

Atherogenic and protective lipoproteins in boys from ten countries differing in dietary carbohydrate intake

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Dietary measures aimed at reducing the risk of coronary heart disease (CHD) imply that carbohydrate intake should be increased, but short term experiments with people indicate that such diets decrease the level of HDL cholesterol and increase the level of fasting triglyceride. In the Framingham Study, high levels of triglyceride and LDL, together with low levels of HDL were associated with a particularly high risk of CHD. Although it is difficult to examine long-term dietary effects with controlled laboratory studies, information can be obtained from studies of populations whose habitual diets differ. In previous studies from this laboratory, a comparison was made between pre-adolescent boys from different countries, thereby reducing the effect of non-dietary environmental variables. We have now carried out a study in such boys from ten countries where the proportion of energy from carbohydrate, and the incidence of death from CHD, vary over wide ranges. Standardized protocols and a common laboratory were used to obtain comparative data. Up until now, the data on triglyceride levels in different countries have been conflicting, although often results are not really comparable. For example, the population may be dissimilar, samples may be non-fasting or the laboratory methods may not have been standardized. In the present study, all these aspects have been controlled. Thus, the data obtained give a reliable picture of triglyceride levels in populations of boys differing in their intake of carbohydrate and incidence of CHD. The major findings are that fasting triglyceride tended to be higher in countries where a high carbohydrate diet and a low serum cholesterol ($r = -0.72$) were found, and that it correlated negatively with both LDL apoB ($r = -0.85$) and HDL cholesterol ($r = -0.85$). No biochemical parameter or risk ratio was more closely correlated with population CHD mortality than total cholesterol. The existence of these differences in pre-adolescent boys supports the concept that intervention in environmental factors could modify known CHD risk factors from an early age.

Introduction

Current dietary advice to reduce the incidence of coronary heart disease (CHD) encourages reduction of fat intake towards 20–30% of total energy requirements [1–3]. Theoretical and practical considerations dictate that the energy deficit be made up with complex carbohydrate rather than protein, alcohol or simple carbohydrate. This may lead to carbohydrate intakes approaching 60% of energy requirements or greater. Most dietary intervention studies related to CHD have assessed the effect of modified fat diets, and so, the long-term effect of high carbohydrate intake on CHD risk factors has not been firmly established.

Laboratory experiments lasting for up to 13 weeks have shown that high carbohydrate diets can elevate triglyceride levels and reduce the concentration of HDL cholesterol, including the HDL₂ subfraction [4–6]. In patients with a tendency towards hypertriglyceridaemia, the carbohydrate-induced hypertriglyceridaemia tended to recede after a few weeks [7]. One long-term trial also showed that carbohydrate-induced hypertriglyceridaemia in normals tended to resolve [8] after about 16 weeks, however, baseline triglyceride levels were not reached completely if the high-fat baseline diet was rich in polyunsaturated fatty acids [6]. The complexity and expense of laboratory experiments of this nature has limited their duration, so the questions surrounding resolution of carbohydrate-induced hypertriglyceridaemia have not been answered.

Indirect evidence from populations with differing habitual diets has so far failed to clarify the situation. Both elevation and reduction of triglyceride levels have been reported in populations with high carbohydrate intakes [9–13, 15]. The reasons for these discrepancies are probably methodological. Populations have not been comparable in terms of age or sex, laboratory methods differed and non-fasting samples were included in some studies. Little was done to control for confounding variables such as alcohol, medication, obesity, glucose tolerance or parasitic and other disease.

Knuiman et al. [14] paid careful attention to these details in their comparison of HDL levels in school boys with different habitual diets. They were able to show that the reduction in HDL which occurs during high carbohydrate intake has a permanent component, but triglycerides were not studied, because the boys had not been fasting.

We have now designed an international study to examine the possibility that there is also a permanent component to the hypertriglyceridaemia which occurs during high carbohydrate diets. The protocol emphasizes strict criteria to insure that only fasting triglyceride results are considered. It also standardizes many aspects of subject selection, laboratory equipment and analysis so that a meaningful comparison may be made between populations with differing diets.

