

## BOYS FROM POPULATIONS WITH HIGH-CARBOHYDRATE INTAKE HAVE HIGHER FASTING TRIGLYCERIDE LEVELS THAN BOYS FROM POPULATIONS WITH HIGH-FAT INTAKE

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West, C. E. (Dept. of Human Nutrition, Agricultural U., P.O. Box 8129, 6700 EV Wageningen, The Netherlands), D. R. Sullivan, M. B. Katan, I. L. Halferkamps, and H. W. van der Torre. Boys from populations with high-carbohydrate intake have higher fasting triglyceride levels than boys from populations with high-fat intake. *Am J Epidemiol* 1990;131:271-82.

Experimentally, high-carbohydrate diets have been shown to elevate triglycerides, but it has not been established whether this rise is permanent or transient. The authors approached this question by studying 719 boys from worldwide populations with marked differences in long-term carbohydrate intake. Fasting serum triglycerides, total cholesterol and high density lipoprotein (HDL) cholesterol concentrations were measured in boys aged 8 and 9 years from 12 countries—eight in Europe, three in Africa, and one in Asia. A standardized protocol was used for obtaining fasting blood and for the preparation, storage and transport of serum, and all measurements were made in one laboratory. Published values were used for the United States. Mean values for lipid levels per country were compared with the percentage of daily energy intake consumed as carbohydrate or fat, as determined by survey. Boys from populations with higher carbohydrate and lower fat intake had lower low density lipoprotein (LDL) cholesterol levels (univariate regression coefficient ( $\pm$  standard error,  $-0.028 \pm 0.009$  mmol/liter for each percent of energy from carbohydrate;  $p < 0.01$ ,  $n = 13$ ), but they also had higher fasting triglycerides ( $0.010 \pm 0.002$  mmol/liter for each percent of energy from carbohydrate;  $p < 0.01$ ,  $n = 13$ ) and lower HDL cholesterol levels ( $-0.022 \pm 0.003$  mmol/liter for each percent of energy from carbohydrate;  $p < 0.001$ ,  $n = 13$ ). These trends agree with results from epidemiologic studies within populations and from controlled dietary trials, and suggest that in normolipidemic healthy subjects, high-carbohydrate, low-fat diets cause higher triglyceride levels than diets that are higher in fats and oils.

carbohydrates; child; fats; lipoproteins, HDL; lipoproteins, LDL; triglycerides

Current dietary advice aimed at reducing the incidence of coronary heart disease encourages reduction in the proportion of

daily energy intake derived from fat (1-3). Since the energy deficit will be made up with carbohydrate rather than protein or

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Abbreviations: HDL cholesterol, high density lipoprotein cholesterol; LDL cholesterol, low density lipoprotein cholesterol; SE, standard error.

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alcohol, this may lead to 60 percent or more of energy requirements being met by carbohydrate. Most long-term dietary intervention studies related to coronary heart disease, however, have assessed the effect of modified fat rather than low-fat diets. Thus, the long-term effect of high carbohydrate intakes on coronary heart disease risk factors is not firmly established.

Experimental and epidemiologic observations have shown that low-fat diets will produce low levels in serum of low density lipoprotein (LDL) cholesterol, the major atherogenic lipoprotein in man. However, laboratory experiments lasting up to 13 weeks have shown that such diets can also elevate serum triglyceride levels and reduce the concentration of high density lipoprotein (HDL) cholesterol, including that in the HDL<sub>2</sub> subfraction (4-6). In patients with a tendency towards hypertriglyceridemia, the carbohydrate-induced hypertriglyceridemia tended to recede after a few weeks (7). One long-term trial (8) also showed that carbohydrate-induced hypertriglyceridemia in healthy persons tended to resolve after about 16 weeks. However, triglyceride levels did not return to those on the baseline diet in those subjects who had consumed a baseline diet rich in polyunsaturated fatty acids (6, figure 5). Because it is difficult and expensive to carry out well-controlled long-term dietary intervention trials, the question surrounding resolution of carbohydrate-induced hyper-

triglyceridemia with time has not been answered.

Indirect evidence from populations with different habitual diets has so far failed to clarify the situation. Both elevations and reductions of triglyceride levels have been reported in populations with high carbohydrate intakes (9-14). The reason for these discrepancies is probably that insufficient attention has been paid to variables such as alcohol intake, medication, obesity, glucose tolerance, or parasitic and other diseases. Furthermore, populations have not been comparable in terms of age or sex, laboratory methods have differed, and non-fasting samples were included in some studies.

