AN INTERNATIONAL STUDY ON THE EFFECT OF DIETARY CARBOHYDRATE ON ATHEROGENIC AND PROTECTIVE LIPOPROTEINS

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ABSTRACT

Dietary measures aimed at reducing the risk of coronary heart disease (CHD) imply that carbohydrate intake should be increased but short-term experiments with humans indicate that such diets decrease the level of high density lipoproteins (HDL) and increase the level of fasting triglycerides. If these changes are permanent, they could detract from the effectiveness of low-fat, high-carbohydrate diets in reducing the incidence of CHD. In the Framingham Study, high levels of triglycerides together with low levels of HDL cholesterol were associated with a particularly high risk of CHD. Although it is difficult to examine long-term dietary changes with controlled laboratory studies, information can be obtained from studies of populations consuming different diets. The aim of the present study is to obtain a reliable picture of triglyceride levels together with the concentration of other lipoprotein constituents between populations differing in their intake of carbohydrate and incidence of CHD. The practical significance of changes in triglyceride metabolism caused by differing intakes of carbohydrate will be able to be evaluated in terms of CHD risk. The study is being carried out in boys aged 8 and 9 years from 14 countries and data is presented for boys from five countries. Although it is early to draw firm conclusions, it does appear that high levels of fasting triglycerides together with low levels of HDL cholesterol are associated with a high intake of carbohydrate. The significance of the results obtained so far are discussed.

INTRODUCTION

In general, dietary advice aimed at minimizing the risk of coronary heart disease (CHD) includes advice to reduce the intake of dietary fat usually to a level of 30% of total energy intake (1, 2). As it is neither practical nor considered desirable to replace dietary fat by either protein or alcohol, it is a corollary of such recommendations that carbohydrate consumption should be increased to provide 50-60% of energy. However short-term experiments with human subjects have shown that high carbohydrate diets can have adverse effects on the concentrations in serum of triglycerides and high density lipoproteins (HDL): triglyceride levels are increased while HDL cholesterol levels and the ratio of cholesterol in the HDL_2 to that in the HDL_3 fractions are reduced (3, 4). Unfortunately, very few long-term experiments have been carried out to study the effect of high carbohydrate diets because of the problems associated with such studies and the costs involved. The long-term study carried out by Antonis and Bersohn (5) does suggest that adaptation may occur but the evidence for such adaptation is really insufficient on which to base dietary advice to populations in general. Information gained from studies of populations habitually consuming different diets has

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been conflicting as both elevation and reduction of serum levels of triglycerides in populations with high intakes of carbohydrate have been reported (6-10). The reason for the differences in the results obtained are probably largely methodological because the populations studied and the methods used have not always been comparable, samples were not always obtained in the fasting state and often there were other confounding variables.

In earlier studies from this laboratory on total and HDL cholesterol levels in non-fasting schoolboys habitually consuming different diets (11-14), careful attention was paid to making the results strictly comparable. The results obtained showed that high carbohydrate intake was associated with low serum HDL levels which is in line with the results obtained in short-term experimental studies. The present study was designed to examine the possibility that the elevation of triglyceride levels in serum obtained from fasting subjects is also seen in subjects habitually consuming high carbohydrate diets. The study is being carried out in 14 countries using a strict protocol for the collection and analysis of samples and the acceptance of data. This paper sets out the results obtained for the analyses carried out so far on samples from five countries.

METHODS

As in the previous studies from this laboratory, pre-adolescent boys are being studied to reduce the effect of non-dietary variables. Samples are being collected from boys after a 14-hour fast in 14 countries throughout the world where the proportion of energy from carbohydrate and the incidence of death from CHD vary over a wide range. Results are presented here for boys from Finland, Hungary, Poland, the Netherlands and the Philippines. The study has been approved by the ethical committee of the Department of Human Nutrition of the Agricultural University and by the appropriate authorities in each of the countries concerned. Approximately 50 healthy boys, evenly distributed in age between 96 and 120 months were randomly selected from four to six typical schools in each country. Information was collected on alchohol, tobacco and medication use, on recent illness, and on food consumed in the last 14 hours. Subjects with a history of recent illness or who were taking medication thought to affect lipid levels were not included in the study.