The results presented in this interim report suggest that boys from populations with a high-carbohydrate diet indeed have a permanent mild elevation of

fasting serum triglycerides. This intensifies the paradox posed by the biochemical changes which accompany high carbohydrate diets, because now two potentially detrimental effects have been described. Our studies also bear relevance to intervention programmes, particularly those involving paediatric populations, because these changes can no longer be assumed to be transitory.

Methods

Countries were selected to provide a cross-sectional representation of anticipated carbohydrate intakes and coronary heart disease mortality rates (Table 1) [16, 17].

The protocol for the study was approved by the ethical committees of the Department of Human Nutrition, Agricultural University, Wageningen, The Netherlands, and the individual countries concerned, as well as the parents of each subject. Approximately 50 healthy boys, evenly distributed between the ages of 96 and 120 months, were randomly selected from four to six 'typical' schools in each country. Height and weight were measured as described previously [18], and clinical history including tobacco, alcohol and medication use, recent illness and confirmation of fasting state were recorded. Subjects with positive history of recent illness or medication thought to affect lipoprotein status were excluded. Serum albumin and C-reactive protein were measured as previously described [18, 23] to exclude subjects with subclinical malnutrition, tropical disease states or infection. (Subjects with C-reactive protein > 10 mg/l or albumin < 36 mg/l were excluded). C-reactive protein is considered superior to the ery-

Table 1

Country	Carbohydrate disappearance (percentage of total dietary energy excluding alcohol)	CHD mortality (deaths/10 ⁵)
Finland	40	321
Hungary	52	259
Italy	51	141
Germany		214
Kenya	71	$< <$
Netherlands	41	176
Philippines	79	49
Poland	55	176
Portugal	58	132
Tanzania	77	$< <$

thocyte sedimentation rate as an indicator of infective and other disease states and is more easily standardized.

The presence of chylomicrons was also assessed on each sample by both electrophoresis on cellulose acetate strips (Cellogel 01A52-25) and by microcentrifugation in haematocrit tubes as described by Schinella et al. [19]. This technique is possibly more sensitive for the detection of chylomicrons than electrophoresis [19]. Triglyceride results were excluded if there was history of failure to fast or if chylomicrons were detected by either method.

Two fasting serum samples were collected a week apart from each subject by standard methods [20]. Our co-ordinating laboratory sent a uniform supply of blood collection and all other necessary equipment to participants. Samples were stored at -20°C before delivery by air express to Wageningen and were in a frozen state on arrival. Further storage was at -20° or -80°C for HDL aliquots. All analyses were performed on previously unfrozen aliquots. HDL cholesterol was measured by the method of Warnick et al. [21] and all cholesterol quantitation was carried out with a Boehringer Monotest kit. Fasting triglyceride was measured with a Boehringer Mannheim 3 component GPO-PAP kit (BM701912) which corrects for free glycerol [22]. Accuracy was monitored with internal laboratory control sera and sera of known lipid and lipoprotein concentration provided by Dr. A. Hainline of the Lipid Standardization Laboratory (Centres for Disease Control, Atlanta GA, U.S.A.). The mean bias for CDC sera was $+0.2\%$ for total cholesterol, -0.3% for HDL cholesterol and -0.4% for triglyceride. Serum albumin was measured with the modified Bromocresol green dye binding method of Robertson [23] calibrated with human serum albumin (Sigma A6019) and standardized with WHO reference serum. All analyses were performed on an Abbott ABA200 bichromatic analyser.

LDL apoprotein B was measured with the radial immunodiffusion method of Sniderman et al. [24]. LDL cholesterol was calculated by means of the Friedewald equation [25].

Results

Mean values for levels of fasting triglyceride, total, HDL and LDL cholesterol and LDL apoprotein B are presented in Table 2. Three countries have not yet been corrected for the possible presence of intercurrent disease by exclusion of subjects with elevated C-reactive protein levels. However, the incidence of such levels is low in such non-tropical countries. One subject was excluded from analysis because a diagnosis of familial hypercholesterolaemia was confirmed on follow-up.

