hypothesis. Results from dietary intervention studies in healthy adults also indicate that when the total cholesterol concentration is high as a result of the diet consumed, the HDL-cholesterol concentration also tends to be high [25,28–31]. Thus dietary factors appear to influence the concentrations of total and HDL-cholesterol in such a way that the ratio of HDL-cholesterol/total cholesterol is relatively independent of the composition of the diet consumed. In the present study the difference in the ratio between the groups of non-vegetarian men and the pooled groups of vegetarian men (macrobiotics, lactovegetarians and semi-lactovegetarians) was 0.06 but this was reduced to 0.03 after correction for differences in body mass index between the groups. Such a calculation could be made as the regression co-efficient in the equation relating the ratio to the body mass index in the pooled groups of vegetarians ($y = -0.0119x + 0.548$, $SE_b = 0.0025$) did not appear to be different to that for the non-vegetarians; in fact it was identical. Thus, half of the difference in the ratio between the vegetarians and the non-vegetarians was mediated through an effect of nutrition and life style factors on the body mass index and half was mediated through an effect independent of the body mass index. Probably other factors such as physical activity [32], smoking habits [33], alcohol consumption [24] and drug use [34] are also important in determining the difference in the HDL-cholesterol/total cholesterol ratio. The ratios HDL-cholesterol/total cholesterol in the groups of boys were very similar (0.33–0.35); this agrees with the observation that there was little difference in body mass index between the groups. The results correspond with those reported earlier for European boys [10,11].

The pattern of distribution of lipoproteins as observed after the density gradient ultracentrifugation of serum in which the lipids were prestained with Sudan Black showed that two HDL bands are present on either side of a density (ρ_{20}, g/ml) of 1.100. This has also been reported by Terpstra et al. [15] and Cheung and Albers [35]. In addition, there is sometimes lipoprotein with a density between 1.060 and 1.075 which probably corresponds to Lp(a). Thus the lower density limit of HDL should be between 1.075 and 1.080. The density limit of 1.100 corresponds to the division between HDL_{2b} (< 1.100) and HDL_{2a} (> 1.100) while the fraction observed with a density greater than 1.100 corresponds to a mixture of HDL_{2a} ($1.100 < \rho_{20} < 1.125$) and HDL_3 ($1.125 < \rho_{20} < 1.210$). The concentration of cholesterol in HDL_2 fractions appeared to vary between groups of men and boys over a wider range than

that in the HDL_3 fraction. Therefore future research in this field should focus especially on the cholesterol in the HDL_2 fraction.

Acknowledgements

We would like to thank all those who participated in the study and those who helped us make contact with the men, especially Mr. M. Callebert (Lima N.V., St. Martens-Latem, Belgium), Mr. H. Overweg and Mrs. M. Riemens-Dijkstra (Netherlands Vegetarian Society) and Dr. J. Van Haaren (Industrial Health Service, Boxmeer). We would also like to thank Dr. A. Verbeek (Faculty of Medicine, Catholic University, Nijmegen) for advice and for carrying out the clinical examination of the macrobiotics in Belgium.

Members of the Department of Human Nutrition who gave invaluable assistance include the dietitians Miss S. Westenbrink, R.D. and Miss L.J.M. Van der Heijden, R.D., the analysts Miss A.E.M.F. Soffers, Mrs. J.C.M. Barendse-van Leeuwen, Mrs. G. Germing-Nouwen, Mrs. E. Koot-Gronsveld, Mr. H.J. Verweij and also Ir. W.A. Van der Wiel-Wetzels, Ir. J.A.M. Baecke, Drs. J. Burema, Miss W.A. Van Staveren, MSc, Dr. M.B. Katan and Professor J.G.A.J. Hautvast.

References

1 Keys, A., Coronary heart disease — The global picture, Atherosclerosis, 22 (1975) 149.
2 The Pooling Project Research Group. Relationship of blood pressure, serum cholesterol, smoking habit, relative weight and E.C.G. abnormalities to incidence of major coronary events — Final report of the Pooling Project, J. Chron. Dis., 31 (1978) 201.
3 Kannel, W.B., Castelli, W.P. and Gordon, T., Cholesterol in the prediction of atherosclerotic disease, Ann. Int. Med., 90 (1979) 85.
4 Miller, N.E., Førde, O.H., Thelle, D.S. and Mjøs, O.D., The Tromsø Heart Study — High density lipoprotein and coronary heart disease: a prospective case-control study, Lancet, i (1977) 965.
5 Gordon, T., Castelli, W.P., Hjørtland, M.C., Kannel, W.B. and Dawber, T.R., High density lipoprotein as a protective factor against coronary heart disease — The Framingham Study, Amer. J. Med., 62 (1977) 707.
6 Castelli, W.P., Doyle, J.T., Gordon, T., Hames, C.G., Hjørtland, M.C., Hulley, S.B., Kagan, A. and Zukel, W.J., HDL-cholesterol and other lipids in coronary heart disease — The Cooperative Lipoprotein Phenotyping Study, Circulation, 55 (1977) 767.
7 Rhoads, G.G., Gulbrandsen, C.L. and Kagan, A., Serum lipoproteins and coronary heart disease in a population study of Hawaii-Japanese men, N. Engl. J. Med., 294 (1976) 293.
8 Kannel, W.B. and Castelli, W.P., Is the serum total cholesterol an anachromism? Lancet, ii (1979) 950.
9 Knuiman, J.T., West, C.E., Hermus, R.J.J. and Hautvast, J.G.A.J., Is serum total cholesterol outmoded? Lancet, ii (1979) 1183.
10 Knuiman, J.T., Hermus, R.J.J. and Hautvast, J.G.A.J., Serum total and high density lipoprotein (HDL) cholesterol concentrations in rural and urban boys from 16 countries, Atherosclerosis, 36 (1980) 529.
11 Anderson, D.W., Nichols, A.V., Span, S.S. and Lindgren, F.T., High density lipoprotein distribution — Resolution and determination of three major components in a normal population sample, Atherosclerosis, 29 (1978) 161.
12 Burstein, M. and Samaille, J., Sur un dosage rapide du cholestérol lié aux α- et β-lipoprotéines du sérum, Clin. Chim. Acta, 5 (1960) 609.
13 Van der Haar, F., Van Gent, C.M., Schouten, F.M. and Van der Voort, H.A., Methods for the estimation of high density cholesterol — Comparison between two laboratories, Clin. Chim. Acta, 80 (1978) 469.

14 Hainline, J.A., Cooper, G.R., Olansky, A.S., Winn, C.L. and Miller, D.T., CDC Survey of High Density Lipoprotein Cholesterol Measurements — A Report, Centre for Disease Control, Atlanta, GA, 1980.
15 Terpstra, A.H.M., Woodward, J.H. and Sanchez-Muniz, F.J., Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation — Visualization by prestaining and rapid separation of serum lipoproteins from small volumes of serum, Anal. Biochem., 111 (1981) 149.
16 Von Röschlau, P., Bernt, E. and Gruber, W., Enzymatische Bestimmung des Gesamt-Cholesterins in Serum, Z. Klin. Chem. Klin. Biochem., 12 (1974) 403.
17 Gear, J.S., Mann, J.I., Thorogood, M., Carter, R. and Jelfs, R., Biochemical and haematological variables in vegetarians, Brit. Med. J., I (1980) 1415.
18 Burslem, J., Schonfeld, G., Howald, M.A., Weidman, S.W. and Miller, J.P., Plasma apoprotein and lipoprotein lipid levels in vegetarians, Metabolism, 27 (1978) 711.
19 Matter, S., Weltman, A. and Stamford, B.A., Body fat content and serum lipid levels, J. Amer. Diet. Ass., 77 (1980) 149.
20 Brennan, P.J., Simpson, J.M., Blacket, R.B. and McGilchrist, C.A., The effects of body weight in serum cholesterol, serum triglycerides, serum urate and systolic blood pressure, Aust. N.Z.J. Med., 10 (1980) 15.
21 Glueck, C.J., Taylor, H.L., Jacobs, D., Morrison, J.A., Beaglehole, R. and Williams, O.D., Plasma high density lipoprotein cholesterol — Association with measurements of body mass, Circulation, 62 (Suppl, IV) (1980) 62.
22 Albrink, M.J., Krauss, R.M., Lindgren, F.T., Groeben, J. von der, Pan, S. and Wood, P.D., Intercorrelations among plasma high density lipoprotein, obesity and triglycerides in a normal population, Lipids, 15 (1980) 668.
23 Sacks, F.M., Castelli, W.P., Donner, A. and Kass, E.H., Plasma lipids and lipoproteins in vegetarians and controls, N. Engl. J. Med., 292 (1972) 1148.
24 Ernst, N., Fischer, M., Smith, W., Gordon, T., Rifkind, B.M., Little, J.A., Mishkel, M.A. and Williams, O.D., The association of plasma high density lipoprotein cholesterol with dietary intake and alcohol consumption, Circulation, 62 (Suppl. IV) (1980) 41.
25 Schonfeld, G., Weidman, S.W., Witztum, J.L. and Bowen, R.M., Alterations in levels and interrelations of plasma apolipoproteins induced by diet, Metabolism, 25 (1976) 261.
26 Connor, W.E., Cerqueira, M.T., Connor, R.W., Wallace, R.B., Malinow, M.R. and Casdorph, H.R., The plasma lipids, lipoproteins and diet of the Tarahumara Indians of Mexico, Amer. J. Clin. Nutr., 31 (1978) 1131.
27 Robinson, D. and Williams, P., High density lipoprotein cholesterol in the Masai of East Africa — A cautionary note, Brit. Med. J., I (1979) 1249.
28 Shepherd, J., Packard, C.J., Patsch, J.R., Gotto, A.M. and Taunton, A.D., Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apoprotein A-1, J. Clin. Invest., 61 (1978) 1582.
29 Brunner, D., Weissbort, J., Fischer, M., Bearman, J.E., Loebl, K., Schwartz, S. and Levin, S., Serum lipid response to a high caloric, high fat diet in agricultural workers during 12 months, Amer. J. Clin. Nutr., 32 (1979) 1342.
30 Brussaard, J.H., Dallinga-Thie, G., Groot, P.H.E. and Katan, M.B., Effects of amount and type of dietary fat on serum lipids, lipoproteins and apoproteins in man, Atherosclerosis, 36 (1980) 515.
31 Ernst, N., Bowen, P., Fischer, M., Schaeffer, E.J. and Levy, R.I., Changes in plasma lipids and lipoproteins after a modified fat diet, Lancet, ii (1980) 111.
32 Haskell, W.L., Taylor, H.L., Wood, P.D., Schrott, H. and Heiss, G., Strenuous physical activity, treadmill exercise test performance and plasma high density lipoprotein cholesterol, Circulation, 62 (Suppl. IV) (1980) 53.
33 Criqui, M.H., Wallace, R.B., Heiss, G., Mishkell, M., Schonfeld, G. and Jones, G.T.L., Cigarette smoking and plasma high density lipoprotein cholesterol, Circulation, 62 (Suppl. IV) (1980) 70.
34 Wallace, R.B., Hunninghake, D.B., Reiland, S., Barrett-Connor, E., Mackenthun, A., Hoover, J. and Wahl, P., Alterations of plasma high density lipoprotein cholesterol levels associated with consumption of selected medications, Circulation, 62 (Suppl. IV) (1980) 77.
35 Cheung, M.C. and Albers, J.J., Distributions of cholesterol and apolipoprotein A-I and A-II in human high density lipoprotein subfractions separated by CsCl equilibrium-gradient centrifugation — Evidence for HDL subfractions with differing A-I/A-II molar ratios, J. Lipid Res., 20 (1979) 200.

5 DETERMINANTS OF TOTAL AND HIGH DENSITY LIPOPROTEIN CHOLESTEROL IN BOYS FROM FINLAND, THE NETHERLANDS, ITALY, THE PHILIPPINES AND GHANA WITH SPECIAL REFERENCE TO DIET

JAN T.KNUIMAN[2], SUSANNE WESTENBRINK[2], LIDWIEN VAN DER HEYDEN[2], CLIVE E.WEST[2], JAN BUREMA[2], JANNA DE BOER[2], JOSEPH G.A.J.HAUTVAST[2], LEENA RÄSÄNEN[3], LIISA VIRKKUNEN[3], JORMA VIIKARI[4], PHOEBE LOKKO[5], J.O.M.POBEE[6], ANNA FERRO-LUZZI[7], ANNA M.FERRINI[7], CHRISTINA SCACCINI[7], STEFANIA SETTE[7], GRACIA M.VILLAVIEJA[8] and JOSEFINA BULATAO-JAYME[8]

[1]Supported by the Netherlands Heart Foundation Grant 78064

[2]Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands

[3]Department of Nutrition, University of Helsinki, Helsinki, Finland

[4]Department of Medicine, University Central Hospital, Turku, Finland

[5]Food Research Institute, Accra, Ghana

[6]Department of Medicine and Therapeutics, University of Ghana Medical School, Accra, Ghana

[7]Istituto Nazionale della Nutrizione, Roma, Italy

[8]Food and Nutrition Research Institute, Manila, The Philippines

ABSTRACT

We have studied the determinants of total and high density lipoprotein (HDL) cholesterol in young boys from five countries characterized by different lifestyles, dietary consumption profiles and mortality rates from coronary heart disease. All measurements including the estimation of dietary intake, physical activity, body mass index and the concentration of total and HDL cholesterol were carefully standardized. The mean concentrations of total and HDL cholesterol were higher in the European boys (4.1 - 4.9 mmol/l and 1.45 - 1.57 mmol/l, respectively) than in boys from Ghana and the Philippines (3.1 - 3.8 mmol/l and 0.93 - 1.10 mmol/l, respectively). A positive correlation was found between the concentration of total cholesterol and the intake of saturated fatty acids in four out of five countries. Within two countries

the concentration of HDL cholesterol was negatively related to the intake of carbohydrate and positively related to the intake of saturated fatty acids, polyunsaturated fatty acids and cholesterol. The concentration of HDL cholesterol was also positively correlated with the physical activity score and negatively with the body mass index. Using the regression coefficients from a multiple regression analysis on the pooled data, it could be calculated that on average 24 percent of the inter-country differences in the levels of total cholesterol is explained by differences in the intakes of saturated fatty acids. Differences between the groups of the different countries in the intakes of carbohydrate explained on average 29 percent of the differences in the concentrations of HDL cholesterol. The results obtained support the hypothesis that higher concentrations of total and HDL cholesterol are associated with western types of diets rich in saturated fatty acids and relatively poor in complex carbohydrates.