In earlier studies from this laboratory (15-17), careful attention was paid to these details when HDL cholesterol levels in schoolboys who consumed different habitual diets were compared. These studies showed that HDL cholesterol levels were strongly and inversely related to carbohydrate intake. This suggests that the reduction in HDL cholesterol which occurs during high carbohydrate intake has a permanent component. Triglycerides were never measured in these earlier studies because blood samples were not taken from the boys in the fasting state.

We now report the findings of a new international collaborative study which focused on the question whether there is also a permanent component to the hypertriglyceridemia resulting from the consumption of high-carbohydrate diets.

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#### MATERIALS AND METHODS

Pre-adolescent boys were studied to reduce differences between countries in non-dietary variables such as obesity, lack of exercise, smoking, and alcohol and drug use, which may confound dietary comparisons in adults (15-17). The countries from which the boys were chosen (table 1) were not selected at random; they were chosen intentionally to provide a wide range of carbohydrate intakes (16-22) and coronary

heart disease mortality rates (23). Field work started in 1984, and sample and data collection was completed in 1985, except for the study in Crete (Greece), which was performed in 1986 (24).

The study was approved by the ethical committees of the Department of Human Nutrition of the Agricultural University and in the individual centers, and parental consent was obtained for each subject. Approximately 50 healthy boys, evenly distributed between the ages of 91 and 125 months, were randomly selected at 4–6 typical schools in each country. Height and weight were measured as described previously (16). Scales were checked locally by determining the weight increase caused by the addition of 20 liters of water from a measuring flask to a large container placed on the scales. A clinical history including tobacco, alcohol and medication use, recent illness and fasting state was recorded. Subjects with a positive history of recent illness or medication thought to affect lipoprotein status were excluded.

Two fasting serum samples were collected one week apart from each subject by standard methods (25), except in Greece where only one sample was collected. Equipment for the collection of blood and the transport of serum was supplied by the coordinating laboratory in Wageningen. Samples were stored at  $-20^{\circ}\text{C}$  until delivery by air express to Wageningen and were in a frozen state on arrival. Further storage was at  $-20^{\circ}\text{C}$ , or at  $-80^{\circ}\text{C}$  for the aliquots on which HDL cholesterol levels were determined. All analyses were performed on previously unfrozen aliquots. HDL cholesterol was measured by the method of Warnick et al. (26) and estimation of cholesterol was carried out with the Boehringer Monotest kit (Boehringer Mannheim, Lelystad, The Netherlands) employing strict standardization (27). Fasting triglycerides were measured with the three-component glycerol-3-phosphate oxidase-p-aminophenazone kit from Boehringer Mannheim (BM 701912), which is not influenced by free glycerol (28). 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, Centers for Disease Control, Atlanta, GA. Mean bias for sera from the Centers for Disease Control was +0.2 percent for total cholesterol,  $-0.3$  percent for HDL cholesterol, and  $-0.4$  percent for triglycerides. Serum albumin was measured with the modified bromocresol green dye binding method of Robertson (29) calibrated with human serum albumin (Sigma A6019, Sigma Chemical Co., St. Louis, MO) and standardized with World Health Organization reference serum obtained from the Central Laboratory for Blood Transfusion, Amsterdam, The Netherlands. All these analyses were performed on an Abbott ABA 200 bichromatic analyser (Abbott Laboratories, N. Chicago, IL). C-reactive protein was measured by Dr. Pepys, Medical Research Council, London, using the EMIT method (Cyva Corporation, Palo Alto, CA). Subjects with C-reactive protein levels above 10 mg/liter or albumin levels less than 36 g/liter were excluded, because such values are indicative of subclinical infection and malnutrition, respectively (table 1). C-reactive protein is considered superior to erythrocyte sedimentation rate as an indicator of infective and other disease states and is more easily standardized (30).

The presence of chylomicrons was assessed both by electrophoresis on cellulose acetate strips (Cellologel 01A52-25, Chemotron, Milan, Italy) and by microcentrifugation in hematocrit tubes (31), which appeared to be much more sensitive. Results for triglycerides were analyzed separately if chylomicrons were detected by either method, and also if the subject admitted having eaten anything since the previous evening (table 2, last column). LDL cholesterol was calculated by means of the Friedewald equation (32). One subject was excluded because of familial hypercholesterolemia.

