Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 1449–1453, March 1983
Medical Sciences

Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia

(triglycerides/chylomicrons/apolipoproteins/exercise/fat tolerance)

JOSEF R. PATSCH, JOAN B. KARLIN, LYNNE W. SCOTT, LOUIS C. SMITH, AND ANTONIO M. GOTTO, JR.

Department of Medicine, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030

Communicated by Donald S. Fredrickson, November 12, 1982

ABSTRACT **Triglyceridemic response to a standard oral fat meal was determined in 28 healthy subjects and related to the levels of several lipids, lipoproteins, and apolipoproteins in the postabsorptive plasma. A fatty test meal was administered orally, and postprandial plasma triglyceride levels were determined. In the fasting blood samples, concentrations of apolipoproteins (apo) A-I, A-II, and B were determined by radioimmunoassay, and those of high density lipoprotein (HDL) subfractions HDL_2 and HDL_3, by zonal ultracentrifugation. The magnitude of triglyceridemic response showed a negative correlation with the plasma levels of HDL_2 ($r = -0.860$, $P < 0.001$), HDL-associated cholesterol ($r = -0.605$, $P < 0.001$), and apoA-I ($r = -0.459$, $P < 0.02$). No correlation was found between the triglyceridemic response and the levels of total cholesterol, HDL_3, and apoA-II. Triglyceridemic response was correlated positively with fasting triglyceride concentrations ($r = 0.450$, $P < 0.02$) and apoB levels ($r = 0.396$, $P < 0.03$). In two subjects followed for 3 yr, when HDL_2 levels rose or fell, the triglyceridemic response decreased or increased, respectively ($r = -0.944$; $r = -0.863$). Our data indicate that normolipidemic individuals with high HDL_2 levels in the plasma are able to clear alimentary fat at a faster rate than normolipidemic subjects with low HDL_2 levels. The pronounced difference in severity and duration of postprandial lipemia among subjects with varying HDL_2 levels may help to explain the negative correlation between the risk of atherosclerosis and HDL cholesterol levels.**

Atherosclerosis is the major underlying cause of death and disability in western society. Virtually all epidemiologic studies to date have shown that enhanced serum cholesterol values at the time of entry into the study are predictors for the development of coronary heart disease (1). The importance of triglyceride as a risk factor is currently a matter of dispute (2, 3). Increases of low density lipoproteins (LDL) predispose to premature coronary heart disease (4), whereas the high density lipoproteins (HDL) have been found to have a powerful inverse correlation with coronary heart disease (5–7). Although a causal relationship can be established both in man and in experimental animals for increases of LDL and atherosclerosis (8), none have yet been established for HDL.

HDL consist of two major subfractions, HDL_2 and HDL_3 (4). The former lipoproteins are larger in size, contain more lipid, and have a lower density (9). HDL_2 contain predominantly apolipoprotein A-I (apoA-I), with relatively little apoA-II, whereas HDL_3 contain appreciable quantities of both of these proteins (10–12). The most striking difference in plasma lipoproteins between males and females lies in the levels of HDL_2, which are roughly 3 times higher in females than in males (4, 8). In hyperalphalipoproteinemia, a familial condition that provides protection against coronary heart disease (13), the primary increase occurs in HDL_2 (14). Physical exercise increases the concentrations of HDL_2, but not of HDL_3 (15).

The metabolism of HDL and HDL_2/HDL_3 ratio are intimately related to the catabolism of the triglyceride-rich lipoproteins, the chylomicrons and very low density lipoprotein (VLDL). In studies carried out *in vitro* (16), in laboratory animals (17, 18), and in man (19), evidence strongly indicates a transfer of surface components from the triglyceride-rich lipoproteins to HDL during triglyceride lipolysis by the enzyme lipoprotein lipase. As the triglyceride core of these particles is hydrolyzed, an excess of surface components is generated containing apoprotein, unesterified cholesterol, and phospholipid. These surface components are thought to be transferred to HDL, thereby increasing the total mass of this class of lipoproteins and effecting a transformation of HDL_3 to HDL_2 (16). A direct *in vivo* experiment in the chicken shows that specific blockage of lipoprotein lipase causes accumulation of VLDL, followed by a decrease of HDL particles, which become smaller and denser (20). According to this concept, HDL_2 would be viewed as one of the end products of the utilization of the triglyceride-rich lipoproteins (16, 21). Thus, the catabolism of triglyceride-rich lipoproteins would serve as a determinant of HDL concentrations and of the HDL_2/HDL_3 ratio.