INTRODUCTION

In our study in boys from 16 countries (1, 2) it appeared that marked differences in the concentrations of total and HDL cholesterol already exist by the age of 7 and 8 years. These differences were closely related to the differences in the mortality rates from coronary heart disease between the countries. Since there is much evidence that serum cholesterol and its partitioning between the different lipoproteins play a key role in the pathogenesis of atherosclerosis and its complications (3 - 11) and because fibrous plaques can be found in the coronary arteries of young people (12 - 15) it was considered worthwhile to carry out an epidemiological study on the determinants of the concentrations of total and HDL cholesterol in children from five countries with different rates of mortality from coronary heart disease (11).

In our study in boys from 16 countries (1, 2) it also appeared that the mean concentration of HDL cholesterol was positively associated with that of total cholesterol ($r=0.90$, $n=26$ regions). On the basis of this it was suggested that the concentrations of total and HDL cholesterol increase under the influence of a "westernized" diet rich in fat and cholesterol and relatively poor in complex carbohydrates. Therefore, the purpose of this study was to examine the relationships of the concentrations of total and HDL cholesterol

with dietary variables in young boys. The consumption of alcohol, the smoking habits, degree of physical activity and the body mass index, all of which might influence the concentrations of total and HDL cholesterol were also studied. The concentrations of albumin and hemoglobin were measured as general indicators of health of the children.

Although a lot of studies on diet and on serum lipids in children have been carried out already (16 - 32), it is difficult to compare the results not only because of differences in data processing and presentation but also because of the use of different methods for the estimation of serum lipids and of dietary intakes and sometimes in the absence of a rigid standardization of the measurements (33 - 35). Therefore, in this study we have tried to standardize all measurements as much as possible in order to enhance the comparibility of the data between the five countries in the study.

METHODS

General

The procedures are described below but more details on this have been described in the working plan (36) that can be obtained on request. The study was carried out in the period February - May 1981.

Before the study was commenced in the five countries a two-week training period and workshop was held in the Netherlands with the leaders of the teams in charge of the nutrition surveys. During this period all aspects of the working plan were thoroughly discussed and special attention was paid to the art of interviewing. In their home countries the survey leaders instructed the other members of the teams that were involved in the surveys.

Selection of population samples

Countries were selected on the basis of the results of our pilot-study in schoolboys from 16 countries (1, 2), the experiences of the investigators and institutes obtained in previous surveys and the possibility of carrying out the study within the constraints of a limited budget. As we aimed at studying the relationships between the concentrations of total and HDL cholesterol and dietary variables over a wide range of values of total and HDL cholesterol concentrations, we selected Ghana for its low concentration of both total and

HDL cholesterol and the Philippines for its low concentration of HDL cholesterol, especially in relation to the total cholesterol concentration, which was found to be comparable to those of the European boys. Within Europe, Italy, the Netherlands and Finland were selected because boys in these countries have low, intermediate and high concentrations, respectively of both total and HDL cholesterol by European standards.

The study was carried out in Helsinki in Finland, in Accra in Ghana, in the Salerno region about 200 km south of Naples in Italy, in the provincial town Ede in the center of the Netherlands and in the San Pablo region about 90 km from Manila in the Philippines.

In all study areas 100 - 135 boys aged between 91 and 122 months participated in the study. The study was restricted to boys of this age category in order to diminish the confounding effect of age and because boys of this age are relatively easily accessible through schools. The boys in each country were selected through at least 4 schools. In Ghana and the Philippines the boys belonged to the socially non-affluent groups. The diets of the socially affluent groups in these countries tend to be entirely different from those of the socially non-affluent groups (37, 38).

Before the boys were asked to participate their parents were contacted by letter or personally. They were told about the aims of the study and the input expected of the parents if they would decide to participate in the food consumption survey. They were also asked to give permission for their sons to participate in the study.

Strict random samples were not required as this was considered unnecessary taking into account that the objective of the study was to examine the relationships between dietary and serum lipid variables rather than to give representative data for particular regions.

Selections of schools and boys was continued until a number of at least 100 boys was reached. Within the schools all boys of the appropriate age for whom permission had been obtained were selected unless the number was higher than could be managed simultaneously by the team of nutritionists involved in the food consumption survey. In that case the boys were selected in alphabetical order until the appropriate number was reached. The study was carried out with subgroups of 20 - 40 boys.

Illness and the use of medicine for treatment or prevention of illness during two weeks preceding the survey were recorded. When the boys were unable to attend school for reasons of illness during the survey, this was also recorded.

Food consumption surveys

The aims of the food consumption surveys were to obtain estimates of the intakes of energy, protein, saturated, monounsaturated and polyunsaturated fatty acids and total fat, carbohydrate and cholesterol both on an individual and on a group level. The individual estimates served for making comparisons within the countries while the average estimates were used for making comparisons between the groups from the different countries.

It is sometimes said that the use of the same method is a prerequisite for making comparisons between the results of different populations, but this is only true if the errors are consistent and apply equally to the population samples being compared. However, it is very doubtful whether the results obtained by different investigators in different circumstances and in populations with different socio-cultural backgrounds will be biased by the same error. Therefore, we saw no objections to using different methods. The record and the recall method seemed to be most feasible taking into account the aims of the study, the experiences of the nutritionists obtained in previous surveys and the socio-cultural backgrounds of the populations. On this basis the recall method was used in Ghana, Italy and the Philippines and the record method in Finland and the Netherlands. Apart from this difference in methods, the food consumption surveys in the different countries had a lot in common. The food consumption of each boy was estimated on 7 days spread over a period of 15 days and such that each day of the week was covered once. The food consumption was recorded or recalled in household measures by the mother with the help of her son and in some instances by the son with the help of his mother. Portion sizes were quantified using dietary scales and calibrated cylinders, cups and spoons. The mothers were requested to provide quantitative detailed description of all ingredients entering in the preparation of all composited dishes consumed by the boys. Particular care was applied to obtain and verify informations on the quality and quantity of cooking fats consumed by the boys.

In Finland and the Netherlands where the record method was used, the food consumption was recorded for periods of two days or, during the weekend, for three days. Each period was immediately followed by a visit of a nutritionist who discussed the record of the preceding two or three days and sought additional information on the type, composition and preparation of the foods consumed. In addition, in Finland plastic models and empty dishes and also local food samples of different sizes were used as aids in quantitative estimations. In Ghana,

Italy and the Philippines where the recall method was used, the mother or the one responsible for providing food for the boy and the boy himself were interviewed six times by a nutritionist on the food consumption of the preceding day or the preceding two days (Saturday and Sunday). In Ghana additional information on portion sizes was collected from food vendors as these people provide much ready to eat food. This is often bought by the boy himself who is provided with money to purchase such food. In Italy, additional information on quantities of foods eaten outside home was collected by estimating the quantities from replicas of the foods consumed. Cartons of different sizes and calibrated cups and spoons were used as memory aids.

Food composition tables

The intakes of energy, protein, fat, saturated, monounsaturated and polyunsaturated fatty acids, carbohydrate and cholesterol of the boys were calculated using the local food composition tables. This was done centrally in Wageningen. Before the food composition tables were used, it was checked whether the data in the tables differed markedly from what could be expected on the basis of other tables on food composition (39 - 47). The carbohydrate content per 100 g was calculated as (100 - protein - fat - water - ash) g. The sum of the saturated, monounsaturated and polyunsaturated fatty acids was checked to see whether it equalled the amount of total fat taking into account a factor relating the amount of fat to the equivalent amount of fatty acids (42). However, this was not done for the Italian food composition table because this table contained data only on the total amount of saturated fatty acids, oleic acid, linoleic acid, linolenic acid and arachidonic acid. For this table most totals for the sums of these individual fatty acids and total saturated fatty acids appeared to be within 75 and 95 percent of the total fat content which is in accordance with expectations. The sum of the energy derived from protein, carbohydrate, fat and alcohol of the foods was also checked to see whether it equalled the total amount of energy. Since the food composition tables of Ghana and the Philippines did not contain data on the fatty acid composition and cholesterol content of foods, food samples were collected. These comprised raw and prepared products rich in fat and cholesterol that were consumed regularly and for which the fatty acid composition and cholesterol content could not be derived from other food composition tables. In addition, in all countries samples were collected of foods which were rich in energy and were consumed regularly and for which the composition reported in the local

food composition tables was unreliable or lacking. The results of the analyses of the food items were described in a special report (48). The percentages of nutrients from products analyzed in Wageningen (Table 1) were on average higher for the Ghanaian and Philippine diets than for the Italian and Finnish diets. This is because more data, especially on the fatty acid and cholesterol composition, were lacking in the Ghanaian and Philippine tables. In order to complete the data on the fatty acid composition and cholesterol content of foods, data from other tables (39 - 47) were used.

TABLE 1

Percentage of nutrient intakes from products analyzed in Wageningen in boys from Finland, Italy, the Philippines and Ghana

Country	Energy	Protein	Carbohydrate	Fat	SFA+	PUFA+	Cholesterol
Finland	11	11	3	22	19	26	12
Italy	21	14	8	49	37	45	11
The Philippines	57	54	60	41	46	38	28
Ghana	83	84	79	92	91	97	69

+: SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids

The energy content of the foods in the different food composition tables appeared to have been calculated with different conversion factors for the energy values of protein, fat and carbohydrate. In the tables from Ghana and the Philippines conversion factors depending on the type of food have been used (49, 50). In the Italian table the factors 4, 9 and 3.75 Kcal/g have been used for protein, fat and carbohydrate, while the corresponding figures are respectively 4, 9 and 4 Kcal/g for the Finnish and Dutch food composition tables. In order to be able to compare the energy intake of the boys on the basis of the same conversion factors and in order to enable calculating the percentages of energy from protein, different types of fat and carbohydrate the factors 4, 9 and 4 Kcal/g were used for protein, fat and carbohydrate, respectively. When the results obtained were compared with the originally used conversion factors, the values obtained were only slightly different and did on average not exceed the 5 percent level.

Physical activity

A questionnaire completed by the boy and his parents was used to calculate a physical activity score (36). This questionnaire was designed in collaboration with the leaders of the survey teams and contained questions on the number of hours spent on sleeping each day, the way and time required to reach school and the activity pattern during leisure time. The frequencies with which the activities in leisure time were carried out could be indicated as often (on 4 - 7 days a week, relative frequency score 5.5), sometimes (on 1 - 3 days a week, relative frequency score 2), rarely (on less than one day a week, relative frequency score, 0.5) and never (relative frequency score 0). Activities were classified into 5 levels varying from 1 for sleeping to 5 for strenuous activities such as running and climbing trees on the basis of the average levels of energy outputs of different activities using data from Durnin et al (51).

The physical activity score was calculated as Σ (time spent on each activity or group of activities x relative level of activity)/Σ (time spent on each activity or group of activities).

Anthropometric measurements and derived parameters

Weight (52), height (52) and four skinfolds on the left side of the body (53, 54) were measured as described previously and within one week after having completed the dietary survey.

During the workshop with the four team leaders from outside the Netherlands it appeared that there were very large between-observer differences for the measurements of skinfolds when compared with the measurements of weight and height. This was probably due to the fact that the level of training of the observers was uneven and therefore produced unreliable data which were not comparable. This was the reason why an alternative estimate based on height and weight was sought to estimate body fatness. For this purpose, the indices weight/height, weight/height^2 and the Benn index (weight/height^p, with p such that the index is independent of height, see reference 55) were calculated and correlated with the sum of four skinfolds.

Drawing blood, preparation, storage and transport of serum

Blood samples were taken (56), after the measurements of height, weight and skinfolds. As a general rule, the boys were not in a fasting condition

because previous studies have shown that time of the day and time since the last meal have no definable effect on the concentrations of total and HDL cholesterol (57, 58). Two tubes of blood were taken. In the first tube, 10 ml of blood was drawn for the preparation of serum for the estimation of total and HDL cholesterol and of albumin. In the second tube, with EDTA as an anti-coagulant, 3 ml of blood was drawn for the estimation of hemoglobin. The estimations of the concentrations of albumin and hemoglobin were used as general indicators of health of the children. The blood needed for the estimations of the concentrations of total and HDL cholesterol and albumin was allowed to clot at room temperature for 1 to 3 hours. Then the serum was separated from the blood cells and stored at -20°C until shipment to the Netherlands in the frozen state by air express, which took place within 4 weeks after blood sampling. All samples arrived at the laboratory in the frozen state and were stored at -80°C until analyzed. All materials needed for the drawing of blood and for the storage and transport of serum were provided by the laboratory in Wageningen.

Blood and serum analysis

The concentration of total cholesterol was measured by a direct method (59) and calibrated using the method of Abell et al (60). Reproducibility for blind control sera provided by the Center for Disease Control, Atlanta, GA, U.S.A. was $\pm$ 0.9 percent and accuracy was within 1.4 percent of the true (target) levels. The concentration of cholesterol in HDL was measured after the precipitation of the low and very low density lipoproteins by heparin - $MnCl_2$ (61, 62). Reproducibility for blind control sera obtained in the Center for Disease Control Survey of HDL Cholesterol Measurements (35) was $\pm$ 2.2 percent and bias with regard to the overall survey mean was on average 0.1 percent.

In all countries the concentration of hemoglobin was determined in whole blood as cyanomethemoglobin. In order to evaluate the bias and the reproducibility of the methods used in the different institutes in the cooperating countries, hemoglobin control samples (Dade, 3186 Düdingen, Switzerland) were sent to these institutes. The bias of the methods used with regard to the control samples was within 3.8 percent for low (5.3 mmol/l), intermediate (6.8 mmol/l) and high (9.9 mmol/l) hemoglobin concentrations. The between-run reproducibility was within 3.8 percent for a number of 4 - 10 runs per country needed to carry out all hemoglobin determinations.