Of the 58 subjects from Benin, 27 boys were dropped because their birth dates had

not been reported. Another seven were excluded because their serum concentration of C-reactive protein exceeded 10 mg/liter, indicating infection or other illness, and one because of a serum albumin level below 36 mg/liter. Of the 88 Tanzanian boys, seven were not eligible because they were too young or too old, four had a low albumin, nine had elevated levels of C-reactive protein, and three had both. In the other countries, the number of boys not eligible was quite modest. In agreement with our previous findings (16), albumin levels of eligible boys from Africa and Asia were slightly lower than those of European boys.

Food consumption data were obtained mostly from recent surveys of children in the countries involved. In the boys in Crete, actual consumption was measured by a 2-day record (24). For Finland, Italy, The Netherlands and The Philippines, food intake was determined by us in very similar cohorts of 114–133 boys 3 years previously (16) using either the record or recall method over a period of 7 days. For Benin, data from our cohort in the neighboring country of Ghana (16) were used in the absence of other data. Data for other countries were taken from studies of similar populations: in the Federal Republic of Germany, using a record method over at least 7 days in a study of 116 boys aged 8 and 9 years in Dortmund (18); in Hungary, a 24-hour dietary recall method in a study of 11 boys and girls aged 8–10 years in Budapest (E. Greiner, State Institute for Nutrition Sciences, Budapest, personal communication, 1986); in Kenya, a 48-hour record method to measure the intake of 65 boys and girls aged 7–12 years in the Machakos area in 1979 (19, 20); in Poland, a 24-hour dietary recall method in a study of about 1,200 boys and girls aged 11–15 years in Warsaw in 1981 (21); and in Portugal, a 24-hour dietary recall method in a study of about 100 boys aged 7–10 years in Lisbon in 1984 as part of the Portuguese nutritional survey (I. Martins, Institut Nacional de Saude, Lisbon, personal communication, 1986). For Tanzania, data from the neigh-

boring country of Kenya were used (19, 20). The nutritional and lipid data for the United States were taken from the Lipid Research Clinics Prevalence Study (22, 33).

The relation of the lipoprotein concentrations with the dietary variables were examined by linear regression using the *Statistical Package for the Social Sciences* (35). We did not calculate correlation coefficients or proportions of variance explained by diet, because the deliberate selection of countries with contrasting intakes would tend to inflate these statistics. Regression coefficients, however, are unaffected by this mode of sampling.

## RESULTS

### *Population characteristics*

Table 1 shows the countries studied, and the contributions of carbohydrate and fat to the diets as a percentage of total daily intake. In four countries, Kenya and Tanzania in East Africa, Benin in West Africa, and the Philippines in Southeast Asia, the proportion of fat in the diet was extremely low, ranging from 12 to 22 percent of energy. In the other nine countries, fat intake ranged between 28 and 45 percent. Table 1 also shows the total numbers of boys seen per country, the number meeting the criteria for participation ("Eligible"), and the number who were reported to be fasting and in addition passed both biochemical tests for the presence of chylomicrons. Data for the United States were taken from published data sources (22, 33).

Table 2 presents the average height, weight, and body mass index for the boys by country. Published data from the North American Lipid Research Clinics Prevalence Study (22, 33) are also included. We felt that this was justified because the standardization employed ensured that lipid levels measured by us were comparable with those produced by the Lipid Research Clinics (34, 36).

Boys from Africa and the Philippines were shorter and lighter than the boys from Europe. Their average weight for height

TABLE 1

Numbers of boys in the study, contribution of energy derived from carbohydrate and fat, and concentrations of albumin and C-reactive protein in serum in boys in 13 countries\*

Country	No. of boys†			Contribution to energy (%)‡		Albumin§ (mg/liter)	C-reactive protein§ (mg/liter)
	Total	Eligible	Negative	Carbohydrate	Fat		
Benin	58	23	11	68	22	40.6 ± 2.3	2.3 ± 2.5
Finland	59	57	56	50	37	45.3 ± 1.8	3.0 ± 1.4
Federal Republic of Germany	47	46	45	48	40	44.5 ± 3.0	1.0 ± 0.0
Greece	97	87	80	44	45	43.4 ± 2.4	2.2 ± 1.8
Hungary	49	42	41	50	34	46.2 ± 1.9	4.0 ± 0.0
Italy	47	44	17	57	28	43.9 ± 2.0	1.1 ± 0.4
Kenya	60	51	13	75	12	41.1 ± 2.8	2.8 ± 1.9
The Netherlands	56	55	54	49	38	44.1 ± 2.9	2.1 ± 0.6
The Philippines	69	62	37	72	16	41.5 ± 2.4	1.7 ± 0.9
Poland	57	57	54	52	37	43.6 ± 1.9	3.7 ± 0.9
Portugal	32	27	26	58	29	44.7 ± 2.9	1.2 ± 0.6
Tanzania	88	65	39	75	12	42.4 ± 2.3	2.1 ± 1.6
United States	-	146	148	50	37	-	-