The purpose of the present study was to determine the association of high levels and low levels of HDL_2, in comparison with HDL_3, with the ability to clear dietary fat from the circulation. We now report that individuals with a high concentration of HDL_2 in their postabsorptive plasma have an increased ability to clear a standard fat load from the circulation, compared to individuals with low levels of HDL_2. Because the major part of a lifetime is spent in the postprandial state, (i.e., the time period between food ingestion and 6–8 hr thereafter), we reason that studies in the fasting state might not necessarily provide the most appropriate information to assess the metabolism of the triglyceride-rich lipoproteins. We observed also that fat tolerance in a given subject can be altered in concert with the subject's levels of HDL_2.

METHODS

Subjects. Twenty-eight healthy volunteers, 23 men and 5 women, ranging in age from 28 to 42 years, were included in this study. Cholesterol and triglyceride levels in postabsorptive plasma were within normal limits, and the weights of most subjects were within ideal body weight range (see Table 1). No subject took

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); apoA-I, apoA-II, apoB, and apoC, apolipoprotein constituents of human plasma lipoproteins.

medication or had clinical or biochemical evidence of cardiac, hepatic, renal, or endocrine disease. All volunteers, except one, were nonsmokers.

Laboratory Procedures. Cholesterol, triglyceride, and HDL-associated cholesterol in plasma were quantified by Lipid Research Program procedures (22). Concentrations of HDL_2 and HDL_3 in postabsorptive plasma were estimated by using a zonal ultracentrifugal procedure (9). Rotor fractions under the HDL_2 and HDL_3 peaks were pooled and analyzed for lipid phosphorus (23), for triglycerides by using Triglyceride C-37 Rapid Stat (Pierce), for cholesterol and cholesteryl esters by using the Boehringer Mannheim enzymatic method (24), and for protein with bovine serum albumin as standard (25). HDL_2 and HDL_3 plasma levels were calculated from the sum of the individual chemical components (9). Cholesterol recovered from the two zonally isolated HDL fractions averaged 94.3% of HDL cholesterol. ApoA-I, apoA-II, and apoB were quantitated by respective double-antibody radioimmunoassay procedures (26–28). Total fecal fat was quantified by the Van de Kamer titrimetric method (29).

Test Meal. The test meal consisted of 350 ml of heavy whipping cream (39.5% fat), two tablespoons of chocolate-flavored syrup, one tablespoon of granulated sugar, and one tablespoon of instant nonfat dry milk. Of the 1,362 calories, 2.8% were derived from protein (9.5 g), 14% from carbohydrate (48 g), and 86% from fat (130 g). Cholesterol content was 480 mg, and the ratio of polyunsaturated/saturated fat was 0.059. Ingredients were mixed but not blended so as to obtain a readily drinkable liquid. The test meal was administered per 2 m^2 of body surface to adjust adequately for major variations in blood volume (30).

Fat Load Procedure. Each individual came as an outpatient in the morning between 8 and 9 a.m. after a 14-hr fast subsequent to ingestion of a light supper and no more than 30 g of ethanol. About 50 ml of blood was collected in EDTA for immediate lipoprotein and lipid analyses from plasma, and 10 ml without anticoagulant, for radioimmunoassay procedures and lipoprotein electrophoresis with serum. Immediately after the postabsorptive blood specimen was drawn (0-hr sample), the test meal was ingested (within 5 min). Subsequent blood sampling (about 10 ml of blood) was performed at 2, 4, 6, and 8 hr after ingestion of the fat meal. During this 8-hr period, only consumption of water or two cups of uncreamed and unsweetened tea or coffee was allowed. The test meal was tolerated well, and no subject reported nausea, vomiting, diarrhea, or signs of gross steatorrhea. In seven subjects with widely varying triglyceridemic response, three consecutive 24 hr stool samples were collected from the time of test meal intake until 72 hr thereafter and were analyzed for total fat. In the first 24 hr, fecal fat ranged from 3.4 to 4.2 g; in the second 24 hr, from 6.3 to 7.9 g; and in the third 24-hr period, from 2.7 to 3.5 g. For the entire 72-hr period after the fat load, fecal fat ranged from 12.9 g to 15.2 g and was not correlated with the magnitude of triglyceridemic response.