The concentration of albumin was estimated by a modified bromcresol green method (63) using Monitrol IE and IIE (Dade, 3186 Düdingen, Switzerland) as calibration and control sera, respectively. The concentration of albumin in these sera was determined by the same method with reference to a standard (IFCC 74/1) obtained from Professor J.R.Hobbs (see reference 64). The values obtained for Monitrol IE and IIE were 40.8 g/l (SD = 0.5, n = 7) and 29.5 g/l (SD = 0.5, n = 7) compared with the values estimated by electrophoresis given by the manufacturer which were 41.0 and 30.3 g/l respectively.

Statistical methods

Dietary variables were computed as individual means over seven days and expressed as energy intake (Kcal/kg) per day, as energy percentage for protein, carbohydrate and fat, saturated, monounsaturated and polyunsaturated fatty acids and as mg/1000 Kcal for the cholesterol intake. Linear correlation coefficients were calculated for the concentrations of total and HDL cholesterol with dietary variables, the body mass index and the physical activity score for each group. The relationships of the concentrations of total and HDL cholesterol and the ratio of HDL cholesterol/total cholesterol with dietary variables, physical activity score and body mass index were examined by calculating linear regression equations using the Statistical Package for the Social Sciences (65). The regression coefficients for the groups from the different countries were estimated from a multiple regression analysis on the pooled data using a model allowing for country specific slopes and intercepts. Indicators of country specific levels were introduced into the regression model followed by dietary variables, body mass index and physical activity score and the country specific slopes for these variables. The introduction of new variables to the model was omitted when this did not result in a significant improvement of the accuracy of estimation of the dependent variable.

Relationships between the food products consumed and the concentrations of total and HDL cholesterol were studied by dividing the groups from each country into quartiles according to their concentrations of total and HDL cholesterol. Comparisons were made by the use of Student's t-test between quartiles 1 and 4 and in addition between quartiles 1 and 3 and 2 and 4 if there was a significant difference between the quartiles 1 and 4.

Although the food intake of each child was estimated over a period of 7 days, the resulting values for the mean intakes of the different nutrients

for each boy are still subjected to a considerable imprecision mainly due to a large variation in the food intake on different days. This error dilutes the true correlation coefficients between dietary variables and other variables (66). In order to quantify the probable influence of this error on the true correlation coefficients, the ratio of total variance minus interindividual variance all over interindividual variance (the "dilution factor", see reference 66) for the correlation coefficients was calculated by a one-way analysis of variance (67), and used as an approximation for the ratio of intraindividual variance over interindividual variance.

RESULTS

Anthropometry, physical activity and smoking

Means and standard deviations of weight, height, weight/height^2 and sum of four skinfolds are given in Table 2. Mean weights for the boys from Finland, Italy and the Netherlands were similar (30 kg) and higher than those for the Ghanaian boys (24 kg) and the Filipino boys (22 kg). The European boys were on average also taller (133 - 138 cm) than the boys from Ghana (126 cm) and the boys from the Philippines (121 cm). The body mass index, expressed as weight/height^2 ranged from 14.9 kg/m^2 in the boys from Ghana to 16.8 kg/m^2 in the Italian boys. The percentage of boys with weight/height^2 below 14.0 kg/m^2, which is close to the 5th percentile of the standards given by Cronk et al (68) was high for the Ghanaian group (26.1) while this percentage was 14.0 for the Filipino boys and varied between 4 and 7 for the European boys. The sum of four skinfolds was also lowest in the Ghanaian boys (18 mm), although it was not estimated for the Filipino boys and highest in the Italian boys (31 mm). The percentage of boys with weight/height^2 beyond 21.0 kg/m^2 which is close to the 95th percentile of the same standards was 7.3 percent in the Italian group and less than 1 percent in the other groups.

Generally, the weight/height^2 correlated slightly higher with the sum of four skinfolds ($r = 0.24$ for the Ghanaian boys and 0.68 to 0.87 for the European boys) than either weight/height ($r = 0.33$ for the Ghanaian boys and 0.68 to 0.86 for the European boys) or the Benn index ($r = 0.26$ for the Ghanaian boys and 0.58 to 0.78 for the European boys). The power p of the Benn index varied from 1.8 for the Ghanaian boys to 3.1 for the Italian boys.

TABLE 2

Age, anthropometry, physical activity and the concentration of albumin, hemoglobin, total and high density lipoprotein (HDL) cholesterol in boys from five countries

Country	Sample size	Age (years)	Weight (kg)	Height (cm)	Weight/height^2 (kg/m^2)	Sum of four skinfolds (mm)	Physical activity score
Finland	133	9.1 ± 0.6	30 ± 5	135 ± 6	16.5 ± 1.8	26 ± 10	2.19 ± 0.15
The Netherlands	117	9.1 ± 0.5	30 ± 5	138 ± 7	15.8 ± 1.3	26 ± 8	2.12 ± 0.19
Italy	109	9.0 ± 0.7	30 ± 7	133 ± 7	16.8 ± 3.0	31 ± 19	2.16 ± 0.20
The Philippines	114	8.8 ± 0.5	22 ± 3	121 ± 6	15.2 ± 1.1	---	2.23 ± 0.18
Ghana	116	8.9 ± 0.6	24 ± 3	126 ± 6	14.9 ± 1.5	18 ± 3	2.43 ± 0.15

TABLE 2 *(continued)*

Country	Albumin (g/l)	Hemoglobin (mmol/l)$^+$	Total cholesterol (mmol/l)$^{++}$	HDL cholesterol (mmol/l)$^{++}$	HDL cholesterol/ total cholesterol
Finland	44 ± 2	8.0 ± 0.4	4.9 ± 0.8	1.57 ± 0.30	0.32 ± 0.07
The Netherlands	44 ± 2	8.3 ± 0.4	4.5 ± 0.7	1.52 ± 0.34	0.34 ± 0.08
Italy	43 ± 3	8.3 ± 0.8	4.1 ± 0.9	1.45 ± 0.31	0.37 ± 0.09
The Philippines	40 ± 2	8.0 ± 0.6	3.8 ± 0.6	0.93 ± 0.24	0.25 ± 0.06
Ghana	38 ± 4	7.3 ± 0.6	3.3 ± 0.7	1.10 ± 0.29	0.34 ± 0.08

All values expressed as mean ± SD

+: 1 mmol/l = 1.61 g/100 ml; ++: 1 mmol/l = 38.7 g/100 ml

Therefore, it was considered that the Benn index was unsuitable for further analysis of the pooled data. Since weight/height^2 was less correlated with height ($r = -0.14$ to 0.33) than weight/height, the former was used in further analysis of the data.

Mean scores for the physical activity pattern in leisure time were similar for the groups of boys from all countries. The slightly higher score for the Ghanaian boys appeared to be the result of a lower number of hours spent on sleeping each day (9.4 compared with 9.8 to 11.0 for the other groups) and a relatively high frequency for activities like climbing trees. The boys did not smoke.

Albumin and hemoglobin

Means and standard deviations for the concentrations of albumin and hemoglobin are given in Table 2. Albumin concentrations were on average similar for the European boys (43 to 44 g/liter) and they were higher than those for the Filipino boys (40 g/liter) and the Ghanaian boys (38 g/liter). The low value for the Ghanaian boys appeared to be the result of a subgroup (13.9 percent) with remarkable low concentrations of albumin (below 32.0 g/liter). In the other groups there were no boys with concentrations of albumin below 32.0 g/liter. Hemoglobin concentrations were on average similar for the boys from all countries (8.0 - 8.3 mmol/liter) except for the Ghanaian boys (7.3 mmol/liter). In the Ghanaian group 9.6 percent of the boys had hemoglobin concentrations below 6.5 mmol/liter, while this was 2.8 percent in the Italian group and nil for the other groups.

HDL and total cholesterol

The mean concentrations of total and HDL cholesterol (Table 2) were higher in the European boys (4.1 - 4.9 mmol/liter and 1.45 - 1.57 mmol/liter) than in the boys from Ghana and the Philippines (3.3 - 3.8 mmol/liter and 0.93 - 1.10 mmol/liter). The differences in the concentrations of total and HDL cholesterol in the subgroups that had been ill within two weeks before the onset of the study or during the study or that had used medical drugs for prevention or treatment of disease were small compared with those that had not been ill or used drugs. Therefore, all data were pooled for the further analysis.

The mean ratio of HDL cholesterol/total cholesterol ranged from 0.32 -

TABLE 3

Energy intake, percentage of energy from protein, carbohydrate, fat and different types of fatty acids and the cholesterol intake in boys aged 8 and 9 years from five countries

Country	Energy[x] (kcal/kg)	Percentage of energy from							Cholesterol (mg/1000 kcal)
		Protein	Carbohydrate	Fat					
				Total	SFA[++]	C_{12-16}[++]	MUFA[++]	PUFA[++]	
Finland	74 ± 13	13.8 ± 1.7	50 ± 5	37 ± 5	17.7 ± 3.2	10.9 ± 2.1	12.3 ± 1.7	4.6 ± 1.7	157 ± 37
The Netherlands	70 ± 11	13.5 ± 1.6	49 ± 5	38 ± 5	15.1 ± 2.3	9.9 ± 1.6	14.2 ± 2.2	6.2 ± 1.6	142 ± 36
Italy[+++]	74 ± 17	13.4 ± 1.7	57 ± 6	28 ± 5	10.4 ± 2.2	---	12.1 ± 2.7	2.9 ± 1.2	159 ± 53
The Philippines	87 ± 21	11.7 ± 2.0	72 ± 7	16 ± 6	9.3 ± 2.8	7.3 ± 2.1	4.0 ± 2.3	1.4 ± 0.9	97 ± 48
Ghana	71 ± 14	9.2 ± 1.6	68 ± 5	22 ± 4	10.5 ± 2.6	9.0 ± 2.2	5.7 ± 1.6	4.0 ± 1.2	48 ± 23

All values expressed as mean ± SD

\+ : Calculated by using the conversion factors 4, 9, 4 kcal/g respectively for protein, fat and carbohydrate

++ : SFA = saturated fatty acids, C_{12-16} = saturated fatty acids with 12, 14 or 16 carbon atoms, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

+++ : For the Italian boys, MUFA = oleic acid and PUFA = linoleic acid + linolenic acid + arachidonic acid

0.37 in the Ghanaian and European boys and was equal to 0.25 in the Filipino boys.

Energy and nutrient intakes

The means and standard deviations of the intakes of energy and the percentages of energy from protein, carbohydrate and different types of fat and the cholesterol intake per 1000 Kcal are given in Table 3. The mean energy intakes per kg of body weight were similar (70 - 74 Kcal/kg) except for that of the Filipino boys (87 Kcal/kg). The contribution of protein, total fat and monounsaturated fatty acids to the energy intake and the cholesterol intake were higher in the European boys than in the boys from Ghana and the Philippines. The intake of carbohydrate was higher in the boys from Ghana and the Philippines than in the European boys. The energy percentage from the saturated fatty acids was higher in the Finnish and Dutch boys than in the other boys, although the differences in the contributions of the C_{12-16} fatty acids were relatively small. The energy percentage from the polyunsaturated fatty acids was highest in the Dutch boys and lowest in the Filipino boys. The intake of alcohol (not mentioned in Table 3) was 2.9 g/day (range, 0 - 20 g/day) in the Italian group and was negligible in the other groups.

Intake of nutrients from the different food groups

The food items used in each of the countries have been classified into 18 groups (see Table 4) according to similarities in composition and biological relationship.

The diets of the European boys contained high proportions of animal products that contributed significantly to the intake of energy, protein, saturated fat and cholesterol, although there was a clear gradient from a lower contribution of animal products in the diets of the Italian boys to a higher contribution in those of the Finnish boys. Cereal products, visible fats and oils, products rich in sugar and, to a lesser extent, roots and tubers were also important sources of macronutrients in the diets of the European boys. The diets of the Filipino and Ghanaian boys contained high proportions of vegetable products such as cereals and also roots and tubers. The diets of the Ghanaian boys also contained fish products, visible fats and oils and soups and stews as the main sources of macronutrients. The Ghanaian soups and stews

TABLE 4

Percentages of energy, protein, carbohydrate, fat, saturated fatty acids (SFA), cholesterol supplied by the different groups of foods in the food intake of the Philippines (Ph) and Ghana (Gh)

Number	Groups	Energy					Protein					Carbohydrate				
		Fi	Ne	It	Ph	Gh	Fi	Ne	It	Ph	Gh	Fi	Ne	It	Ph	Gh
1	Cereals used as staples such as maize, rice, macaroni and bread	21	19	40	54	35	19	19	34	41	37	36	30	60	66	42
2	Cereals used as a delicacy such as cakes, pastries and cookies	1	7	6	4		1	3	3	3		2	7	7	4	
3	Starchy roots, tubers and fruits such as cassave, potato, plantain, banana	4	7	3	5	31	3	4	2	1	8	7	11	4	6	42
4	Legumes such as peas, beans and peanuts	1	2	1	2	3	2	3	3	3	6	1	1	1	1	1
5	Nuts and seeds				1											
6	Vegetables except for those from group 3	1	1	1	1		1	2	3	1		2	2	2	1	
7	Fruits except for those from group 3	8	5	3	2	1	3	1	1	1		15	11	4	3	1
8	Meat products except for those from group 9	12	12	7	6	1	24	22	22	15	4	2	1			
9	Brain, liver and kidney						2		1							
10	Egg products	1	1	2	1		3	3	5	3	1					
11	Fish and shellfish	1		1	5	5	4	1	5	22	31					
12	Milk products except cheese and butter	20	17	8	2		30	30	10	2		14	16	5	1	
13	Cheeses	5	4	6			8	8	11	1		2		1		
14	Low PUFA (< 10 percent w/w) oils and fats	6	1	2	2	3										
15	Intermediate PUFA (between 10 and 30 percent w/w) oils and fats	3	7	9												
16	High PUFA (> 30 percent w/w) oils and fats	4	3	1												
17	Products rich in sugar except for those from group 2	10	11	10	9	2	1	2	1	1		19	20	15	11	4
18	Soups, stews and ready-made meals		1		7	19		1		6	13		1		6	10

Contributions equal to zero have been omitted

monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and 8 and 9 year-old boys from Finland (Fi), the Netherlands (Ne), Italy (It),

Nutrients																										
Fat						SFA						MUFA						PUFA						Cholesterol				
Fi	Ne	It	Ph	Gh		Fi	Ne	It	Ph	Gh		Fi	Ne	It	Ph	Gh		Fi	Ne	It	Ph	Gh		Fi	Ne	It	Ph	Gh
3	4	3	13	11			5	1	14	1		1	2	2	2	1		11	10	4	13	57				1		
1	7	5	6			1	9	6	8			1	7	3	3			3	6	5	6				7	5	1	
1	4	2	3	5			5		3	3		1	4	1	1	2		1	1	12								1
	4		4	8			2		2	5			4		6	13			10	2	14	9						
	1	1	3	1			1		5	1			1	1	1				1	1								
			1						1												1							
																					1							
22	22	13	26	1		18	21	14	18	1		31	28	12	46	3		11	10	11	27			23	21	18	19	10
			1															1			1			7	1	1	2	
2	2	5	4			1	1	7	2			2	3	4	6	1		3	1	5	4			25	34	48	36	11
1		1	12	11		1		1	15	14		1			7	7		4	1	1	7	6		4	1	5	28	69
25	13	13	4			34	18	21	4			22	14	7	3			1		2				20	16	8	1	
8	7	13	1			11	11	21	1			7	6	7	1			4	2	4				7	8	10	1	
16	3	5	11	11		21	4	7	14	17		13	3	4	6	7		4	1	3	10	4		13	2	2		
8	18	31		1		6	12	11		1		10	18	51		2		14	28	30		1		2	8			
10	9	3				4	4	1				9	6	2				41	25	15	3				1			
3	3	5	2			3	5	7	3			2	3	5	2			1		3	1					3		
	2		10	50			2		9	56			1		15	64			2		8	22			2		11	8

usually contain large amounts of palm oil; different types of vegetables, some meat or fish and sometimes peanut paste (groundnut soup). The diets of the Filipino boys also contained meat and fish products and products rich in sugar as the major sources of macronutrients.