\* Data, except those for the United States, are for 719 boys studied at primary schools in 12 countries. These studies were carried out in 1984 and 1985, except for the study in Greece, which was done in 1986.

† Total, no. of boys from whom blood was taken; eligible, excluding boys outside age range 91–125 months or with serum C-reactive protein levels above 10 mg/liter or albumin levels less than 36 g/liter; negative, eligible boys in whose serum no chylomicrons could be detected by any of the methods employed.

‡ For sources of dietary data, see Materials and methods.

§ Eligible boys only.

|| Data for the United States are taken from the Lipid Research Clinics Prevalence Study performed between 1972 and 1976 (white males, visit 2 random sample, aged 5–9 years (22, 33)). The numbers of eligible and negative boys refer to the numbers for whom data are given for the plasma concentration of cholesterol and triglycerides, respectively. No data were available for albumin and C-reactive protein.

ranged from 90.2 percent of the Harvard standard (37) for the Tanzanian boys to 97.3 percent for the Filipino boys. Thus, they were thinner than boys from affluent countries, but they could not be considered malnourished.

Table 2 also presents the mean lipid and lipoprotein values per country. Total cholesterol and HDL cholesterol values agreed well with those found in our two previous international studies, 3 and 6 years earlier (15, 16). The trend in LDL cholesterol values was very similar to that in total cholesterol, with the highest values being found in Finland and the lowest in the non-European countries.

Serum triglycerides were highest in the boys from the four developing countries in Africa and Asia. This tendency was undiminished if every serum sample that might have contained traces of chylomicrons was eliminated (table 2, last column).

There was a positive association between LDL cholesterol and body mass index between countries (regression coefficient or slope ± standard error (SE)  $0.22 \pm 0.08$  mmol/liter per  $\text{kg}/\text{m}^2$ , significantly different from 0,  $p = 0.017$ ). The associations of HDL cholesterol and triglycerides with body fatness were opposite to those usually seen *within* populations: the univariate regression coefficient with body mass index ( $\pm$  SE) was  $0.16 \pm 0.04$  mmol/liter per  $\text{kg}/\text{m}^2$  for HDL cholesterol and  $-0.07 \pm 0.03$  for triglycerides ( $n = 13$ ; all regression coefficients significantly different from 0 at the  $p < 0.05$  confidence limit).

#### Associations of serum lipids with diet

Figures 1 and 2 show the relation of the levels of fasting triglycerides, HDL cholesterol, and LDL cholesterol with carbohydrate and fat consumption. Fasting triglyc-

TABLE 2  
*Anthropometric data and serum lipids and lipoproteins in boys in 13 countries\**