Data Analysis and Reduction. Triglyceride levels in the 0-, 2-, 4-, 6-, and 8-hr plasma specimens were plotted (see Fig. 1B). The magnitude of triglyceridemic response to the fat meal was quantified in two ways. (*i*) The area under the triglyceride values was calculated as defined by two lines—i.e., one connecting the individual triglyceride values and one originating at the 0-hr level parallel to the abscissa. This area (mg/dl of plasma·8-hr triglyceride area) was calculated by the trapezoidal rule (31) normalized to the 0-hr level. (*ii*) The increase of triglyceride levels from the 0-hr sample to the mean of the two highest triglyceride values observed postprandially was calculated according to

$$\text{triglyceride increase (mg)} = \frac{n_{max} + n_{2nd}}{2} - n_0$$

where n_{max} is the highest, n_{2nd} the second highest, and n_0 the 0-hr triglyceride value. Linear regression analysis was performed using PROPHET, an interactive network computer system with statistical capability, subsidized by the National Institutes of Health (32).

RESULTS

Lipemia and Levels of Plasma Lipoprotein Components. The triglyceridemic responses of two study subjects, one with low and the other with a high concentration of HDL_2, are shown in Fig. 1. HDL_2 and HDL_3 from the postabsorptive or fasting plasma are given in Fig. 1*A* and are contrasted with the postprandial triglyceride values in Fig. 1*B*. The triglyceridemic response was very pronounced in the individual with a low level of HDL_2 (Fig. 1*A Upper* and Fig. 1*B*), whereas it was minor in the subject with a high concentration of HDL_2 (Fig. 1*A Lower* and Fig. 1*B*). Two methods for quantification of triglyceridemic response were (*i*) the area under the triglyceride curve and (*ii*), the magnitude of the triglyceride increase. The results were very similar with either method of quantification ($r = 0.960$). Because of the high degree of correlation, for further regression analyses, only the area under the triglyceride curve (method A) was used.

The concentrations of lipids, apolipoproteins, HDL_2, and HDL_3 in the postabsorptive plasma and the triglyceridemic response to the fatty test meal in the 28 subjects are summarized in Table 1. The magnitude of the triglyceridemic response was inversely related to the fasting plasma concentration of apoA-I, HDL-associated cholesterol, and HDL_2. The correlation was strongest for HDL_2 (Fig. 2). No significant correlations existed between the triglyceridemic response and the fasting concentration of cholesterol, apoA-II, or HDL_3. A significant positive

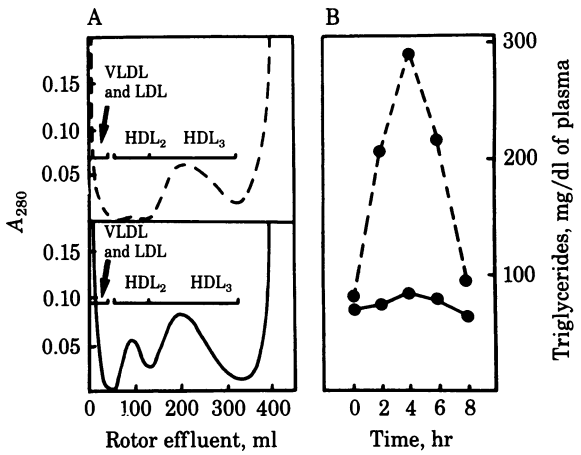

FIG. 1. (*A*) Isolation of HDL_2 and HDL_3 from the postabsorptive plasma of two study subjects by zonal ultracentrifugation. Fractions pooled for chemical analysis and quantitative estimation of HDL subfraction concentrations (9) are indicated. (*B*) Postprandial triglyceride (TG) levels after the ingestion of the test meal in the two study subjects. ---, TG values in the volunteer whose HDL subfraction distribution is shown in *A Upper*; the calculated triglyceridemic response was 876 mg/dl of plasma·8-hr TG area. ——, TG values of the volunteer whose HDL distribution is shown in *A Lower*; the triglyceridemic response was 70 mg/dl·8-hr TG area. The reproducibility of the fat load test when HDL levels were relatively constant was evaluated in three individuals and was 164 vs. 112, 366 vs. 382, and 1,023 vs. 972 mg/dl·8-hr TG area, respectively.