The main sources of cholesterol in the diet for all the boys were meat and egg products, while fish was also an important source for the Ghanaian and Filipino boys. Milk products also contributed significantly to the cholesterol intake of the European boys.

Cereals and starchy roots and tubers provided 73 to 85 percent of the carbohydrate intake in the Ghanaian and Filipino boys, while they provided 65 percent in the Italian boys and about 40 percent in the Finnish and Dutch boys.

Associations between dietary variables, weight/height^2, physical activity score and the concentrations of total and HDL cholesterol

Higher mean values for both total and HDL cholesterol concentrations were associated with higher mean intakes of protein, different types of fat and cholesterol and with lower intakes of carbohydrate (see Table 3).

In Table 5 the Pearson correlation coefficients between dietary variables, weight/height^2, physical activity score and the concentrations of total and HDL cholesterol have been summarized. The associations between the concentration of total cholesterol and the energy percentages from total fat, from saturated and monounsaturated fatty acids and from cholesterol/1000 Kcal tended to be positive, while that between the concentration of total cholesterol and the percentage of energy from polyunsaturated fatty acids was negative in the Finnish and Ghanaian group and positive in the Filipino boys. The correlation coefficients for the associations between the concentration of total cholesterol and the energy percentage from the C_{12-16} fatty acids were almost identical (less than 0.02 difference) to those between the concentration of total cholesterol and the energy percentage from saturated fatty acids.

The concentration of HDL cholesterol was negatively related (Table 5) to the energy percentage from carbohydrate in the Italian and Filipino boys and it was generally positively related to the energy percentages from saturated fatty acids, from polyunsaturated fatty acids and cholesterol/1000 Kcal in the Filipino, Italian and Dutch boys. The correlation coefficients for the C_{12-16} fatty acids were similar to those for the total of saturated fatty acids.

TABLE 5

Pearson correlation coefficients for the associations of dietary variables, weight/height^2 and physical activity score with the concentrations of total and high density lipoprotein (HDL) cholesterol in boys from five countries

Countries	Energy	Percentage of energy from					Cholesterol/ 1000 kcal	Alcohol	Weight/ height^2	Physical activity score
		Protein	Carbohydrate	Fat	SFA+	MUFA+	PUFA+				
Total cholesterol											
Finland	-0.11	0.00	-0.12	0.10	0.22¶	0.07	-0.17¶	0.26	-0.10	-0.10	-0.13
The Netherlands	0.09	0.01	-0.26¶	0.26¶	0.26¶	0.26	0.10	0.23¶	0.09	-0.12	-0.13
Italy	-0.03	0.08	-0.12	0.08	0.16¶	0.00	-0.04	0.09	0.06	0.01	-0.04
The Philippines	-0.03	0.14	-0.21¶	0.19¶	0.16¶	0.16¶	0.28¶	0.09	----	0.06	-0.02
Ghana	-0.03	-0.08	0.04	-0.01	0.07	-0.08	-0.17¶	-0.03	----	0.16¶	-0.13
HDL cholesterol											
Finland	0.00	-0.01	0.01	0.02	0.04	0.09	-0.06	-0.11	-0.15	-0.09	0.16¶
The Netherlands	-0.15	-0.02	-0.12	0.13	0.10	0.00	0.23¶	-0.02	0.08	-0.05	-0.01
Italy	-0.14	0.04	-0.18¶	0.15	0.22¶	0.12	-0.02	0.18¶	0.08	-0.21¶	-0.04
The Philippines	0.14	0.32¶	-0.40¶	0.37¶	0.28¶	0.39¶	0.39¶	0.37¶	----	0.04	0.00
Ghana	-0.05	-0.01	0.04	-0.04	0.01	-0.14	0.00	-0.03	----	-0.02	0.25¶
HDL cholesterol/total cholesterol											
Finland	0.06	-0.05	0.11	-0.07	-0.13	0.02	0.07	-0.27¶	-0.06	-0.01	0.22¶
The Netherlands	0.07	-0.04	0.12	-0.10	-0.13	-0.21¶	0.14	-0.13	-0.01	0.03	0.10
Italy	-0.10	-0.01	-0.07	0.04	0.01	0.09	0.00	0.06	0.09	-0.18¶	0.02
The Philippines	0.20¶	0.25¶	-0.31¶	0.28¶	0.21¶	0.33¶	0.24¶	0.30¶	----	0.01	-0.05
Ghana	-0.04	0.07	0.04	-0.07	-0.06	-0.14	0.11	-0.02	----	-0.12	0.37¶

+ : SFA = saturated fatty acids, MUFA = monounsaturated fatty acids, PUFA = polyunsaturated fatty acids

¶ : Significantly different from zero ($p < 0.05$)

TABLE 6

Multiple regression analysis of the concentrations of total cholesterol, HDL percentages from saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), physical activity score (PAS) in boys from five countries and with Ghana or

Independent variables	Total cholesterol			
Intercept of reference country	3.7		(3.5)	
Difference in intercept compared with reference country for				
Finland	1.2¶	± 0.1	(0.24¶	± 0.11)
The Netherlands	0.9¶	± 0.1	--	--
Italy	0.7¶	± 0.1	(-0.24	± 0.14)
The Philippines	0.4¶	± 0.1	--	--
Regression coefficients				
SFA	0.045¶	± 0.011	(0.048¶	± 0.018)
PUFA	--	--	--	--
CARB	--	--	--	--
CHOL	--	--	(0.002	± 0.001)
Weight/height^2	--	--	--	--
PAS	-0.34	± 0.18	--	--

Differences in intercepts and regression coeffcients expressed as mean ± standard

¶: $P < 0.05$

+: The regression coefficients for the physical activity score of other groups (-0.157¶), Italy (-0.216¶) and The Philippines (-0.223¶)

cholesterol or the ratio of HDL cholesterol/total cholesterol on the energy carbohydrate (CARB) and on the cholesterol intake (CHOL), weight/height^2 and the Netherlands (between brackets) as the reference country

Dependent variables							
HDL cholesterol				HDL cholesterol/total cholesterol			
1.83		(2.15)		-0.13		(0.24)	
0.36¶	± 0.06	(0.07	± 0.04)	0.27	± 0.15	(-0.020	± 0.011)
0.29¶	± 0.06	--	--	0.39¶	± 0.15	--	--
0.30¶	± 0.05	(0.01	± 0.05)	0.56¶	± 0.14	(0.022	± 0.011)
-0.14¶	± 0.04	--	--	0.46¶	± 0.14	--	--
--	--	--	--	--	--	--	--
--	--	--	--	0.0055¶	± 0.0022	--	--
-0.0074¶	± 0.0023	(-0.0064	± 0.0033)	--	--	--	--
--	--	--	--	--	--	--	--
-0.015¶	± 0.007	(-0.020¶	± 0.008)	-0.0040¶	± 0.0018	--	--
--	--	--	--	0.206¶+	± 0.047	(0.047	± 0.024)

error

were lower than for the reference country: Finland (-0.105), The Netherlands

Associations between the ratio of HDL cholesterol/total cholesterol and dietary variables were generally not statistically significant except for the Filipino boys.

Generally, weight/height^2 was not significantly associated with the concentrations of total or HDL cholesterol or the HDL cholesterol/total cholesterol ratio. The physical activity score was positively related to the concentration of HDL cholesterol and the ratio of HDL cholesterol/total cholesterol in the Finnish and Ghanaian boys.

In a multiple regression analysis on the pooled data for the boys from all countries (Table 6) the concentration of total cholesterol was positively related to the percentage of energy from saturated fatty acids and negatively to the physical activity score, although this latter relationship was only of borderline statistical significance. There was no evidence from the data for different slopes of the regression lines describing the relationships between the concentration of total cholesterol and the percentage of energy from saturated fatty acids or the physical activity score. Since other nutritional variables that were entered in the regression model did not significantly improve the estimation of the dependent variable, a model of five horizontal planes separated by differences in intercept as given in Table 6 resulted for estimating the concentrations of total cholesterol in the five groups.

In a similar multiple regression analysis for the concentration of HDL cholesterol it was found that there was a negative relationship between the HDL cholesterol concentration and the percentage of energy from carbohydrate and weight/height^2. For the ratio of HDL cholesterol/total cholesterol it was found that there was a positive relationship with the energy percentage from polyunsaturated fatty acids and the physical activity score and a negative relationship with weight/height^2.

As it may be argued that the regression coefficients from the analysis on the pooled data are biased because of the prevalence of parasites that may interfere with cholesterol metabolism in the Ghanaian and Filipino boys, the multiple regression analysis was also carried out using the data from the European boys only. The results of this analysis are given between brackets in Table 6 and were basically similar to the results for all boys pooled.

Using the regression coefficients from Table 6, the explained difference in both total and HDL cholesterol from Italy, the reference country for this purpose, can be calculated (Figures 1 and 2). It can be seen that for total cholesterol, the proportion of energy from saturated fatty acids explains

53 percent of the difference between Italy and the Netherlands, while it explains none of the difference between Italy and Ghana (Figure 1). Physical activity explains a small proportion of the difference; e.g. about 10 percent of the difference between Italy and Ghana. A similar picture is observed for the contribution of the proportion of energy from carbohydrate and body mass index to the difference from Italy in HDL cholesterol concentration of the other countries (Figure 2). When similar calculations are made taking other countries as reference, it can be calculated that on average 24 percent (range, minus 11 to 53 percent) of the inter-country differences in the mean concentrations of total cholesterol is explained by differences in the intakes of saturated fatty acids. Differences between the groups of the different countries in the intakes of carbohydrate explained on average 29 percent (range, minus 15 to 85 percent) of the differences in the concentrations of HDL cholesterol.

The ratios of residual variance (total variance - interindividual variance) over interindividual variance are given in Table 7. From these ratios it can be calculated (66) that correlation coefficients are probably "diluted" by a factor two or three.

TABLE 7

Lambda values (ratio of total variance minus interindividual variance all over interindividual variance) per nutrient for boys from five countries

Nutrients	Finland	The Netherlands	Italy	The Philippines	Ghana
Energy	2.0	4.2	2.8	1.4	2.8
Percentage of energy from					
Protein	4.5	3.3	5.0	2.8	3.5
Carbohydrate	2.8	3.2	2.3	1.5	4.6
Fat	2.1	1.3	2.7	1.9	5.7
Saturated fatty acids	1.2	4.1	3.0	2.2	6.3
Monounsaturated fatty acids	2.7	1.9	3.1	2.7	6.4
Polyunsaturated fatty acids	2.3	1.9	2.3	2.2	2.8
Cholesterol/1000 Kcal	4.6	10.1	6.1	3.3	4.1

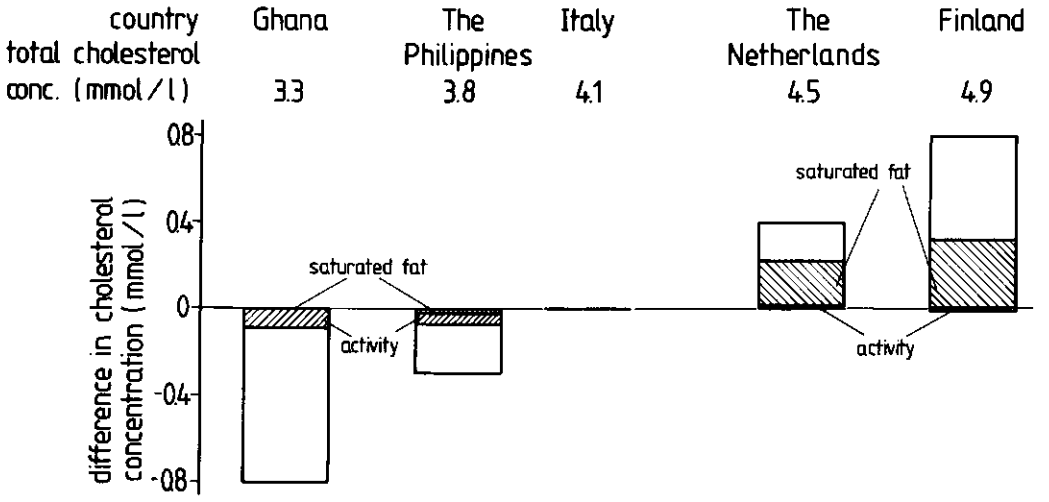

Fig. 1. Factors contributing to the difference between the total cholesterol concentration observed in the boys from Italy and the mean values observed in boys from Ghana, the Philippines, the Netherlands and Finland.