The results show that total and LDL cholesterol and LDL apoprotein B are negatively related to the proportion of carbohydrate in the diet in different coun-

Table 2

Concentration of lipids and lipoprotein components in serum from 8- and 9-year-old boys from 10 countries

Country	Total cho- lesterol mmol/l (N)	LDL cho- lesterol mmol/l (N)	LDL apo B mg/dl (N)	HDL cho- lesterol mmol/l (N)	Fasting triglyceride mmol/l (N)
Finland	4.97 ± 0.99(55)	3.14 ± 0.93(48)	78 ± 13(55)	1.54 ± 0.33(55)	0.61 ± 0.28(48)
West					
Germany ^a	4.72 ± 0.65(41)	2.94 ± 0.57(33)	83 ± 15(41)	1.51 ± 0.26(41)	0.58 ± 0.18(33)
Hungary	4.61 ± 0.77(45)	2.77 ± 0.80(34)	71 ± 10(45)	1.46 ± 0.24(45)	0.70 ± 0.23(34)
Poland	4.39 ± 0.68(40)	2.74 ± 0.64(35)	68 ± 10(40)	1.26 ± 0.21(40)	0.71 ± 0.26(35)
Nether-					
lands	4.36 ± 0.60(52)	2.65 ± 0.46(40)	70 ± 9(51)	1.35 ± 0.24(52)	0.58 ± 0.23(40)
Italy ^a	4.40 ± 0.59(33)	2.75 ± 0.56(13)	85 ± 20(34)	1.45 ± 0.20(33)	0.46 ± 0.11(13)
Portugal ^a	4.37 ± 0.51(28)	2.73 ± 0.39(25)	69 ± 13(29)	1.40 ± 0.32(28)	0.56 ± 0.15(25)
Philip-					
pines	3.94 ± 0.65(32)	2.54 ± 0.65(15)	61 ± 11(32)	0.94 ± 0.20(32)	0.86 ± 0.29(15)
Tanzania	3.55 ± 0.69(31)	2.17 ± 0.27(14)	58 ± 12(32)	0.89 ± 0.28(31)	0.76 ± 0.27(14)
Kenya	2.96 ± 0.52(38)	1.59 ± 0.46(9)	58 ± 13(39)	0.81 ± 0.18(38)	0.89 ± 0.19(9)

Results are expressed as mean ± S.D.

^aIncludes data from subjects whose levels of C-reactive protein are yet to be determined.

tries, as might be expected. Fasting triglycerides tended to be higher in countries where high carbohydrate diet and low serum cholesterol were found.

The results also confirm the positive relationship between HDL and total cholesterol in interpopulation comparisons, as has been described by Knuiman [14]. On this occasion, we were also able to illustrate a strong positive relationship between LDL cholesterol and HDL cholesterol in such studies ($r = 0.88$). In addition, we draw attention to the negative relationship between HDL cholesterol and fasting triglycerides ($r = -0.85$) at the interpopulation level. This relationship is well described in intrapopulation studies, but not previously in interpopulation studies.

Table 3 compares the correlation of biochemical parameters, including risk ratios such as total/HDL cholesterol or LDL/HDL, with CHD mortality between populations.

Discussion

The results of our study are paradoxical because they show that populations with low incidence of CHD mortality, who consume the favoured high complex carbohydrate diet, have two lipoprotein features which are considered detrimental,

Table 3

Correlation of the concentration of lipids and lipoprotein components in boys aged 8 and 9 years in ten countries and the CHD mortality in those countries

Variable ^a	Correlation with CHD mortality ^b
Total cholesterol	0.89
LDL cholesterol	0.82
LDL apoprotein B	0.70
HDL cholesterol	0.88
Fasting triglyceride	-0.55
Total cholesterol/HDL	-0.70
LDL cholesterol/HDL	-0.52

^aConcentration of lipids or lipid components in serum or the ratio of such concentrations

^bSee Table 1 for rate of CHD mortality in the various countries.

namely elevated triglyceride and reduced HDL. We confirm Knuiman's finding that HDL both in boys [14] and adult men [26] exhibits a paradoxically positive relationship with CHD mortality between populations. This effect correlated with differences in diet [27].