Country	Height (cm)	Weight (kg)	Body mass index (kg/m <sup>2</sup> )	Serum cholesterol (mmol/liter)			Serum triglycerides (mmol/liter)	
				Total cholesterol	HDL cholesterol	LDL cholesterol	All subjects	Negative only
Benin	124.5 ± 1.2	22.8 ± 0.6	14.7 ± 0.2	3.03 ± 0.13	0.81 ± 0.06	1.88 ± 0.12	0.75 ± 0.06	0.58 ± 0.06
Finland	135.3 ± 0.9	31.4 ± 0.8	17.0 ± 0.3	5.07 ± 0.11	1.54 ± 0.04	3.21 ± 0.10	0.70 ± 0.04	0.66 ± 0.04
Federal Republic of Germany	139.2 ± 0.9	31.9 ± 0.8	16.4 ± 0.3	4.72 ± 0.10	1.48 ± 0.04	2.95 ± 0.09	0.61 ± 0.03	0.59 ± 0.03
Greece	133.4 ± 0.7	32.2 ± 0.6	18.0 ± 0.3	4.43 ± 0.07	1.39 ± 0.03	2.77 ± 0.07	0.61 ± 0.04	0.59 ± 0.04
Hungary	137.9 ± 0.8	30.9 ± 0.8	16.2 ± 0.3	4.68 ± 0.11	1.46 ± 0.03	2.91 ± 0.10	0.70 ± 0.04	0.66 ± 0.04
Italy	137.2 ± 1.1	35.9 ± 1.4	18.8 ± 0.5	4.47 ± 0.10	1.44 ± 0.04	2.76 ± 0.08	0.58 ± 0.03	0.50 ± 0.03
Kenya	123.8 ± 0.8	22.9 ± 0.4	14.9 ± 0.1	2.94 ± 0.08	0.79 ± 0.03	1.66 ± 0.06	1.08 ± 0.06	0.81 ± 0.07
The Netherlands	139.7 ± 0.8	31.0 ± 0.6	15.8 ± 0.2	4.34 ± 0.08	1.35 ± 0.03	2.72 ± 0.07	0.60 ± 0.04	0.56 ± 0.03
The Philippines	123.8 ± 0.9	23.8 ± 0.5	15.4 ± 0.2	4.09 ± 0.08	0.98 ± 0.03	2.73 ± 0.08	0.85 ± 0.04	0.77 ± 0.04
Poland	134.7 ± 0.9	31.3 ± 0.9	17.1 ± 0.3	4.53 ± 0.10	1.31 ± 0.04	2.88 ± 0.09	0.74 ± 0.04	0.73 ± 0.04
Portugal	133.1 ± 1.1	30.3 ± 1.0	17.0 ± 0.4	4.44 ± 0.09	1.38 ± 0.06	2.79 ± 0.08	0.58 ± 0.04	0.56 ± 0.03
Tanzania	125.7 ± 0.6	22.8 ± 0.3	14.4 ± 0.1	3.54 ± 0.09	0.90 ± 0.03	2.28 ± 0.08	0.81 ± 0.04	0.72 ± 0.04
United States†	127.2 ± 0.7	26.1 ± 0.4	16.0 ± 0.2	4.01 ± 0.05	1.44 ± 0.03	2.39 ± 0.05	0.59 ± 0.02	0.59 ± 0.02

\* Means ± standard errors for all eligible subjects. For triglycerides, values are also given for boys who showed no evidence of chylomicrons with any of the tests used (*negative*). Data, except for those for the United States, are for 616 boys studied at primary schools in 12 countries. These studies were carried out in 1984 and 1985, except for the study in Greece, which was done in 1986.

† Data for the United States are taken from the Lipid Research Clinics Prevalence Study (22, 33) performed between 1972 and 1976, and refer to 146 white males aged 5-9 years. Values for cholesterol and triglycerides are for plasma.