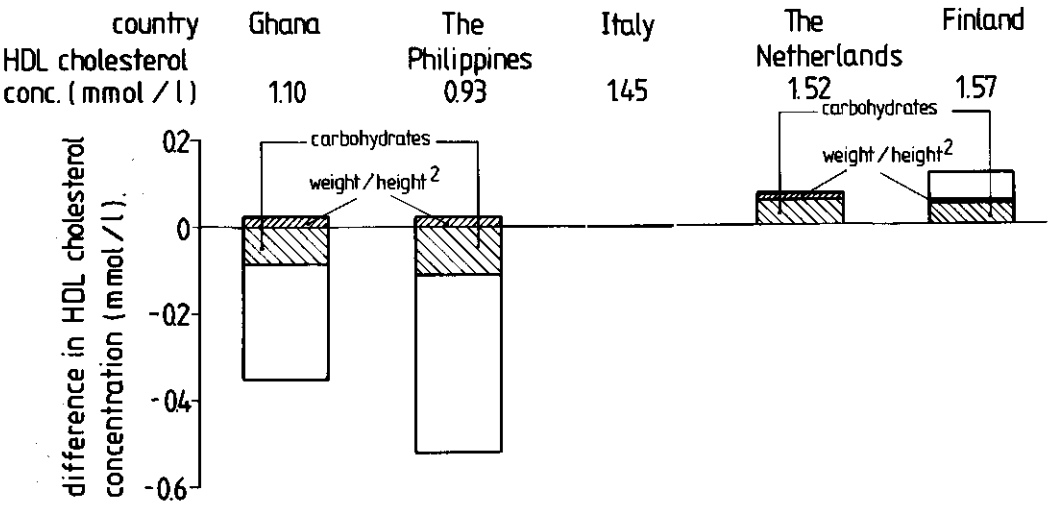

Fig. 2. Factors contributing to the difference between the mean HDL cholesterol concentration observed in the boys from Italy and the mean values observed in boys from Ghana, the Philippines, the Netherlands and Finland.

Food consumption in low, medium and high quartiles of total and HDL cholesterol groups

In Table 8 a summary is given of the groups of foods whose contribution to the energy intake showed significant differences between the low {1} and high {4} quartiles and, in case of a significant difference between these two quartiles, also other significant differences between the quartiles of total cholesterol. In the Finnish boys, higher concentrations of total

TABLE 8

Mean daily contribution of different groups of foods to the energy intakes (percentages) in low {1} , medium {2} and {3}, and high {4} quartiles of cholesterol levels of boys from five countries

Groups+	Quartiles	Finland	The Netherlands	The Philippines
1	1 and 4		22.4 and 15.9¶¶	56.9 and 49.9¶¶
	1 and 3		22.4 and 17.5¶¶	
4	1 and 4			0.9 and 2.2¶¶
	1 and 3			0.9 and 1.8¶¶
9	1 and 4		0.06 and 0.27¶¶	
	1 and 3		0.06 and 0.23¶¶	
10	1 and 4	0.9 and 1.4¶¶		
	1 and 3	0.9 and 1.2¶		
11	1 and 4	0.8 and 1.3¶¶		
13	1 and 4	6.0 and 4.4¶		0.05 and 0.55¶¶
	1 and 3	6.0 and 3.9¶¶		
14	1 and 4	4.2 and 7.1¶¶	0.7 and 1.5¶	
16	1 and 4	5.4 and 3.3¶¶		
	1 and 3	5.4 and 3.0¶¶		

+: The numbers of the groups correspond to those in Table 4

¶: $P < 0.05$; ¶¶: $P < 0.01$

cholesterol were associated with higher intakes of egg products, fish products, oils and fats poor in polyunsaturated fatty acids and with lower intakes of cheese and fats and oils rich in polyunsaturated fatty acids. As there were

TABLE 9

Mean daily contribution of different groups of foods to the energy intake (percentages) in low {1}, medium {2} and {3}, and high {4}, quartiles of HDL cholesterol levels of boys from five countries

Groups[+]	Quartiles	Countries				
		Finland	The Netherlands	Italy	The Philippines	Ghana
1	1 and 4				59.0 and 50.4¶¶	
	2 and 4				55.0 and 50.4¶	
2	1 and 4	2.0 and 1.0¶			3.5 and 5.4¶	
	1 and 3	2.0 and 1.0¶				
4	1 and 4				1.1 and 2.4¶	
	2 and 4				1.1 and 2.4¶¶	
5	1 and 4		0.2 and 0.8¶¶			
	2 and 4		0.2 and 0.8¶¶			
6	1 and 4	1.5 and 1.0¶¶				
	2 and 4	1.3 and 1.0¶				
7	1 and 4	9.0 and 7.0¶¶				0.9 and 0.4¶
	1 and 3					0.9 and 0.4¶
	2 and 4					1.0 and 0.4¶¶
8	1 and 4				3.0 and 7.3¶¶	
	1 and 3				3.0 and 6.7¶¶	
	2 and 4				4.6 and 7.3¶¶	
10	1 and 4			1.8 and 2.6¶		
	1 and 3			1.8 and 2.5¶¶		
13	1 and 4				0.05 and 0.34¶¶	
14	1 and 4		0.4 and 2.2¶¶			
	1 and 3		0.4 and 1.6¶¶			
17	1 and 4		11.4 and 9.6¶			

+: The numbers of the groups correspond to those in Table 4

¶: $P < 0.05$; ¶¶: $P < 0.01$

no significant differences between the first and fourth quartiles for the Ghanaian and Italian boys no data for these groups are included in the table. In the Dutch boys, there were associations between higher concentrations of total cholesterol with higher intakes of organs rich in cholesterol, with higher intakes of oils and fats poor in polyunsaturated fatty acids and with lower intakes of group 1 cereals. In the Filipino boys there were associations with lower intakes of group 1 cereals and with higher intakes of legumes and milk products.

Higher concentrations of HDL cholesterol were associated (Table 9) with lower intakes of group 2 cereals, vegetables and fruits in the Finnish boys, with lower intakes of fruits in the Ghanaian boys and with higher intakes of egg products in the Italian boys. In the Dutch boys higher concentrations of HDL cholesterol were associated with higher intakes of nuts and seeds and oils and fats poor in polyunsaturated fatty acids, while in the Filipino boys this was associated with lower intakes of group 1 cereals and with higher intakes of group 2 cereals, legumes, meat products, egg products and milk products from group 13.

DISCUSSION

The purpose of this study was to examine the relationships of the concentrations of total and HDL cholesterol with dietary variables in boys both within and between countries taking into account differences in physical activity and body mass index.

As far as mean differences in the concentrations of total cholesterol between groups are concerned we would suggest that an important part of these differences should be ascribed to differences in dietary intake profiles. This is based on the following considerations. Firstly, higher mean values for total cholesterol were associated with higher mean intakes of protein, different types of fat and cholesterol and with lower intakes of carbohydrate. Secondly, the correlations found within countries between dietary variables, especially the intake of saturated fatty acids, and the concentrations of total cholesterol give further support to this suggestion. It may be argued that the correlations were generally low, but this is probably due to the unfavourable ratio of (total minus interindividual variance)/(interindividual variance) (66, 69). Furthermore, it should be realized that the study was

carried out in relatively homogeneous subsamples within each country. The associations beteen the intake of saturated fatty acids and the concentrations of total cholesterol remained statistically significant after adjustment was made for country-specific intercepts and other variables that contributed significantly in improving the estimation of the concentration of total cholesterol (Table 6). Thirdly, it has been shown in controlled trials that diets rich in saturated fatty acids increase the concentration of total cholesterol (70 - 72).

Interestingly, the physical activity score was negatively related to the concentration of total cholesterol, although the difference from zero was only of borderline significance (Table 6). This probably results from the fact that the estimate of the physical activity score is subject to a considerable error due to the daily variation in physical activity patterns and the nature of the questionnaires used. The value of such a questionnaire for calculating a precise estimate of the physical activity score can be questioned (73).

From the apparent regression coefficients relating the two independent variables to the concentration of total cholesterol and also from the range of mean values of the groups for these two variables themselves, the proportion of energy from saturated fatty acids explains a higher proportion of the difference in the mean serum cholesterol levels observed in the five countries than the physical activity score (Figure 1). It should be remembered however that these apparent regression coefficients would differ from the actual regression coefficients, because of the errors involved in the determination of the values of the two independent variables and also of total cholesterol (74). These errors arise not only from the inability of the methods to accurately determine the parameter at the time it was measured, but also from the inability of a limited number of estimations to give a reliable measure of the long-term value of the parameter under study. The within-country estimates of the regression coefficients as determined by an analysis of the pooled data are likely to be more valid estimates of the effects of dietary and other variables on the concentration of total cholesterol than regression coefficients calculated from the mean values, because such estimates are more independent of inter-country confounding factors. However, as discussed above, such estimates probably underestimate the contribution of various factors to the difference in total cholesterol levels, observed in the five countries. On the other hand regression coefficients calculated using the mean values are likely to provide an overestimate.

For the differences in the mean concentrations of HDL cholesterol we would also suggest that an important part of these differences should be ascribed to differences in dietary intake profiles. This follows from a comparison of the carbohydrate intakes of the groups and the corresponding HDL cholesterol levels, the relationships found in some of the groups and the results of the multiple regression analysis. Studies carried out previously support our findings (75, 76).

The body mass index was also negatively related to the concentration of HDL cholesterol and the regression coefficients found were similar to those found for adult men (77, 78).

In the multiple regression analysis on the concentrations of total and HDL cholesterol, it was found that the proportion of energy from saturated fatty acids and from carbohydrate, respectively were the only dietary variables that were significantly related to the concentrations of total or HDL cholesterol. However, it should not be concluded from these findings that the percentages of energy from saturated fatty acids or from carbohydrate are the only dietary variables that affect the concentration of total or HDL cholesterol, respectively. The limited size of the samples studied, the inaccuracy of the variables under estimation and the type of multiple regression analysis that was used would not justify such a conclusion. Furthermore, the dietary variables were sometimes highly correlated with each other, especially the correlation coefficients beteen the proportions of energy from carbohydrate and that from total fat or saturated fatty acids were high in all groups ($r = -0.7$ to -0.9). Therefore, only one of these two variables was selected into the regression model through the procedure used. However, on a forced introduction, for example, of the proportion of energy from saturated fatty acids instead of that from carbohydrate into the regression model for HDL cholesterol, the percentage of variation explained in the HDL cholesterol concentration was only slightly lower than with the energy percentage of carbohydrate in the regression model.

The ratio of HDL cholesterol/total cholesterol was positively related to the physical activity score in the Ghanaian and Finnish boys and this is in agreement with the results of previous studies (79, 80). The energy percentage from polyunsaturated fatty acids was positively related to the ratio in the Filipino boys and in all boys when pooled. This suggests that the lowering effect of polyunsaturated fatty acids on the concentration of total cholesterol is lower than on that of HDL cholesterol. Some dietary trials have shown

pronounced effects of diets rich in polyunsaturated fatty acids and relatively poor in cholesterol on both total and HDL cholesterol (81 - 84). However, in other trials using diets rich in polyunsaturated fatty acids and rich in cholesterol, the effect on HDL cholesterol was smaller than that on total cholesterol resulting in a more favourable ratio (85, 86). It is possible that the low concentrations of HDL cholesterol and the low ratios of HDL cholesterol/total cholesterol of the Filipino boys are partly the result of their high intake of carbohydrate and their extremely low intakes of polyunsaturated fatty acids and cholesterol.

High cholesterol subgroups (Table 8) tended to have higher intakes of products rich in saturated fatty acids and cholesterol and poor in complex carbohydrates and polyunsaturated fatty acids. One notable exception to this was the higher consumption of cheese products in the low cholesterol groups of the Finnish boys and this was also found in an earlier study by Räsänen et al (19). The explanation for this and for other discrepancies will probably be found in the interrelationships between the intakes of particular food items. For example, it appeared from the Multicenter-study in 8-year old Finnish boys (18) that a high consumption of cheese is typical for children from the higher social and educational groups. In relation to the average, these families also consume more soft vegetable margarines, oils and low-fat milk products and less butter and high-fat milk products.

Low HDL subgroups (Table 9) tended to have higher intakes of products rich in complex carbohydrate and lower intakes of products rich in saturated fatty acids and cholesterol.

Therefore, the results of this study support the hypothesis that the effects of dietary components on total and HDL cholesterol are similar, although it is possible that a higher intake of products rich in polyunsaturated fatty acids has a more pronounced effect on the concentration of total cholesterol than on that of HDL cholesterol.

In general terms the concentration of both total and HDL cholesterol are higher, when the diet is rich in saturated fat and lower when the diet is rich in carbohydrate. As the carbohydrate in the diet of the Filipino boys contained a high proportion of complex carbohydrate, it may well be that such carbohydrates are also effective in lowering total and HDL cholesterol levels, like the simple sugars for which high concentrations have been found to be associated with lower HDL cholesterol levels (75). As fiber is associated with the complex carbohydrates, fiber also may play a role in the regulation

of the level of total and HDL cholesterol in serum (87).

It has been assumed that both fat and carbohydrate are metabolized to triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins (VLDL), respectively) and that after the removal of much of the triglyceride in the extrahepatic tissues, the surface material of the remnant particles is converted to HDL (88). However, this is not supported by our data or by kinetic studies in which the production and clearance of HDL has been measured in subjects on carbohydrate-rich diets. Thus Huff et al (89) found that increased production of HDL was associated with delayed clearance, while Melish et al (90) found that the increased VLDL apoprotein B size was due only to a decrease in its removal rate. Therefore it appears that in man much of the ingested carbohydrate is not converted to triglyceride-rich lipoproteins in the liver but is metabolized directly. The assumption that carbohydrate is converted to triglyceride in the liver was based on studies in the rat (91).