Studies such as those in Bogalusa have not shown a close correlation between diet and lipoprotein risk factors within populations [28]. Different racial groups did not have sufficiently different dietary habits to explain interracial differences within the same community [29]. However, as Gordon points out [30], there are many difficulties inherent in the collection and interpretation of dietary data from free living populations. The same difficulties apply to the demonstration that lipoprotein differences were correlated with differences in nutrient intake between populations [27], however, such studies probably benefit from the dilution of intraindividual variation in both diet and lipoproteins, which occurs in interpopulation comparisons.

Our results intensify the LDL-HDL paradox, because they establish that serum triglyceride levels are also permanently elevated by high carbohydrate diets. We have considered two possible interpretations. The first one, which we favour, is that the habitual diet has its primary effect on LDL cholesterol, which is the main determinant of HDL levels and of incidence of CHD. In the statistical analysis, HDL and triglycerides then act as surrogate variables for LDL, which is the real determinant of differences in coronary heart disease death rates between populations. Individual susceptibility to CHD, modulated by HDL or triglyceride-rich lipoprotein could be obscured by the comparison of whole population means.

The reduction of HDL and increase of fasting triglyceride could have detrimental effects on the atherosclerotic process, which are far outweighed by the benefit of reduction in LDL cholesterol.

Alternatively, it is possible that reduced HDL and elevated triglyceride might represent an altered metabolic state which is beneficial in the atherosclerotic process. Ginsberg's finding that VLDL triglyceride synthesis increases, but transformation to LDL decreases on high carbohydrate diet [31] supports such a concept. Our finding of a negative relationship between fasting triglyceride and LDL apoprotein B between populations reinforces this and suggests that a high carbohydrate diet does not cause the combination of hyperapobetalipoproteinaemia in hypertriglyceridaemia, which Sniderman considers an independent risk factor for CHD [32].

Our demonstration that high VLDL and low HDL are permanent rather than transitory features of high carbohydrate diets makes it mandatory that the full significance of such changes be determined. If these changes occur in prepubertal boys, as we have demonstrated, then they may be anticipated in intervention programmes aimed at this age group. Although a generally beneficial effect may be anticipated because low incidence of CHD is associated with low LDL-C and TC, further benefit may be derived from modifications which preserve HDL and minimise triglyceride.

The mechanism for reduction of HDL during high carbohydrate diets is unclear; both an increase in catabolism [33] and a decrease in synthesis have been described [34]. The reciprocal relationship between triglyceride and HDL levels between or within individuals has been attributed to differences in the rates of lipolysis [33]. Our demonstration of a reciprocal relationship between fasting triglycerides and HDL levels of populations with different diets is a striking coincidence which suggests a greater degree of integration of lipoprotein pathways than is currently postulated.

Table 3 emphasises that HDL no longer has a strong negative relationship with CHD in interpopulation studies, and therefore cannot enhance the interpopulation assessment of CHD risk provided by total or LDL cholesterol. We have assessed several other parameters for such a purpose, but it is doubtful whether a better predictor for interpopulation studies is currently available. This emphasizes the importance of total cholesterol as the primary determinant of CHD. Surprisingly, the correlation of LDL cholesterol with CHD is weaker than that with total cholesterol. Fasting triglyceride, or its use to calculate LDL cholesterol has not improved the ability of serum lipids to predict interpopulation CHD risk in our studies so far, however, its measurement will be helpful in determining the metabolic basis and clinical significance of the paradoxical changes in HDL cholesterol levels. It is also possible that the lower correlation is due to a non-linear relationship between LDL cholesterol levels and CHD risk, in which case calculated LDL cholesterol may still be very useful in relation to a cut-off point for increased CHD risk.

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