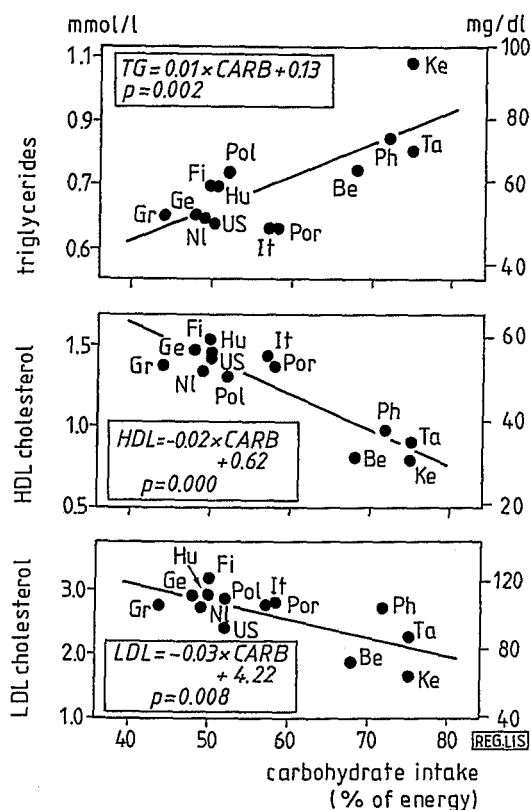


FIGURE 1. Relations between proportion of daily energy obtained from carbohydrate (CARB) and the concentration in serum of triglycerides (TG), high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol in boys from 13 countries. The lipid data were obtained for 616 boys studied at primary schools in 1984 and 1985 (Greece, 1986). The values represent means of all eligible boys per country. The data on serum lipids for the United States are taken from the Lipid Research Clinics Prevalence Study and were obtained between 1972 and 1976 (146 white males, visit 2 random sample, aged 5-9 years (33)). The regression lines and equations give the lipid level, expressed in mmol/liter, as a function of the carbohydrate intake, expressed in percent of total daily caloric intake. To convert mmol/liter into mg/100 ml, multiply cholesterol values by 38.7 and triglyceride values by 88.5. Abbreviations: Be, Benin; Fi, Finland; Ge, Federal Republic of Germany; Gr, Greece; Hu, Hungary; It, Italy; Ke, Kenya; NL, The Netherlands; Ph, The Philippines; Pol, Poland; Por, Portugal; Ta, Tanzania; US, United States.

erides were higher in countries with high-carbohydrate, low-fat diets where low serum cholesterol levels were also found. HDL cholesterol and LDL cholesterol were both negatively related to the proportion of

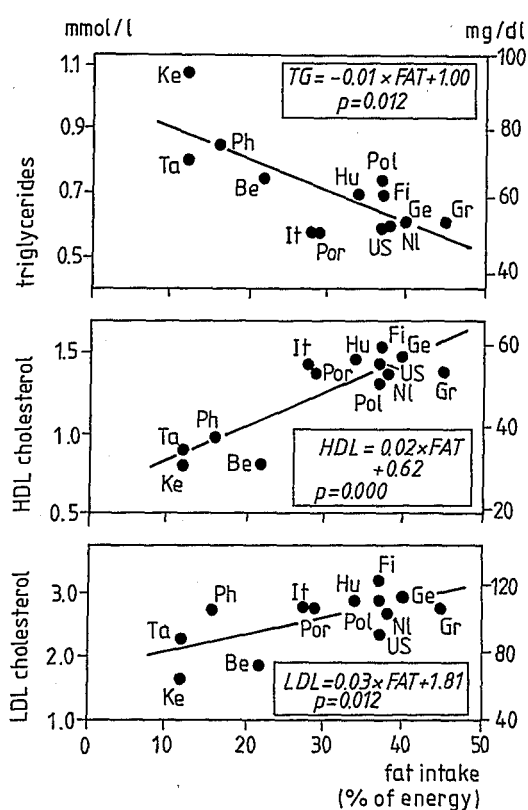


FIGURE 2. Relations between proportion of daily energy obtained from fat and the concentration in serum of triglycerides (TG), high density lipoprotein (HDL) cholesterol, and low density lipoprotein (LDL) cholesterol in boys from 13 countries. The lipid data were obtained for 616 boys studied at primary schools in 1984 and 1985 (Greece, 1986). The values represent means of all eligible boys per country. The data on serum lipids for the United States are taken from the Lipid Research Clinics Prevalence Study and were obtained between 1972 and 1976 (146 white males, visit 2 random sample, aged 5-9 years (33)). The regression lines and equations give the lipid level, expressed in mmol/liter, as a function of the fat intake, expressed in percent of total daily caloric intake. To convert mmol/liter into mg/100 ml, multiply cholesterol values by 38.7 and triglyceride values by 88.5. Abbreviations: Be, Benin; Fi, Finland; Ge, Federal Republic of Germany; Gr, Greece; Hu, Hungary; It, Italy; Ke, Kenya; NL, The Netherlands; Ph, The Philippines; Pol, Poland; Por, Portugal; Ta, Tanzania; US, United States.

energy from carbohydrate and positively related to the proportion of energy from fat in the diet (figure 1). The regression coefficient or slope ( $\pm$  SE) for the association with carbohydrate intake was  $-0.022 \pm$

0.003 mmol/liter for each percent of energy for HDL cholesterol,  $-0.028 \pm 0.009$  for LDL cholesterol, and  $0.010 \pm 0.002$  for triglycerides (all slopes significantly different from 0 at the  $p < 0.01$  level). For the association with fat intake, the regression coefficients (slopes) were  $0.021 \pm 0.004$  mmol/liter per percent of energy for HDL cholesterol,  $0.027 \pm 0.009$  for LDL cholesterol, and  $-0.010 \pm 0.003$  for triglycerides (all coefficients significantly different from 0 at the  $p < 0.05$  confidence level). For some countries, the microcentrifugation test (31) suggested the presence of chylomicrons in the serum of a large proportion of subjects (table 1). We found later that frozen storage, as employed here during and after transport from the field, induced a positive microcentrifugation test in sera that had been found to be negative before freezing. The reverse (sera that did contain chylomicrons testing negative after freezing) was never seen (L. Kilsdonk, et al., Dept. of Human Nutrition, Agricultural University, Wageningen, personal communication, 1987). However, even after elimination of the triglyceride values of all boys who tested positive by the microcentrifugation test, the association of triglycerides with carbohydrate intake was still clearly present; the univariate regression coefficient ( $\pm$  SE) between the mean serum triglyceride concentration and the percentage of energy from carbohydrates now was  $0.005 \pm 0.002$  mmol/liter per percent of energy ( $p < 0.05$ ,  $n = 13$ ).