Table 1. Postabsorptive lipid, lipoprotein, and apolipoprotein levels and magnitude of triglyceridemic response in 28 study subjects*

	Levels, mg/dl of postabsorptive plasma								Triglyceridemic response†	
	Chol‡	TG‡	HDL-Chol	HDL_2	HDL_3	apoA-I	apoA-II	apoB	TG-area, mg/dl·8 hr	TG-increase, mg
Mean	183	78	56	54	212	147	33	96	527	209
± 1 SD	29	21	12	37	35	27	6	22	337	76
Range	146–248	43–121	34–86	5–143	142–310	109–228	23–52	68–151	45–1,110	15–282

Chol, total cholesterol; TG, triglyceride; HDL-Chol, high density lipoprotein cholesterol.

*The complete set of data for the 28 individual study subjects is available on request. Weight index (body weight/average body weight of adults) (33) was 0.91 ± 0.09 (mean ± 1 SD).

†For computation see *Methods*.

‡Plasma levels of these lipids were under the reported 90th percentile of normal age- and sex-matched subjects (34).

correlation existed between the magnitude of the triglyceridemic response and the fasting concentrations of apoB and triglyceride (Fig. 2). The triglyceridemic response showed a significant negative correlation with reported weekly running mileage ($r = -0.474$, $P < 0.02$) but did not correlate with relative body weight, age, or reported daily consumption of alcohol.

In this study, HDL_2 was correlated negatively with fasting triglyceride values ($r = -0.501$, $P < 0.01$). HDL_2 levels showed a positive correlation with HDL cholesterol ($r = 0.743$, $P < 0.001$) and with apoA-I ($r = 0.589$, $P < 0.002$) but not with apoA-II; and HDL_3 levels were correlated with apoA-II ($r = 0.427$, $P < 0.03$) but not with HDL cholesterol or apoA-I. The data are internally consistent in that, above a certain threshold level, the HDL cholesterol level is directly related to HDL_2 concentration (35). Increase of HDL_2 also increases apoA-I but not apoA-II, which is found predominantly in HDL_3 (10–12).

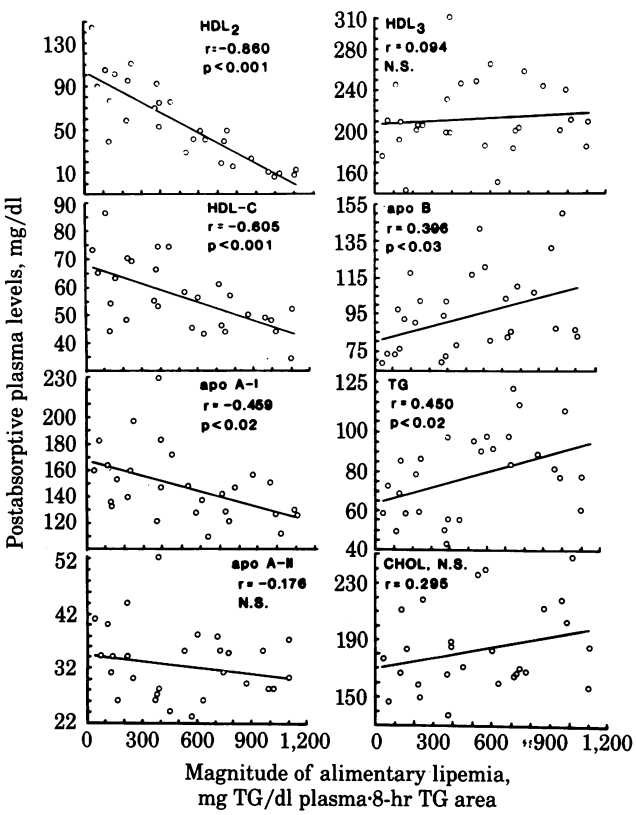

FIG. 2. Linear regression analyses comparing the postabsorptive plasma levels of HDL_2, HDL-associated cholesterol (HDL-C), apoA-I, apoA-II, HDL_3, apoB, triglycerides (TG), and total cholesterol (CHOL) (ordinates) with the magnitude of the triglyceridemic response (abscissae). N.S., not statistically significant.