The diets of the Ghanaian and Filipino boys had a larger contribution of products from vegetable origin than those of the other groups and these diets were associated with the lowest concentrations of total cholesterol despite a relatively low intake of polyunsaturated fatty acids. However, 14 percent of the Filipino boys and 26 percent of the Ghanaian boys had a body mass index below 14.0 kg/m^2 and there was a considerable percentage of Ghanaian boys with low albumin and hemoglobin concentrations. This suggests that the energy, protein and probably also the intake of other nutrients of part of the Ghanaian and Filipino boys was insufficient or that diseases like parasite infections had a particular influence. The diets of the Italian boys with about 13 percent of energy from proteins, 57 percent of energy from carbohydrate and 28 percent of energy from fat and with only moderate amounts from meat and milk products (7 and 14 energy percent, respectively) were associated with lower concentrations of total and HDL cholesterol than those of the Dutch and Finnish boys who obtained 12 percent of the energy from meat products and 21 - 25 percent of energy from milk products. The slightly lower HDL cholesterol concentration of the Italian boys does probably not indicate a higher risk of coronary heart disease because their fat intake was lower and their carbohydrate intake was higher than those of the Dutch and Finnish boys (78, 92, 93). Therefore, we would suggest that an Italian-like diet as described in this study would be beneficial for the cholesterol carrying lipoproteins of Dutch and Finnish boys. In practical terms this would mean that the consumption of cereals should be increased and that of saturated fat containing products like meat and milk

products should be decreased.

Apart from dietary factors, it should be emphasized that higher levels of physical activity and optimum body weight also favourably influence the concentration of total and HDL cholesterol.

In conclusion, our hypothesis that dietary factors influence the concentration of total and HDL cholesterol in a similar way has found support in this study. Diets with relatively high proportions of food of vegetable origin, together with a considerable degree of physical activity and optimal body fatness might be beneficial for the prevention of the development of atherosclerosis in childhood.

ACKNOWLEDGEMENTS

The authors wish to thank Dr M.B.Katan for valuable advice and discussion throughout the project, Ms A.E.M.Soffers, Ms J.C.M.Barendse-van Leeuwen, Ms G.Germing-Nouwen, Mr H.J.Verwey, Mr F.J.M.Schouten, Mr P.Van den Bovenkamp, Ms J.G.Kosmeyer-Schuil, Ms J.Bos, Ms E.A.M.Koot-Gronsveld, Ms A.Jaartsveld, Ms C.A.M.Sijstermans-Mertens, Ms W.A.van der Wiel-Wetzels, Ms G.J.C.van Oosten-Van der Goes and Mr B.A.Scholte for technical assistance in Wageningen and all those people who assisted with the project in Finland, Ghana, Italy, the Netherlands and the Philippines.

REFERENCES

1. Knuiman JT, West CE, Hermus RJJ, Hautvast JGAJ. Is serum cholesterol outmoded? Lancet ii, 1183 (1979).
2. Knuiman JT, Hermus RJJ, Hautvast JGAG. Serum total and high density lipoprotein (HDL) cholesterol concentrations in rural and urban boys from 16 countries. Atherosclerosis 36, 529 (1980).
3. Epstein FH. Nutrition, atherosclerosis and coronary heart disease. Evidence from epidemiological observations. Atherosclerosis Rev 5, 149 (1979).
4. Keys A. Coronary heart disease in seven countries. Circulation 41 and 42 (suppl 1), 1 (1970).
5. Kannel WB, McGee D, Gordon T. A general cardiovascular risk profile. The Framingham Study. Am J Card 38, 46 (1976).
6. Miettinen M, Turpeinen O, Karvonen MJ, Elosuo F, Paavilainen E. Effect of cholesterol-lowering diet on mortality from coronary heart disease and other causes. Lancet ii, 835 (1972).
7. Dayton S, Pearce ML, Goldman H, et al. Controlled trial of a diet high in unsaturated fat for prevention of atherosclerotic complications. Lancet ii, 1060 (1968).
8. Smith EB, Slater RS. Relationship between low density lipoprotein in aortic intima and serum lipid levels. Lancet i, 463 (1972).
9. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. Am J Med 62, 707 (1977).
10. Miller NE, Førde OH, Thelle DS, Mjos OD. The Tromsø heart study. High density lipoprotein and coronary heart disease. A prospective case-control study. Lancet i, 965 (1977).
11. Knuiman JT, West CE, Hautvast JGAG. Infant and child nutrition: the effects on serum lipids and the consequences in later life. Biblthca Nutr Dieta 31, 131 (1982).
12. Enos WJ, Beijer JC, Holmes FH. Pathogenesis of coronary disease in American soldiers killed in Korea. JAMA 158, 912 (1955).
13. McNamara J, Malot MA, Stremple JF, Cutting RT. Coronary artery disease in combat casualties in Vietnam. JAMA 216, 1185 (1971).
14. Vihert AM. Atherosclerosis of the aorta in five towns. Bull World Health Org 53, 501 (1976).
15. Rissanen V. Aortic and coronary atherosclerosis in a Finnish autopsy series of violent deaths. Annal Acad Scient Fenn, Series A, V. Medica (1972).
16. Van der Haar F, Kromhout D. Food intake, nutritional anthropometry and blood chemical parameters in 3 selected Dutch schoolchildren populations. Med Landbouwhogeschool, Wageningen, The Netherlands. (1978).
17. Antonini AC, Dal Palù C. Pordenone study on the precursors of atherosclerosis in childhood. Italy: Savioprint (1980).

18. Viikari J, Åkerblom HK, Nikkari T, et al. Multicenter study of atherosclerosis precursors in Finnish children - pilot study of 8-year-old boys. Ann Clin Research 14, 103 (1982).

19. Räsänen L, Wilska M, Kantero R, Näntö V, Ahlström A, Hallmann N. Nutrition survey of Finnish rural children. IV. Serum cholesterol values in relation to dietary variables. Am J Clin Nutr 31, 1050 (1978).

20. Frank GC, Berenson GS, Webber LS. Dietary studies and the relationship of diet to cardiovascular disease risk factor variables in 10-year-old children. Am J Clin Nutr 31, 328 (1978).

21. Nichols AB, Ravenscroft C, Lamphear DE, Ostrander L. Daily nutritional intake and serum lipid levels. The Tecumseh study. Am J Clin Nutr 29, 1384 (1976).

22. Askevold R, Høstmark AT, Vellar OD, Von Kraemer Bryn M, Glattre E. Serum cholesterol and triglyceride levels in Norwegian adolescent schoolchildren. Acta Paed Scand 67, 157 (1978).

23. Dyerberg J, Hjörne N. Plasma lipid and lipoprotein levels in childhood and adolescence. Scand J Clin Lab Invest 31, 473 (1973).

24. Golubjatnikov R, Paskey T, Inhorn SL. Serum cholesterol levels of Mexican and Wisconsin schoolchildren. Am J Epid 96, 36 (1972).

25. Lauer RM, Connor WE, Leaverton PE, Reiter MA, Clarke WR. Coronary heart disease risk factors in schoolchildren. The Muscatine Study. J Pediatr 86, 679 (1975).

26. Mendoza S, Nucete H, Zerpa A, et al. Lipids and lipoproteins in 13 - 18 year-old Venezuelan and American schoolchildren. Within and cross-cultural comparisons. Atherosclerosis 37, 219 (1980).

27. Morrison JA, de Groot I, Edwards BK, et al. Lipids and lipoproteins in 927 schoolchildren, ages 6 to 17 years. Pediatrics 62, 990 (1978).

28. Okuni M, Hayaski K, Kiryu S, Yamauchi K. Risk factors of arteriosclerosis in Japanese children. Jap Circ J 44, 69 (1980).

29. Savage PJ, Hamman RF, Bartha G, et al. Serum cholesterol levels in American (Pima) Indian children and adolescents. Pediatrics 58, 274 (1976).

30. Scrimshaw NS, Balsam A, Arroyave G. Serum cholesterol levels in school-children from three socio-economic groups. Am J Clin Nutr 5, 629 (1957).

31. Tamir I, Heiss G, Glueck CJ, Christensen B, Kwiterovich P, Rifkind BM. Lipid and lipoprotein distributions in white children ages 6 - 19 yr. The Lipid Research Clinics Program Prevalence Study. J Chron Dis 34, 27 (1980).

32. Weidman WH, Elveback LR, Nelson RA, Hodgson PA, Ellefson RD. Nutrient intake and serum cholesterol levels in normal children 6 to 16 years of age. Pediatrics 61, 354 (1978).

33. Castelli WP, Cooper GR, Doyle JT, et al. Distribution of triglyceride and total, LDL and HDL cholesterol in several populations: a cooperative lipoprotein phenotyping study. J Chron Dis 30, 147(1977).

34. Whitehead TP, Brownings DM, Gregory A. A comparative survey of the results of analysis of blood serum in clinical chemistry laboratories in the U.K. J Clin Path 26, 435 (1973).

35. Hainline A, Cooper GR, Olansky AS, et al. CDC Survey of high density lipoprotein cholesterol measurement: a report. U.S. Department of Health and Human Services, Public Health Service, Center for Disease Control, Atlanta, GA, U.S.A. (1980).

36. Knuiman JT, Westenbrink S, Van der Heyden L, West CE, Hautvast JGAJ. International study on food consumption and serum lipids in boys. Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands (1981).

37. Ostwald R, Gebre-Medhin M. Westernization of diet and serum lipids in Ethiopians. Am J Clin Nutr 31, 1028 (1978).

38. Pongpaew P, Saovakontha S, Schelp F, et al. Serum lipid pattern in urban and rural Thai population. J Nutr Sci Vitaminol 24, 289 (1978).

39. Anderson BA, Kinsella JA, Watt BK. Comprehensive evaluation of fatty acids in foods. II. Beef products. J Am Diet Assoc 67, 35 (1975).

40. Anderson BA. Comprehensive evaluation of fatty acids in foods. VII. Pork products. J Am Diet Assoc 69, 44 (1976).

41. Exler J, Weihrauch JL. Comprehensive evaluation of fatty acids in foods. VIII. Finfish. J Am Diet Assoc 69, 243 (1976).

42. Paul AA, Southgate DAT. McCance and Widdowson's The Composition of Foods. 4th ed. London: Her Majesty's Stationery Office (1978).

43. Watt BK, Merill AL. Composition of foods. Agriculture Handbook no. 8. Revised ed. 1975. Washington DC 20402: US Government Printing Office (1975).

44. Reeves JB, Weihrauch JL. Composition of foods. Agriculture Handbook no. 8-4. Revised ed. 1979. Washington DC 20402: US Government Printing Office (1979).

45. Platt BS. Tables of representative values of foods commonly used in tropical countries. Special Report Series no. 302. London: Her Majesty's Stationery Office (1975).

46. Nederlandse Voedingsmiddelentabel. 29th ed. The Hague: Voorlichtingsbureau voor de Voeding (1976).

47. Hautvast JGAJ. Commissie Uniforme Codering voedingsenquêtes; ontwikkeling van een systeem om gegevens van voedingsenquêtes met behulp van de computer te verwerken. Voeding 36, 356 (1975).

48. Westenbrink S, Knuiman JT, Van der Heyden L, et al. Nutrient composition of 167 food items from Ghana, the Philippines, Italy and Finland. Wageningen: Department of Human Nutrition, Agricultural University, Report No 82-27 (1983).

49. Eyeson KK, Ankrah EK. Composition of foods commonly used in Ghana. Accra: Food Research Institute. Council for Scientific and Industrial Research (1975).

50. Abdon IC, Del Rosario IF, comps. Food composition tables. Handbook 1, 5th revision. Manila: Food and Nutrition Research Institute (1980).

51. Durnin JVGA, Passmore R. Energy, work and leisure. London: Heinemann Educational Books LTD (1967).

52. Knuiman JT, West CE. The concentration of cholesterol in serum and in various serum lipoproteins in macrobiotic, vegetarian and non-vegetarian men and boys. Atherosclerosis 43, 71 (1982).

53. Weiner JS, Lourie JA. In: Human Biology: a Guide to Field Methods. I.B.P. Handbook no. 9. Oxford: Blackwell Scientific Publications (1969).

54. Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged 16 to 72 years. Br J Nutr 32, 77 (1974).

55. Benn RT. Some mathematical properties of weight-for-height indices used as measures of adiposity. Brit J Prev Soc Med 25, 42 (1971).

56. National Institute of Health, Manual of Laboratory Operations. Lipid Research Clinics Program, Vol 1 (Lipid and Lipoprotein Analysis). DHEW Publ. no (NIH) 75. Bethesda MD: National Institute of Health (1974).

57. Mayer KH, Stamler J, Dyer AR, et al. Epidemiologic findings on the relationship of time of day and time since last meal to five clinical variables: serum cholesterol, hematocrit, systolic and diastolic blood pressure, and heart rate. Prev Med 7, 22 (1978).

58. Henderson LO, Saritelli AL, LaGarde E, et al. Minimal within-day variation of high density lipoprotein cholesterol and apoprotein A-1 levels in normal subjects. J Lip Res 21, 95 (1980).

59. Katan MB, van der Haar F, Kromhout D, Schouten FJM. Standardization of serum cholesterol assays by use of serum calibrators and direct addition of Liebermann-Burchard reagent. Clin Chem 28, 683(1982).

60. Abell AA, Levy BB, Broady BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and a demonstration of its specificity. J Biol Chem 195, 357 (1952).

61. Burstein M, Samaille J. Sur un dosage rapide du cholesterol lié au alpha- et beta lipoproteins du serum. Clin Chim Acta 5, 609 (1960).

62. Van der Haar F, Van Gent CM, Schouten FM, et al. Methods for the estimation of high density cholesterol, comparison between two laboratories. Clin Chim Acta 88, 469 (1978).

63. Corcoran RM, Durnan SM, Albumin determination by a modified bromcresol green method. Clin Chem 23, 765 (1977).

64. International Federation of Clinical Chemistry. Committee on standards. Provisional recommendation (1978) on specification for human serum albumin standard. Clin Chim Acta 98, 175F (1979).

65. Kim J, Kohout FJ. Special topics in general linear models. In: Nie NH, Hadlai Hull C, Jenkins JG, et al., eds. Statistical Package for the Social Sciences (SPSS). 2nd ed. New York: McGraw-Hill Book Company (1975).

66. Liu K, Stamler J, Dyer A. McKeever J, McKeever P. Statistical methods to assess the role of intra-individual variability in obscuring the relationship between dietary lipids and serum cholesterol. J Chron Dis 31, 399 (1978).

67. Kim J, Kohout FJ. Analysis of variance and covariance: subprograms anova and oneway. In: Nie NH, Hadlai Hull C, Jenkins JG, et al., eds. Statistical Package for the Social Sciences (SPSS). 2nd ed. New York: McGraw-Hill Book Company (1975).