#### DISCUSSION

Our results show that healthy school boys aged 8 and 9 years from countries in Africa and Asia, with a high carbohydrate intake, have higher fasting serum triglyceride levels than boys from more affluent countries. The question is whether we should interpret this association as being causal, or whether it is a chance association due to one of the many other differences between developed and developing countries.

#### *Possible confounders*

The following possible confounders deserve consideration.

*Sample handling, transportation, and analysis.* The field work in Africa and Asia was performed by trustworthy colleagues, using tubes, needles, etc., supplied by us. As far as we could determine, the freezing chain from the field to the laboratory in Wageningen was unbroken. Also, separate tests (unpublished data) showed that repeated freezing and thawing of samples caused no change in triglyceride values. Thus, storage and handling of sera do not explain the differences in triglycerides between affluent and developing countries. Analytic bias was eliminated by central analysis. Samples arrived in random order, and accuracy was strictly controlled throughout.

*Compliance with fasting.* A major concern in setting up this new study was to obtain sera that were totally free from chylomicrons. In retrospect, uniform poor compliance with the requirements for fasting would have tended to reduce the difference in triglyceride values between Europe and Africa/Asia rather than artefactually produce it, because breakfasts in developing countries contain very little fat. The microcentrifugation test employed by us was so stringent that no serum with even a trace of chylomicrons would pass. On the other hand, later tests (L. Kilsdonk, et al., personal communication, 1987) showed that, after frozen storage, quite a few bona fide fasting sera would test positive, especially those with higher triglyceride concentrations. This may be one reason why the number of samples testing positive was higher in the tropical countries: fasting levels were higher there. Thus, the true fasting triglyceride levels probably lie between the values given in table 2 for negative subjects and the values for all eligible subjects. Because both values were significantly higher in the high-carbohydrate populations, any



intermediate value would also be significantly higher.

*Diseases and malnutrition.* Subjects with intercurrent or chronic diseases were eliminated by a medical examination plus determination of C-reactive protein, an acute-phase protein considered to be a good indicator of subclinical infection and inflammation, and of albumin, which is considered to be a general indicator of protein-energy malnutrition. Although less well-fed than their European counterparts, the eligible boys from the tropics were in good condition. Indeed, except for times of natural or man-made disasters, malnutrition tends to be present mainly in infants and toddlers, and those children who live through this earlier period unscathed are usually reasonably well nourished (37).

*Body fatness and activity.* The boys from the tropics, although not malnourished, were definitely thinner than the boys from affluent countries. Our earlier study (16) showed that they are also slightly more active. Both factors would tend to counteract the high triglycerides and low HDL cholesterol levels found here. Thus, if the boys from the high-carbohydrate populations had had the same body mass indexes as those of the populations with a high fat intake, their triglycerides would probably have been even higher.

*Genetic factors.* We have no figures for the prevalence of genetic hypertriglyceridemia in Africa and Asia. Black boys in North America, whose ancestors probably came largely from Africa, have slightly lower rather than higher triglycerides than white North American boys (33, tables 13 and 17). This makes a racial explanation somewhat less plausible.

*Secondary hypertriglyceridemia.* Several metabolic abnormalities, such as diabetes and nephrotic disease, can cause hypertriglyceridemia. In the absence of a full clinical examination, some such cases might have escaped the attention of the examining physician. However, these diseases are quite rare in childhood, and the higher

mean triglycerides in the African and Asian boys were caused by a mild elevation across the board rather than by sporadic cases with pathologically high values.