Changes in HDL_2 Levels and Fat Tolerance. Two of our study subjects were subjected to rigorous changes in the level of physical exercise and were investigated repeatedly in a follow-up study over 3 years. The findings on one of the two volunteers are illustrated in Fig. 3. Subject J.V. was studied first in December 1978 and showed low average HDL cholesterol (46 mg/dl) and low HDL_2 levels (Fig. 3*A*, curve I). At this time, alimentary lipemia after the test meal was pronounced (Fig. 3*B*, curve I). Then, the volunteer undertook a program of physical activity, which consisted of running an increasing mileage and pace, as described in the legend to Fig. 3. HDL distribution was evaluated regularly at intervals of about 3 months. A definite increase in the subject's HDL cholesterol and HDL_2 levels was noticed first in June 1979, approximately 7 months after initial testing and onset of exercise (data not shown). By 1980, with continued exercise, HDL_2 levels increased further (Fig. 3*A*, curve II) at which time the triglyceride response to the fat meal was considerably attenuated (Fig. 3*B*, curve II). The subject continued his progressive physical exercise program until July 1981, when he developed a right inguinal hernia which required surgery. At this time, the volunteer's highest HDL_2 levels were recorded (Fig. 3*A*, curve III) and his lowest triglyceridemic response was observed and was confirmed in a fat-load test repeated after 5 days (Fig. 3*B*, curve III). Four and five weeks after cessation of exercise, HDL_2 levels had decreased drastically (Fig. 3*A*, curve IV). At this time, triglyceridemic response to the fat meal was pronounced (Fig. 3*B*, curve IV) and was similar to that recorded at the beginning of the trial. Resumption of running for 10 weeks resulted in elevation of HDL_2 (Fig. 3*A*, curve V) and in an improved fat tolerance (Fig. 3*B*, curve V). For this subject, HDL_2 levels and triglyceridemic responses recorded on seven occasions over 3 years showed a negative correlation that was statistically significant ($n = 7$, $r = -0.944$, $P < 0.002$). HDL cholesterol also showed a significant negative correlation ($r = -0.936$, $P < 0.002$). Correlations between alimentary lipemia and levels of apoA-I and fasting triglycerides did not reach statistical significance but showed regression lines similar to those observed for the entire aggregate of study subjects. Similar observations were recorded in the second subject (R.P.), who entered the study as a runner with high HDL_2 levels and was followed also from 1978 to 1982. A leg injury in 1980 and exploratory surgery on a testicle in 1981 forced complete temporary cessation of exercise, each followed by a marked decrease of HDL_2 levels within 4 weeks. In this study, triglyceridemic response—recorded on six occasions—showed also a significant negative correlation with HDL_2 levels ($n = 6$, $r = -0.863$, $P < 0.03$). With HDL cholesterol, the association did not reach the level of significance.