Two samples of serum were collected from each boy (15) using equipment supplied from the laboratory in Wageningen and were stored at $-20^{o}C$ before being transported by air to the Netherlands where they arrived in the frozen state. On arrival, samples for lipoprotein analysis were stored at $-80^{o}C$ while other samples were stored at $-20^{o}C$. A number of tests are being carried out on the serum samples to ensure that only results from healthy boys will be included in the data analysed. Low levels of serum albumin (16) has been used as a measure of subclinical malnutrition. When the final results of the study are reported, data from boys with elevated levels of C-reactive protein indicative of subclinical infection will also be excluded from analysis. In addition to asking the boys if they were fasting the absence of chylomicrons in serum, as determined by electrophoresis on cellulose on acetate strips and by staining with Nile Blue (17), was also used to check if the boys were fasting when the blood samples were taken. Triglyceride values will not be used unless a subject was fasting when the blood was taken. Cholesterol was determined enzymatically in total serum and in HDL precipitated with Mg^{2+}-dextran sulphate (18) using the kit (CHOD-PAP Mono test) supplied by Boehringer Mannheim (FRG) and serum calibrators (20). Mean bias for external control sera (Centres for Disease Control, Atlanta, GA) was +0.2% for total cholesterol and -0.3% for HDL cholesterol in this period. The concentration of triglyceride was measured by an enzymatic method not affected by free glycerol (21) using a kit supplied by Boehringer Mannheim. Mean bias for external control sera provided by the Centres for Disease Control was -0.4% during this period. The concentration of LDL apoprotein B was measured by radial immunodiffusion (22).

RESULTS

The concentration of total and HDL cholesterol are presented in Table 1 while the concentration of triglycerides and of cholesterol and apoprotein B in LDL are presented in Table 2.

Table 1. The concentration of total and HDL cholesterol in fasting boys aged 8 and 9 years from five countries.

Country	n	Cholesterol, mmol/l Total	Cholesterol, mmol/l HDL	HDL cholesterol/ Total cholesterol
Finland	59	4.99 ± 1.00	1.52 ± 0.36	0.31 ± 0.06
Hungary	49	4.60 ± 1.04	1.46 ± 0.26	0.32 ± 0.06
Poland	57	4.44 ± 0.89	1.29 ± 0.30	0.29 ± 0.07
The Netherlands	56	4.29 ± 0.75	1.34 ± 0.28	0.31 ± 0.05
The Philippines	33	3.87 ± 0.82	0.94 ± 0.25	0.25 ± 0.06

Results expressed as mean ± SD

The concentration of LDL cholesterol has been calculated using the Friedwald equation (23) although the validity of using this method in different racial groups has yet to be confirmed. Data on triglyceride levels was excluded on the basis of chylomicrons being present in their serum from the following number of samples: Finland, 8; Hungary, 10; Poland, 5; The Netherlands, 8; and The Philippines, 17. In addition, results from two boys were excluded as one had hypoalbuminaemia while the other was diagnosed as having familial

Table 2. The concentration of triglycerides, LDL cholesterol and LDL apoprotein B in fasting boys aged 8 and 9 years from five countries.

Country	Triglycerides mmol/l(n)	LDL cholesterol mmol/l	LDL apo B mg/dl
Finland	0.64 ± 0.36(59)	3.17	78 ± 13(59)
Hungary	0.67 ± 0.27(48)	2.84	72 ± 14(49)
Poland	0.70 ± 0.31(55)	2.83	68 ± 14(57)
The Netherlands	0.56 ± 0.30(54)	2.69	70 ± 9(56)
The Philippines	0.90 ± 0.33(30)	2.54	60 ± 11(33)

Results expressed as mean ± SD except for LDL cholesterol which is calculated using the Friedwald equation (24).[1]

[1] The values in parentheses indicate the number of subjects for which data are included. The individual triglyceride values for each boy are the mean obtained from two samples taken one week apart except when one of the samples was shown to contain chylomicrons.