*Bias in the dietary data.* The dietary data were not obtained at the same time from exactly the same samples of boys as the lipid values. However, the differences in diet composition and serum lipids between the countries studied here are so large that small differences in time or in region sampled will not cause marked changes in the position of a country in the distribution of lipid or diet values, when viewed internationally. For instance, in Finland there has been an intensive, and successful, campaign to change the diet, and through it, the lipid levels of the population. Nevertheless, the Finnish boys still topped our list of serum total and LDL cholesterol levels (table 2), just as they did in our first study in 1978 (15).

#### *Carbohydrates and serum triglycerides*

The most plausible interpretation of our data appears to be that differences in the proportion of carbohydrates in the diet were responsible for the difference in triglyceride levels between boys from Europe and boys from tropical countries.

This conclusion is in agreement with results from controlled experiments (4-6, 17), and it suggests that at least part of the carbohydrate-induced elevation of triglycerides seen in controlled trials is permanent and not temporary, as described in other studies (7, 8). Our findings are also consistent with other recent epidemiologic data. In Israel, Rubinstein et al. (38) studied new immigrant Ethiopian Jews (Falashes), who probably had a high-carbohydrate diet. The Falashy boys had higher triglycerides and lower HDL cholesterol and LDL cholesterol levels than resident Israeli children. Wiedermann et al. (39) compared the Bambara, a tribe from Mali in West Africa that obtains 80 percent of its energy from millet, with a matched healthy Viennese population. Again, the African children had higher

triglycerides. In both studies, the reverse was seen at older ages, with the adults from the more affluent populations showing higher triglycerides than the African adults; the latter were also much leaner. We have suggested elsewhere (17) that differences in HDL cholesterol between adult populations in various parts of the world are determined by a balance between, on the one hand, the dietary fuel mix (with fat raising and carbohydrates depressing HDL cholesterol), and, on the other hand, the extent of caloric excess and the accompanying degree of obesity and lack of activity (which both lower HDL cholesterol). If a similar balance holds for triglycerides, then the triglyceride-elevating effect of a higher body mass index evidently overrode the triglyceride-lowering effect of a lower carbohydrate intake in the adult Israelis (38) and Viennese (39) compared with their African counterparts.

#### *Implications for coronary heart disease risk*

Formal proof is still lacking that manipulation of HDL cholesterol or serum triglyceride levels through diet or drugs will influence the risk for coronary heart disease. However, evidence from a variety of sources does suggest that either or both are causally related to coronary heart disease. HDL cholesterol is a strong predictor of coronary heart disease risk in epidemiologic studies (40, 41). Risk is high in people with genetically low HDL cholesterol levels (42). In clinical trials, the change in HDL cholesterol levels caused by drug treatment was predictive of changes in atherosclerosis progression (43) and of incidence of coronary heart disease (44, 45) independent of changes in LDL cholesterol. Although the status of serum triglycerides as an independent risk factor is still not established, the combination of high levels of triglycerides with low levels of HDL cholesterol in adults is associated with a high risk of coronary heart disease (46). In the present ecologic comparison, the opposite relation was found in children: boys from populations

with a low coronary heart disease mortality had high triglycerides and low HDL cholesterol levels. As the children grow up, inter-population differences in HDL cholesterol and triglycerides may decrease because factors such as obesity and lack of physical activity may work to increase triglycerides and reduce HDL cholesterol in affluent populations. Nevertheless, even in adult men, HDL cholesterol levels are still somewhat lower in developing countries with a low rate of coronary heart disease than in affluent populations (17, 47). In such countries, however, both boys and adult men also have lower LDL cholesterol and total cholesterol levels (see figure 1 and reference 47). Possibly, the favorable effect of a low LDL cholesterol concentration caused by low-fat diets overrides the detrimental effects of low HDL cholesterol and high triglyceride concentrations induced by the same diets. Alternatively, undefined other factors may be responsible for the low rates of coronary heart disease in tropical countries.

Theoretically, replacement of saturated fats by unsaturated oils rather than by carbohydrate-rich foods might lower total cholesterol levels without lowering HDL cholesterol or elevating triglyceride levels (48-50). One must guard against the obesity-promoting effects of such diets (51), which could negate their favorable effects on HDL cholesterol and triglycerides. However, our data do support the notion (4, 52) that high-carbohydrate diets may cause adverse changes in triglycerides and HDL cholesterol in patients with forms of hypertriglyceridemia not caused by obesity. For such patients, a slightly more liberal allowance of dietary oils may produce a more favorable lipoprotein pattern than a diet in which total fat intake is limited to only 20 percent of daily energy intake.

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