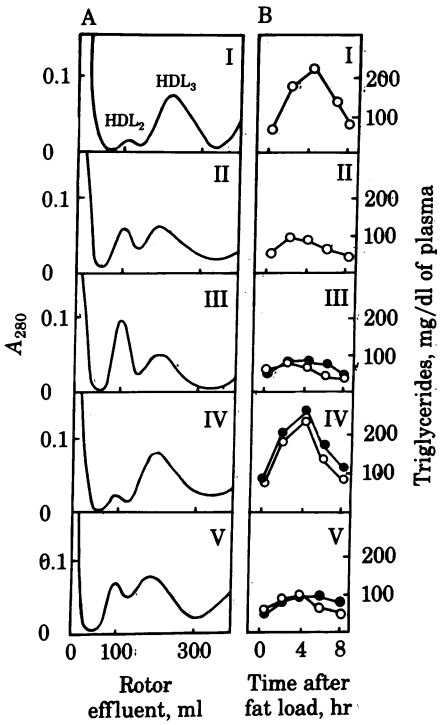

FIG. 3. Changes in HDL_2 levels and fat tolerance in a single subject during an observation period of 3 years. (*A*) HDL subfractions. (*B*) Corresponding triglyceridemic response. In December 1978, subject J.V. had a sedentary life style, with a HDL-associated cholesterol level of 46 mg/dl of plasma; HDL_2, 44 mg/dl; and HDL_3, 185 mg/dl (*A*, curve I); triglyceridemic response was 670 mg/dl·8-hr TG area (*B*, curve I). Volunteer J.V. started at this time a physical exercise program consisting of running 20–25 miles (1 mile = 1.61 km) per week. In July 1980, the exercise level was 30 miles of running per week at 7.5 min/mile. At this time, HDL cholesterol was 58 mg/dl; HDL_2, 95 mg/dl; and HDL_3, 178 mg/dl (*A*, curve II); triglyceridemic response was 192 mg/dl·8-hr TG area (*B*, curve II). Throughout spring and early summer of 1981, J.V. ran 40–45 miles/week at a pace of 6.5 min/mile. In July 1981, injury forced complete cessation of physical exercise. At the time of injury, HDL cholesterol was 76 mg/dl; HDL_2, 142 mg/dl; and HDL_3, 173 mg/dl (*A*, curve III); triglyceridemic response was 35 mg/dl·8-hr TG area (88 mg/dl·8-hr TG area when repeated after 4 days) (*B*, curve III). Four weeks after cessation of physical exercise, HDL cholesterol was 51 mg/dl and 7 days later was 46 mg/dl. At these respective times, the levels of HDL_2 were 45 and 36 mg/dl (*A*, curve IV) and of HDL_3 were 211 and 210 mg/dl, and the triglyceridemic responses were 534 (○) and 733 (●) mg/dl·8-hr TG area (*B*, curve IV). About 6 weeks after injury, the subject resumed running. In November 1981, the exercise level was ≈30 miles/week. In January 1982, HDL cholesterol had increased to 63 mg/dl, HDL_2 was 85 mg/dl, and HDL_3 was 198 mg/dl (*A*, curve V); triglyceridemic response was 221 mg/dl·8-hr TG area and, when repeated after 1 week, 184 mg/dl·8-hr TG area (*B*, curve V). During the entire observation period, the subject reported no changes in dietary habits, and alcohol intake was constant at 30 g/day. The volunteer's body weight was constant between 60.5 and 61.5 kg.

DISCUSSION

The rate of clearance of the triglyceride-rich dietary remnant particles at the endothelial surface has been postulated as a highly important factor in the development of atherosclerosis (36, 37). The work described here links the chylomicron remnant hypothesis with the known inverse correlation between the concentrations of HDL and coronary heart disease (5–7). The essence of our findings is that individuals with normal fasting plasma lipids but high levels of HDL_2 in their blood catabolize chylomicrons at a faster rate than do individuals with normal fasting lipids and low HDL_2 levels. The correlation between activity of lipoprotein lipase and levels of HDL-associated cholesterol (21) supports the hypothesis that the long-term level of triglyceride flux from chylomicrons and VLDL is one determinant of HDL concentrations in the blood.

A number of cautionary statements are appropriate. Although we have demonstrated a strong statistical relationship between dietary triglyceride clearance and HDL_2 levels in 28 normolipemic subjects, the selection of subjects could have introduced an element of bias. Many volunteers were members of the staff of the Division of Atherosclerosis and Lipoprotein Research at Baylor. Only five of the subjects were females because use of oral contraceptives precluded participation in the study. The findings in females, however, were consistent with those in males and the overall group. Our study subjects were of similar age, ranging from 28 to 42 years. This is also a point of consideration because some reports have noted a decline of fat tolerance with age (38). Most of our study subjects had a body weight index of less than 1.0, and we found no statistically significant correlation between the triglyceridemic response and relative body weight. However, two of three subjects with an index greater than 1.0 had high triglyceridemic response; these two subjects were sedentary and had low levels of HDL_2. The group of subjects studied varied considerably in reported levels of physical activity. Some were sedentary, others exercised on the weekend, others on a regular basis, and one was a marathon runner. HDL_2 levels and reported running mileage showed a significant positive correlation ($r = 0.617$, $P < 0.001$), and thus, the levels of HDL_2 varied also widely throughout the group. However, a few sedentary subjects had relatively high concentrations of HDL_2 and good fat tolerance, while some runners had relatively low HDL_2 concentrations and pronounced lipemia. In the two subjects who were followed for 3 years, the increase of HDL_2 because of running was transient. On cessation of exercise, the HDL_2 dropped to pre-exercise levels within 4 weeks.