hypercholesterolaemia. The results obtained for total and HDL cholesterol are similar to those reported earlier (11, 12, 14) except that the concentration of total cholesterol in the boys from Hungary is somewhat higher than the 4.11 mmol/l measured in boys aged 7 and 8 years in May 1979 (11, 12). Positive relationship between total cholesterol and HDL cholesterol reported earlier from this laboratory (11, 12) is confirmed (r = +0.93). It is interesting to note that the mean serum concentration of triglyceride in the boys from The Philippines, albeit from 25 boys, is higher than that for the boys from the other countries. These high concentrations of triglyceride and associated with low concentrations of HDL cholesterol which is in contrast with the results obtained in patient studies on diets differing in carbohydrate intake. The proportion of energy derived from carbohydrate is much higher in the boys from the Philippines than for the other countries (see Table 3). The observation that higher levels of triglyceride are associated with lower levels of LDL apoprotein B would suggest that carbohydrate-induced hypertriglyceridaemia is not associated with hyperapobetaproteinaemia.

Table 3. Proportion of energy derived from carbohydrate in boys and mortality from coronary heart disease (CHD) in five countries.

Country	Proportion of energy from carbohydrate, %	age-adjusted mortality from CHD deaths per 100 000 per year
Finland	50[1]	264
Hungary	52[2]	240
Poland	52[3]	77
The Netherlands	49[1]	183
The Philippines	72[1]	27

1. Data derived from boys aged 8 and 9 years in 1981 by Knuiman et al (14).
2. Data derived from national food balance sheets for 1972-1974 by FAO (25).
3. Data derived from boys aged 11 years in 1982 (26).
4. Data for 1976 (27).

DISCUSSION

The preliminary results obtained from five countries participating in an international study to examine the concentration of cholesterol and triglycerides in serum and of various lipoprotein constituents indicate that it is possible to collect samples from fasting subjects. In the earlier international studies from this laboratory (11-14), no attempt was made to determine the concentration of triglycerides in serum because it was thought that it was not possible to ensure that all subjects were fasting. From the five countries, it was necessary to reject data for serum triglycerides for nine per cent of the samples while in the Philippines, it was necessary to reject data from twenty six per cent of the samples. As we anticipate this to be a consistent problem in developing countries, we are planning to collect samples from more boys in these countries. In addition, we expect more problems of subclinical malnutrition and infection in developing countries. One subject had to be excluded on the basis of low serum albumin levels while at this stage, data is not available for C-reactive protein which will enable subclinical infection to be monitored. One subject had to be excluded on the basis of having familial hypercholesterolaemia which is consistent with the known gene frequency of this disease which is of the order of 0.2-0.5%.

The data obtained on fasting subjects is important for a number of reasons. It is interesting to see that the positive relationship between countries of HDL cholesterol levels with total cholesterol levels reported earlier (11, 12) is observed with fasting samples. Such a result was expected however because previous studies (28, 29) 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. From the data obtained so far, the lower HDL levels and the elevated levels of fasting triglycerides in serum associated with increased carbohydrate intake may reflect metabolic processes which reduce rather than increase CHD risk (see Table 3). The negative relationship between fasting triglycerides and LDL apoprotein B suggests that dietary carbohydrates do not cause the combination of hyperapobetalipoproteinaemia and hypertriglyceridaemia which has been identified by Sniderman and colleagues (30) and an independent risk factor for CHD. Indeed, the lower levels of LDL apoprotein B are consistent with the findings of Ginsberg and coworkers (31) that carbohydrate increases VLDL triglyceride synthesis without affecting VLDL apoprotein B synthesis: catabolism of VLDL apoprotein B was found to be increased whilst the rate of transformation to LDL apoprotein B was decreased. There is an indication that the relationships with mortality from CHD of both serum HDL cholesterol and triglyceride levels between populations found here are opposite to those found within populations such as in the Framingham study (32, 33). However, firm conclusions will have to await completion of the analyses.

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