68. Cronk CE, Roche AF. Race- and sex-specific reference data for triceps and subscapular skinfolds and weight/stature^2. Am J Clin Nutr 35, 347 (1982).

69. Jacobs DR, Anderson JT, Blackburn H. Diet and serum cholesterol. Do zero correlations negate the relationship ? Am J Epid 110, 77 (1979).

70. Keys A, Anderson JT, Grande F. Serum cholesterol response to changes in the diet. I. Iodine value of dietary fat versus 2S-P. Metab Clin Exptl 14, 747 (1965).

71. Grande F, Anderson JT, Keys A. Diets of different fatty acid composition producing identical serum cholesterol levels in man. Am J Clin Nutr 25, 53 (1972).

72. Hegsted DM, McGandy RB, Myers ML, Stare FJ. Quantitative effects of dietary fat on serum cholesterol in man. Am J Clin Nutr 17, 281 (1965).

73. Durnin JVGA, Ferro-Luzzi A. Conducting and reporting studies on human energy intake and output: suggested standards. Am J Clin Nutr 35, 624 (1982).

74. Snedecor GW, Cochran WG. Statistical methods. 6th ed. Ames: The Iowa State University Press, p. 164 (1976).

75. Ernst M, Fischer M, Smith W, et al. The association of plasma high density lipoprotein cholesterol with dietary intake and alcohol consumption. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 41 (1980).

76. Schonfeld G, Weidman SW, Witztum JL, et al. Alterations in levels and interrelations of plasma apolipoproteins induced by diet. Metabolism 25, 261 (1976).

77. Glueck CJ, Taylor HL, Jacobs D, et al. Plasma high density lipoprotein cholesterol: associations with measurements of body mass. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 62 (1980).

78. Knuiman JT, West CE, Burema J. Serum total and high density lipoprotein (HDL) cholesterol concentrations and body mass index in adult men from 13 countries. Am J Epid 116, 631 (1982).

79. Hartung GH, Foreyt JP, Mitchelli RE, et al. Relation of diet to high density lipoprotein cholesterol in middle-aged marathon runners, joggers, and inactive men. N Engl J Med 302, 357 (1980).

80. Haskell WL, Taylor HL, Wood PD, et al. Strenuous physical activity, treadmill exercise test performance and plasma high density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 53 (1980).

81. Shepherd J, Packerd CJ, Patsch JR, Gotto AM, Taunton OD. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and metabolism of apoprotein A-1. J Clin Invest 61, 1582 (1978).

82. Schaefer EJ, Levy RI, Ernst ND, Van Sant D, Brewer B. 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 34, 1758 (1981).

83. Ernst M, Fisher M, Bowen P, Schaefer EJ, Levy RI. Changes in plasma lipids and lipoproteins after a modified fat diet. Lancet ii, 111 (1980).

84. Vessby B, Boberg J, Gustafsson I, Karlström B, Lithell H, Lindqvist A. Reduction of high density lipoprotein cholesterol and apolipoprotein A-1 concentrations by a lipid-lowering diet. Atherosclerosis 35, 21 (1980).

85. Brussaard JH, Katan MB, Groot PHE, Havekes LMH, Hautvast JGAJ. Serum lipoproteins of healthy persons fed a low-fat diet or a polyunsaturated fat diet for three months. A comparison of two cholesterol-lowering diets. Atherosclerosis 42, 205 (1982).

86. Lewis B, Hammett F, Katan M, et al. Towards an improved lipid-lowering diet: additive effects of changes in nutrient intakes. Lancet ii, 1310 (1981).

87. Kay RM. Dietary fiber. J Lip Research 23, 221 (1982).

88. Tall AR, Small DM. Body cholesterol removal: role of plasma high density lipoproteins. Adv Lip Research 17, 1 (1980).

89. Huff MW, Nestel PJ. Metabolism of apolipoproteins CII, $CIII_1$, $CIII_2$ and VLDL-B in human subjects consuming high carbohydrate diets. Metabolism 31, 493 (1982).

90. Melish J, Ngoc-Anh L, Ginsberg H, et al. Dissociation of apoprotein B and triglyceride production in very low density lipoprotein. Am J Physiol 239, E354 (1980).

91. Geelen MJH, Harris RA, Beynen AC, McCune SA. Short-term hormonal control of hepatic lipogenesis. Diabetes 29, 1005 (1980).

92. Knuiman JT, West CE. HDL cholesterol in men from thirteen countries. Lancet ii, 367 (1981).

93. Knuiman JT, West CE, Hautvast JGAJ. Role of diet to HDL cholesterol and coronary disease: serum total and HDL cholesterol in boys and men from developing and developed countries. Am Heart J 103, 447 (1982).

6 GENERAL DISCUSSION

The concentration of total cholesterol in young boys

The results of the first study described in this thesis (Chapter 2) showed that the concentrations of total cholesterol in Dutch boys were similar to those of boys from Austria, Denmark, Ireland and Sweden. However, the concentrations of the Dutch boys were considerably lower than those of the Finnish boys and higher than those of African and Asian boys. Although the samples taken from the different countries were not representative, the concentrations of total cholesterol were highly correlated with the mortality rates of coronary heart disease of the different European countries (1). This suggests that young boys from Finland and the Netherlands for example have a higher risk of developing coronary heart disease than boys from countries such as Greece and Portugal.

Relationships between the concentration of HDL cholesterol and occurrence of coronary heart disease

From a statistical point of view, the negative relationship between the concentration of HDL cholesterol and the occurrence of coronary heart disease as found within populations (2 - 5) is not incompatible with the absence of a negative, or even the presence of a positive, relationship between the concentration of HDL cholesterol and the occurrence of coronary heart disease when different countries are compared (6 - 7). This is illustrated in Figure 1. An epidemiological explanation for such relationships comes from a consideration of the determinants of total and HDL cholesterol. It is now well established that changes in total and HDL cholesterol induced by diet tend to be parallel (8 - 11). If diet is the only determinant of the concentrations of total and HDL cholesterol one would expect a positive relationship not only between the concentration of total cholesterol and the occurrence of coronary heart disease but also between the concentration of HDL cholesterol and the occurrence of coronary heart disease. However, there does exist a negative relationship between HDL cholesterol and the occurrence of coronary heart disease within populations (2 - 5). Therefore, this negative relationship between the concentration of HDL cholesterol and the occurrence of coronary heart disease results from the influence of other determinants on HDL cholesterol such as body mass index (7, 12), physical activity (13)

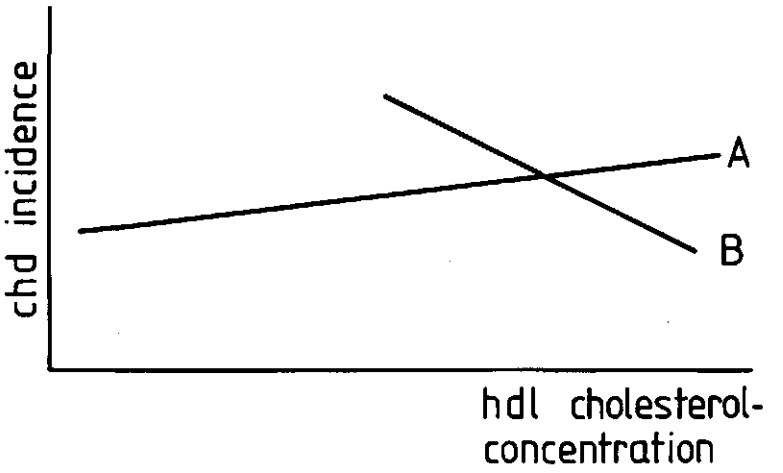

Fig. 1. Relationship between the concentration of HDL cholesterol and the incidence of coronary heart disease (CHD).
A: when different populations are compared.
B: within populations.

and smoking (14). These non-nutritional factors influence the concentrations of total and HDL cholesterol in opposing ways. Other factors such as alcohol consumption (15), use of drugs (16) and genetic factors may also be important. It is possible that the relative effect of the different determinants of total and HDL cholesterol, given both by the magnitude of the determinant itself and the relative strength and direction of its contribution, will determine whether there is a negative relationship or not between HDL cholesterol and the occurrence of coronary heart disease.

Consequences for the prediction of coronary heart disease

Since the concentration of HDL cholesterol is positively influenced by a more westernized diet, it is tempting to suggest that the significance of HDL cholesterol as a risk predictor for coronary heart disease depends on the nature of the diet being consumed. High concentrations of HDL cholesterol associated with a high intake of saturated fat probably reflect a higher capacity to handle large amounts of saturated fat. Thus people in an affluent population with high HDL cholesterol concentrations would have a relatively low risk of coronary heart disease compared with people with a low HDL cholesterol concentration. On the other hand, people with low intakes of saturated fat, such as those in the developing countries and vegans and macrobiotics (17 - 20), have low levels of HDL cholesterol, and also of total

cholesterol, but these people would not have an enhanced risk of coronary heart disease.

The ratio of HDL cholesterol/total cholesterol is probably relatively independent of the nature of the diet consumed (6 - 11, 17 - 20). This would explain, at least partly, why the mean values for the ratios of the groups of boys and men from the different countries are only slightly different. However, this also implies that different risks of coronary heart disease are associated with similar ratios of HDL cholesterol/total cholesterol. On the basis of these considerations we would suggest that a linear combination of total and HDL cholesterol would ultimately appear to give the best and most generally applicable prediction of coronary heart disease risk as far as the contribution from serum lipids is concerned.

Body mass index and the concentration of HDL cholesterol

In the studies of the boys (Chapter 5) and of the adult men (Chapters 3 and 4), negative relationships were found between the body mass index and the concentration of HDL cholesterol. It appeared that per unit increase in body mass index there was an average decrease in the concentration of HDL cholesterol of about -0.020 mmol/liter. Although this appears to be low, it should be kept in mind that small differences in the concentration of HDL cholesterol can change the risk of coronary heart disease significantly (4).

Dietary variables and the concentrations of total and HDL cholesterol

The relationships between food intake or the pattern of food intake and the concentrations of total and HDL cholesterol were examined in the studies described in the Chapters 4 and 5. The hypothesis under study was that both the concentrations of total and HDL cholesterol increase under the influence of a more westernized diet rich in saturated fat and poor in complex carbohydrates. According to this hypothesis the concentrations of both total and HDL cholesterol in the macrobiotic boys and men (Chapter 4) should have been lower than those of all other groups. Although the concentration in the macrobiotic boys was somewhat lower than those of the other boys, the HDL cholesterol concentration of the macrobiotic men was identical to that of the non-vegetarian men. This may be partly due to the unfavourable body mass index of the non-vegetarian men compared with that of the macrobiotic men. However, when the Dutch non-vegetarian men were compared with other European

men of the same age (Chapter 3), than it appeared that the levels in the Dutch non-vegetarian men were relatively low, especially when their concentrations of total cholesterol were taken into account.

In the study described in Chapter 5 it was found that both the concentrations of total and HDL cholesterol were higher in the boys consuming more saturated fat and less carbohydrate. This is not only true for the comparisons of groups from different countries, but it also holds, at least partly, for the groups of boys within the different countries. Taking the results of all studies together, it appears that considerable evidence has been collected to support the hypothesis that both the concentrations of total and HDL cholesterol increase under the influence of a more westernized diet.

Practical consequences for the prevention of the development of atherosclerosis in childhood

The main conclusions of the studies described in this thesis are that dietary factors influence the concentrations of total and HDL cholesterol in a similar way and that a relatively low level of HDL cholesterol is not likely to be associated with an increased risk of coronary heart disease provided that the intake of saturated fat is relatively low and that of carbohydrate is relatively high. Therefore, it is not unlikely that attempts to lower the concentration of total cholesterol will also result in a decrease of the concentration of HDL cholesterol. However, when this decrease in HDL cholesterol concentration is not associated with a decrease in the HDL cholesterol/total cholesterol ratio, dietary intervention will probably be beneficial in the prevention of atherosclerosis. On the other hand, higher levels of physical activity and optimal levels of body fatness will have a favourable influence on the ratio of HDL cholesterol/total cholesterol and may therefore be beneficial for the prevention of the development of atherosclerosis in childhood.

The studies described in this thesis have been carried out in males for whom the risk of coronary heart disease is higher than in females. However, it may be worthwhile to carry out similar studies in females to establish if the relationships described here are applicable to both sexes.

REFERENCES

1. Knuiman JT, West CE, Hautvast JGAJ. Infant and child nutrition: the effects on serum lipids and the consequences in later life. Biblthca Nutr Dieta 31,

131 (1982).

2. Miller NE, Førde OH, Thelle DS, Mjøs OD. The Tromsø Heart Study - High density lipoprotein and coronary heart disease: a prospective case-control study. Lancet i, 965 (1977).

3. Castelli WP, Doyle JT, Gordon T, Hames CG, Hjørtland MC, Hulley SB, Kagan A, Zukel WJ. HDL cholesterol and other lipids in coronary heart disease - The Cooperative Lipoprotein Phenotyping Study. Circulation 55, 767 (1977).

4. Gordon T, Castelli WP, Hjørtland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease - The Framingham Study. Amer J Med 62, 707 (1977).

5. Rhoads GG, Gulbrandsen CL, Kagan A. Serum lipoproteins and coronary heart diease in a study population of Hawaii-Japanese men. N Engl J Med 294, 293 (1976).

6. Knuiman JT, Hermus RJJ, Hautvast JGAJ. Serum total and high density lipoprotein (HDL) cholesterol concentrations in rural and urban boys from 16 countries. Atherosclerosis 36, 529 (1980).

7. Knuiman JT, West CE, Burema J. Serum total and high density lipoprotein (HDL) cholesterol concentrations and body mass index in adult men from 13 countries. Am J Epid 116, 631 (1982).

8. Brunner F, Weissbort J, Fischer M, Bearman JE, Loebl K, Schwartz S, Levin S. Serum lipid response to a high caloric, high fat diet in agricultural workers during 12 months. Am J Clin Nutr 32, 1342 (1979).

9. Ernst W. Bowen P, Fischer M, Schaefer EJ, Levy RI. Changes in plasma lipids and lipoproteins after a modified fat diet. Lancet ii, 111 (1980).