We examined the relative strength of the statistical correlations for the inverse relationship between the degree of postprandial triglyceridemia and the concentration of HDL. The strongest inverse relationship existed between the degree of triglyceride increase after the meal and the plasma concentration of HDL_2 particles. The second strongest inverse relationship was with HDL cholesterol and the third was with the concentration of apoA-I in plasma. A correlation of interest in our study is that the magnitude of the triglyceridemic response correlated positively with the fasting concentration of plasma triglycerides. This was noted also by Nestel (39), who demonstrated that both fasting triglyceride levels and extent of alimentary lipemia correlate negatively with the rate of removal of chylomicrons from the blood. We are unable to present a satisfactory explanation at this time for the positive correlation between the extent of alimentary lipemia and concentration of total serum apoB. Our radioimmunoassay method does not discriminate between the various apoB forms present in chylomicrons, VLDL, and LDL (40). Our measurements were performed by using fasting sera of normolipidemics; therefore, most of the apoB measured was probably associated with LDL. Interestingly, the high HDL_2 levels found in hyperalphalipoproteinemia are associated with low LDL levels (13).

Several hypotheses have been proposed to account for the inverse relationship between HDL and coronary heart disease. Glomset (41) has suggested that HDL acts as a reversed cholesterol transport mechanism; thus, HDL would serve as a scavenger of tissue cholesterol, including that from the arterial wall. Carew *et al.* (42) have reported that HDL competes with LDL for binding to LDL receptors. A third possibility is that HDL in some unknown way may increase the rate of clearance of the triglyceride-rich lipoproteins. An alternative hypothesis, not

exclusive of any of the above, is that the inverse correlation between HDL and coronary heart disease could be a secondary phenomenon. According to this hypothesis, individuals with high levels of HDL-associated cholesterol and HDL_2 are less prone to coronary disease because of their rapid clearance of the triglyceride-rich particles from the circulation, especially their postprandial triglyceride-rich fractions. From this perspective, high concentrations of HDL_2 are a result of the phenomenon which protects against atherosclerosis rather than the cause of it. We would caution, however, that the validity of the relationship between HDL_2 levels, the extent of postprandial lipemia, and the protection against atherosclerosis and coronary heart disease awaits careful testing in larger population studies.

The authors are grateful to many of their colleagues for their enthusiastic cooperation as volunteers; to D. Ehler, T. C. Chen, and I. Thandi for technical assistance; to Sarah Myers, Debbie Mason-Delz, and Rosetta Ray for assistance in the preparation of the manuscript; and to Susan McNeely-Kelly for providing the line drawings. This work was supported by Grants HL-24759, HL-17269, HL-15648, and HL-27341 and by a grant from the American Egg Board.