10. Brussaard JH, Dallinga-Thie G, Groot PHE, Katan MB. Effects of amount and type of dietary fat on serum lipids, lipoproteins and apoproteins in man. Atherosclerosis 36, 515 (1980).

11. Shepherd J, Packard CJ, Patsch JR, Gotto AM, Taunton AD. Effects of dietary polyunsaturated and saturated fat on properties of apoprotein A-1. J Clin Invest 61, 1582 (1978).

12. Glueck CJ, Taylor HL, Jacobs D, et al. Plasma high density lipoprotein cholesterol: associations with measurements of body mass. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 62 (1980).

13. Haskell WL, Taylor HL, Wood PD, et al. Strenuous physical activity, treadmill exercise test performance and plasma high density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 53 (1980).

14. Criqui MH, Wallace RB, Heiss G, et al. Cigarette smoking and plasma high density lipoprotein cholesterol. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 70 (1980).

15. Ernst M, Fischer M, Smith W, et al. The association of plasma high density lipoprotein cholesterol with dietary intake and alcohol consumption. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 41 (1980).

16. Wallace RB, Hunninghake DB, Reiland S, et al. Alterations of plasma high density lipoprotein cholesterol levels associated with consumption of

selected medications. The Lipid Research Clinics Program Prevalence Study. Circulation 62 (suppl IV), 77 (1980).

17. Connor WE, Cerqueira MT, Connor RW, Wallace RB, Malinow MR, Casdorph HR. The plasma lipids and lipoproteins and diet of the Tarahumara Indians of Mexico. Amer J Clin Nutr 31, 1131 (1978).

18. Burslem J, Schonfeld G, Howald MA, Weidman SW, Miller JP. Plasma apoprotein and lipoprotein lipid levels in vegetarians. Metabolism 27, 711 (1978).

19. Sacks FM, Castelli WP, Donner A, Kass EH. Plasma lipids and lipoproteins in vegetarians and controls. N Engl J Med 292, 1148 (1975).

20. Knuiman JT, West CE. The concentration of cholesterol in serum and in various serum lipoproteins in macrobiotic, vegetarian and non-vegetarian men and boys. Atherosclerosis 43, 71 (1982).

SUMMARY AND CONCLUSIONS

At present it is assumed that atherosclerosis begins in childhood and that this process may ultimately result in the manifestations of coronary heart disease later in life. For this reason it is relevant to study the distribution of risk indicators for coronary heart disease (CHD) in children from different countries and to seek possible determinants of these risk indicators.

In Chapter 1 a general overview is given of coronary heart disease and its determinants. The reasons and objectives for research on CHD and the risk indicators for CHD are also discussed.

In Chapter 2 the results are presented of a study on the distributions of mean total and HDL cholesterol concentrations in boys aged 7 and 8 years from urban and rural regions in 16 countries. A standardized protocol was used for the collection of samples with the analyses being carried out in one laboratory. The results of this study showed that the concentrations of total cholesterol in Dutch boys are similar to those of boys from Denmark, Austria, Ireland and Sweden but are lower than those of Finnish boys and higher than those of African and Asian boys. The mean concentrations of HDL cholesterol of the boys appeared to increase linearly with that of total cholesterol. This would indicate that both the concentrations of total and HDL cholesterol increase under the influence of a westernized diet. It would also indicate that the mean concentration of HDL cholesterol would be positively related to the incidence of coronary heart disease when different populations are compared, provided that the findings in boys could be extrapolated to adults.

Chapter 3 deals with the concentrations of total and HDL cholesterol in two age-categories of adult men from thirteen countries. The concentrations of total and HDL cholesterol were on average higher in the groups of men from the European countries than in those from Asia and Africa. Although the tendency for a concomitant increase of mean HDL cholesterol and total cholesterol concentrations was less clear in the adult men than it was in the boys, there was no tendency for lower HDL cholesterol concentrations in men with higher total cholesterol concentrations. The body mass index appeared to be positively related with the concentrations of total cholesterol and negatively with that of HDL cholesterol.

Chapter 4 deals with the concentrations of total and HDL cholesterol in macrobiotic, vegetarian and non-vegetarian men and boys. The concentrations of both total and HDL cholesterol were lower in the macrobiotic men and boys

than in the other groups except for the concentration of HDL cholesterol in the non-vegetarian men. The variation between groups in the concentration of HDL cholesterol appeared to be largely due to variations in the concentration of cholesterol in the HDL_2 fraction ($1.063 < \rho_{20} < 1.125$).

In Chapter 5 the results are described of a more in depth study on the determinants of total and HDL cholesterol in boys from Finland, the Netherlands, Italy, the Philippines and Ghana. Positive correlations were found between the intake of fat, saturated fatty acids, monounsaturated fatty acids and dietary cholesterol and the concentrations of total and HDL cholesterol within several groups. Using the regression coefficients from a multiple regression analysis on the pooled data, it could be calculated that on average 24 percent of the inter-country differencesin the levels of total cholesterol is explained by differences in the intakes of saturated fatty acids. Differences between the groups of the different countries in the intakesof carbohydrate explained on average 29 percent of the differences in the concentrations of HDL cholesterol. The results support the hypothesis that higher concentrations of total and HDL cholesterol are associated with western types of diets which are rich in saturated fatty acids and relatively poor in complex carbohydrates.

Chapter 6 contains a general discussion of the various studies. The main conclusions were the following:

- young boys from countries like Finland, the Netherlands, Denmark, Austria and Sweden are likely to be at a higher risk of developing coronary heart disease than boys from Greece or Portugal and boys from Asian and African countries;
- the negative relationship between HDL cholesterol concentration and mortality or incidence from coronary heart disease as found within populations is not incompatible with the absence of a negative or even the presence of a positive relationship between HDL cholesterol and mortality from coronary heart disease when different countries are compared;
- the changes induced by diet in the concentrations of total and HDL cholesterol tend to be parallel;
- high concentrations of HDL cholesterol associated with a high intake of animal fat probably reflect a higher capacity to handle large amounts of dietary fat;
- low concentrations of HDL cholesterol associated with a high intake of animal fat probably reflect a lower capacity to handle large amounts of dietary fat or when associated with a low intake of fat a normal capacity to handle

dietary fat;

- diets with relatively high proportions of food from vegetable origin, especially those relatively rich in complex carbohydrates and relatively poor in saturated fat, together with a considerable proportion of physical activity and an optimum level of body fatness might be beneficial for the prevention of the development of atherosclerosis in childhood.

Thus it has been shown that epidemiological studies can play an important role in elucidating the relationship between diet and coronary heart disease. In particular, it has been possible to develop hypotheses on the significance of the concentration of HDL cholesterol as a risk factor for coronary heart disease.

SAMENVATTING EN CONCLUSIES

Thans wordt verondersteld dat atherosclerose reeds in de kinderjaren begint en dat dit proces uiteindelijk leidt tot de manifestatie van coronaire hartziekten op latere leeftijd. Het is daarom relevant om de verdelingen van risicoindicatoren voor coronaire hartziekten (CHZ) bij kinderen uit verschillende landen te bestuderen en om mogelijke determinanten van deze risicoindicatoren op te sporen.

Hoofdstuk 1 geeft een algemeen overzicht van CHZ en de determinanten van CHZ. Ook worden argumenten gegeven voor onderzoek op het gebied van CHZ en er wordt aandacht besteed aan de risicoindicatoren voor CHZ.

Hoofdstuk 2 behandelt de resultaten van een studie met betrekking tot de verdelingen van de gemiddelde concentraties van totaal en HDL cholesterol van 7 en 8-jarige jongens uit de rurale en urbane gebieden van 16 landen. Een gestandaardiseerd protocol werd gebruikt voor de verzameling van monsters en de analyses werden uitgevoerd in een laboratorium. Uit de resultaten van deze studie bleek dat de concentraties van totaal cholesterol van nederlandse jongens op hetzelfde niveau liggen als die van jongens uit Denemarken, Oostenrijk, Ierland en Zweden. De concentraties van nederlandse jongens zijn echter lager dan die van finse jongens en hoger dan die van afrikaanse en aziatische jongens. Hogere gemiddelden voor de HDL cholesterol concentraties werden gevonden bij die groepen van jongens die ook hogere concentraties van totaal cholesterol in hun bloed hadden. Dit zou er op kunnen wijzen dat zowel de concentraties van totaal als van HDL cholesterol toenemen onder invloed van een verwesterde voeding. Het zou er ook op kunnen wijzen dat de gemiddelde HDL cholesterol concentratie positief gecorreleerd is met de incidentie van CHZ wanneer populaties met elkaar worden vergeleken. Hierbij wordt dan wel verondersteld dat de bevindingen bij de jongens geëxtrapoleerd kunnen worden naar volwassenen.

Hoofdstuk 3 behandelt de concentraties van totaal en HDL cholesterol in twee leeftijdscategorieën volwassen mannen uit 13 landen. De totaal en HDL cholesterol concentraties waren gemiddeld hoger bij de europese mannen dan bij de afrikaanse en aziatische mannen. Hoewel de positieve lineaire correlatie tussen totaal en HDL cholesterol minder duidelijk was bij de volwassen mannen dan bij de jongens, was er geen aanwijzing dat lagere HDL cholesterol concentraties bij deze groepen gepaard gingen met hogere totaal cholesterol concentraties. De Quetelet index bleek positief gecorreleerd te zijn met de totaal cholesterol concentratie en negatief met de HDL concentratie.

Hoofdstuk 4 behandelt de concentraties van totaal en HDL cholesterol van macrobiotische, vegetarische en niet-vegetarische mannen en jongens. Zowel de totaal als de HDL cholesterol concentraties van de macrobiotische mannen en jongens waren lager dan die van de andere groepen met uitzondering van de HDL cholesterol concentratie van de niet-vegetarische mannen. De HDL_2-fractie ($1{,}063 < \rho_{20} < 1{,}125$) was verantwoordelijk voor het belangrijkste deel van de variatie in de HDL cholesterol concentratie tussen de groepen.

In Hoofdstuk 5 worden de resultaten beschreven van een meer gedetailleerde studie met betrekking tot de determinanten van totaal en HDL cholesterol van jongens uit Finland, Nederland, Italië, de Filippijnen en Ghana. Er werden positieve correlaties gevonden van de opname van vet, verzadigd vet en enkelvoudig onverzadigd vet en cholesterol in de voeding met de concentraties van totaal en HDL cholesterol binnen enkele groepen. Gebruikmakend van de regressiecoëfficiënten uit een multiple regressie analyse van de gepoolde data, kon worden berekend dat gemiddeld 24 procent van de verschillen in totaal cholesterol tussen de groepen uit de verschillende landen kon worden verklaard uit verschillen in de opname van verzadigde vetzuren. Voor de verschillen in HDL cholesterol tussen de groepen gold dat 29 procent hiervan kon worden verklaard uit verschillen in de opname van koolhydraten. De resultaten ondersteunen de hypothese dat hogere concentraties van totaal en HDL cholesterol geassocieerd zijn met westerse voedingen die relatief rijk zijn aan verzadigde vetzuren en relatief arm aan complexe koolhydraten.

Hoofdstuk 6 bevat een algemene discussie van de diverse studies.
De belangrijkste conclusies waren als volgt:

- jonge jongens uit landen zoals Finland, Nederland, Denemarken, Oostenrijk en Zweden hebben waarschijnlijk een hoger risico op het krijgen van een coronaire hartziekte dan jongens uit Griekenland, Portugal, Afrika en Azië;
- de negatieve relatie tussen de concentratie van HDL cholesterol en mortaliteit of incidentie van CHZ zoals deze gevonden is binnen populaties is niet onverenigbaar met de afwezigheid van een negatieve of zelfs de aanwezigheid van een positieve relatie tussen de concentratie van HDL cholesterol en mortaliteit van CHZ wanneer verschillende landen met elkaar worden vergeleken;
- de concentraties van totaal en HDL cholesterol hebben de neiging in dezelfde richting te veranderen wanneer veranderingen in de voeding worden aangebracht;
- hoge HDL cholesterol concentraties die gepaard gaan met een hoge opname van dierlijk vet reflecteren wellicht een groter vermogen om met grote hoeveelheden voedingsvet om te springen;

- lage concentraties van HDL cholesterol die gepaard gaan met een hoge opname van dierlijk vet reflecteren wellicht een kleiner vermogen om met grote hoeveelheden voedingsvet om te springen of, wanneer deze lage concentraties gepaard gaan met een lage opname van vet, een normaal vermogen om met voedingsvet om te gaan;
- voedingen met een relatief groot aandeel plantaardige voedingsmiddelen, in het bijzonder die relatief rijk zijn aan complexe koolhydraten en relatief arm aan verzadigd vet, gecombineerd met een behoorlijke portie lichamelijke activiteit en een optimaal lichaamsgewicht kunnen wellicht bijdragen tot de preventie van de ontwikkeling van atherosclerose tijdens de kinderleeftijd.

Op basis van het in deze studie verzamelde materiaal mag geconcludeerd worden dat epidemiologische studies een belangrijke rol kunnen spelen in het ophelderen van relaties tussen voeding en CHZ. In het bijzonder, is het mogelijk gebleken om hypotheses te ontwikkelen met betrekking tot de betekenis van de HDL cholesterol concentratie als risicoindicator voor CHZ.

CURRICULUM VITAE

De auteur werd geboren op 10 augustus 1948 te Nijmegen. Na het behalen van het M.U.L.O., leerling-analist en H.B.S.-diploma in respectievelijk 1964, 1966 en 1968 begon hij in september 1968 met zijn studie aan de Katholieke Universiteit van Nijmegen. In 1976 slaagde hij voor het doctoraal-examen met Biofysische Chemie als hoofdvak en Microbiologie als bijvak. In september 1975 begon hij met zijn studie aan de Landbouwhogeschool. In 1977 slaagde hij voor het kandidaatsexamen (cum laude) en in 1979 voor het doctoraalexamen met Voedingsleer als hoofdvak en Fysische chemie als bijvak. Van januari tot juli 1979 was hij als docent/coördinator verbonden aan de International Course in Food Science and Nutrition te Wageningen. Van juli 1979 tot juni 1982 verrichtte hij met financiële steun van de Nederlandse Hartstichting het in dit proefschrift beschreven project bij de Vakgroep Humane Voeding van de Landbouwhogeschool.