1. Department of Health, Education and Welfare (1971) *Arteriosclerosis: A report by the National Heart and Lung Institute Task Force on Arteriosclerosis, NIH Publ.* No. 72-219 (NIH, Bethesda, MD) Vol. 2.
2. Hulley, S. B., Rosenman, R. H., Bawol, R. D. & Brand, J. R. (1980) *N. Engl. J. Med.* **302,** 1383–1389.
3. Lippel, K., Tyroler, H., Eder, H., Gotto, A. M., Jr., & Vahouny, G. (1981) *Arteriosclerosis* **1,** 406–417.
4. Gofman, J. W., DeLalla, O., Glazier, F., Freeman, N. K., Lindgren, F. T., Nichols, A. V., Strisower, B. & Tamplin, A. R. (1954) *Plasma* **2,** 413–484.
5. Miller, G. J. & Miller, N. E. (1975) *Lancet* **i,** 16–20.
6. Rhoads, G. G., Gulbrandsen, C. L. & Kagan, A. (1976) *N. Engl. J. Med.* **294,** 293–298.
7. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. & Dawber, T. R. (1977) *Am. J. Med.* **62,** 707–714.
8. Havel, R. J., Goldstein, J. L. & Brown, M. S. (1980) in *Metabolic Control and Disease*, eds. Bondy, P. K. & Rosenberg, L. E. (Saunders, Philadelphia), pp. 393–494.
9. Patsch, J. R., Sailer, S., Kostner, G., Sandhofer, F., Holasek, A. & Braunsteiner, H. (1974) *J. Lipid Res.* **15,** 356–366.
10. Kostner, G. M., Patsch, J. R., Sailer, S., Braunsteiner, H. & Holasek, A. (1974) *Eur. J. Biochem.* **45,** 611–621.
11. Cheung, M. C. & Albers, J. J. (1979) *J. Lipid Res.* **20,** 200–207.
12. Patsch, W., Schonfeld, G., Gotto, A. M., Jr., & Patsch, J. R. (1980) *J. Biol. Chem.* **255,** 3178–3185.
13. Glueck, C. J. (1976) *Artery* **2,** 196–197.
14. Patsch, W., Kuisk, I., Glueck, D. & Schonfeld, G. (1981) *Arteriosclerosis* **1,** 156–161.
15. Wood, P. D. & Haskell, W. D. (1979) *Lipids* **14,** 417–427.
16. Patsch, J. R., Gotto, A. M., Jr., Olivecrona, T. & Eisenberg, S. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4519–4523.
17. Redgrave, T. G. & Small, D. M. (1979) *J. Clin. Invest.* **64,** 162–171.
18. Tall, A. R., Green, P. M. R., Glickman, R. M. & Riley, J. W. (1979) *J. Clin. Invest.* **64,** 977–989.
19. Havel, R. J., Kane, J. P. & Kashyap, M. L. (1973) *J. Clin. Invest.* **52,** 32–38.
20. Behr, S. R., Patsch, J. R., Forte, T. & Bensadoun, A. (1981) *J. Lipids Res.* **22,** 443–451.
21. Nikkila, E. A. (1978) in *High Density Lipoproteins and Atherosclerosis*, eds. Gotto, A. M., Jr., Miller, N. E. & Oliver, M. F. (Elsevier, Amsterdam), pp. 177–191.
22. Department of Health, Education and Welfare (1974) *Lipid Research Clinics Program Manual of Laboratory Operations, NIH Publ.* No. 75-628 (NIH, Bethesda, MD).
23. Bartlett, G. R. (1959) *J. Biol. Chem.* **234,** 466–468.
24. Patsch, W., Sailer, S. & Braunsteiner, H. (1976) *J. Lipid Res.* **17,** 182–185.
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
26. Karlin, J. B., Juhn, D. J., Scanu, A. M. & Rubenstein, A. H. (1978) *Eur. J. Clin. Invest.* **8,** 19–26.
27. Karlin, J. B., Juhn, D. J., Starr, J. I., Scanu, A. M., Edelstein, C. & Rubenstein, A. H. (1980) *J. Lipid Res.* **21,** 30–37.
28. Goldberg, R. B., Karlin, J. B., Juhn, D. B., Scanu, A. M., Edelstein, C. & Rubenstein, A. H. (1980) *J. Lipid Res.* **21,** 902–912.
29. Van de Kamer, J. H., Ten Bokkel Huinink, H. & Weyers, H. A. (1948) *J. Biol. Chem.* **177,** 347–355.
30. Griffin, G. E., Abbott, W. E., Pride, M. P., Muntwyler, E., Mautz, F. R. & Griffith, L. (1945) *Ann. Surg.* **121,** 352–360.
31. Johnson, R. E. & Kickemeister, F. L. (1964) in *Calculus with Analytical Geometry* (Allyn and Bacon, Boston), 3rd Ed.
32. *Department of Health and Human Services Prophet Statistics* (1980) *NIH Publ.* No. 80-2169 (NIH, Bethesda, MD).
33. Society of Actuaries (1970) in *Documenta Geigy*, Scientific Tables, eds. Diem, K. & Lentner, C. (Ciba-Geigy, Basel), p. 711.
34. Department of Health and Human Services (1980) *The Lipid Research Population Study Data Book, The Prevalence Study, NIH Publ.* No. 80-1527 (NIH, Bethesda, MD).
35. Anderson, D. A., Nichols, A. V., Pan, S. S. & Lindgren, F. T. (1978) *Atherosclerosis* **29,** 161–179.
36. Moreton, J. R. (1950) *J. Lab. Clin. Med.* **35,** 373–384.
37. Zilversmit, D. B. (1979) *Circulation* **60,** 473–485.
38. Angervall, G. (1964) *Acta Med. Scand.* **176,** Suppl. 424, 1–84.
39. Nestel, P. J. (1964) *J. Clin. Invest.* **43,** 943–949.
40. Kane, J. P., Hardman, D. A. & Paulus, H. E. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 2465–2469.
41. Glomset, J. A. (1968) *J. Lipid Res.* **9,** 155–167.
42. Carew, T. F., Koschinsky, T., Hayes, S. P. & Steinberg, D. P. (1976) *Lancet* **i,** 1315–1317.