Comparison of gradient gel electrophoresis and zonal ultracentrifugation for quantitation of high density lipoproteins

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Abstract The study was conducted to compare gradient gel electrophoresis (GGE) and zonal ultracentrifugation for quantitation of human plasma high density lipoproteins (HDL). Plasma samples were obtained from seven normal subjects consuming a high fat diet (65% total calories) followed by a high carbohydrate diet (65% total calories). HDL were fractionated into HDL_2 and HDL_3 by zonal ultracentrifugation and lipid and protein mass were determined. HDL were also fractionated by GGE and the results were compared to the zonal method. Zonally isolated HDL_2 represented a homogeneous particle population that was equivalent to HDL_{2b} as determined by GGE. By the zonal method, HDL_2 accounted for 27 ± 4% (mean ± SEM) of total HDL mass in subjects on the high fat diet as compared to 16 ± 2% in subjects fed the high carbohydrate diet; by GGE, the HDL_{2b} values were 27 ± 4% and 14 ± 1%, respectively. The coefficient of correlation (n = 25) for the two methods was 0.894 ($P < 0.001$). — **McNerney, C. A., M. L. Kashyap, R. L. Barnhart, and R. L. Jackson.** Comparison of gradient gel electrophoresis and zonal ultracentrifugation for quantitation of high density lipoproteins. *J. Lipid Res.* 1985. **26:** 1363–1367.

Supplementary key words lipoprotein metabolism • HDL_2 • HDL_3

High density lipoproteins are a heterogenous group of lipoprotein particles ranging from 55 to 120 Å in molecular diameter and from 1.063 to 1.21 g/ml in flotation density (for review, ref. 1). Interest in understanding the structure and metabolism of HDL is related to the fact that HDL-cholesterol, and particularly HDL_2-cholesterol, has a highly significant inverse correlation with coronary heart disease (2). Classically, HDL are separated into HDL_2 and HDL_3 by analytical ultracentrifugation (3, 4). Since HDL_2 are more enriched in lipid, they are less dense and migrate to a lower solvent density during analytical ultracentrifugation. Preparatively, human plasma HDL_2 and HDL_3 have been fractionated with fixed angle (5), vertical (6, 7), swinging bucket (8, 9), and zonal (10) rotors. Gradient gel electrophoresis (GGE) has also been used for HDL fractionation (11), a method of separation based on differences in particle diameter. Because of the capacity to accommodate large numbers of samples, GGE has advantages over ultracentrifugal methods. In the present study, we have fractionated plasma HDL by zonal ultracentrifugation and GGE and have compared the two methods for quantitation of HDL.

MATERIALS AND METHODS

Healthy subjects (ages 23–30 yr), two female and five male, were studied as in-patients in the General Clinical Research Center at the University of Cincinnati College of Medicine. All subjects received an isocaloric basal diet (12) for 1 week before beginning the study diets. The study diets were 2 weeks of a high fat (65% fat, 15% carbohydrate) followed by 4 weeks of a high carbohydrate (65% carbohydrate, 15% fat). For both diets, cholesterol was 400 mg/day, the P/S ratio of the fat was 0.40, fiber was 14 g/1000 calories, and the carbohydrate was two-thirds complex and one-third simple sugars. All meals were prepared from solid food in 2-day cycles as described previously (12). At weekly intervals, fasting blood (60 ml) was collected into tubes containing EDTA (0.15%, final concentration). After centrifugation of red cells, 5 ml of plasma was removed for lipid analysis. To the remainder were added sodium azide, aprotinin (Sigma), and dithiobis-2-nitrobenzoic acid to final concentrations of 0.01% (by wt), 50 kallikrein inhibitory units/ml, and 1 mM, respectively.

Abbreviations: GGE, gradient gel electrophoresis; HDL, high density lipoproteins; LDL, low density lipoproteins.

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Plasma cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol were determined in duplicate by standardized AutoAnalyzer II procedures at the University of Cincinnati Lipid Research Center Laboratory (13). HDL were fractionated into HDL_2 and HDL_3 by zonal ultracentrifugation in a salt gradient of NaBr (d 1.00–1.40 g/ml) as described by Patsch et al. (10). Zonal ultracentrifugation was performed in a Beckman Ti-14 zonal rotor with a Beckman L5-65B centrifuge. Plasma (10 ml) was subjected to ultracentrifugation at 41,000 rpm for 24 hr at 10°C and was removed from the rotor with a Beckman gradient pump; 7-ml fractions were collected. The absorbance at 280 nm was determined and those fractions corresponding to HDL_2 and HDL_3 were pooled, dialyzed against a standard buffer containing 10 mM Tris-HCl, pH 7.4, 0.9% NaCl, 1 mM EDTA, and 0.01% NaN_3, and concentrated by ultrafiltration (Amicon, PM 30 filter). HDL_2 and HDL_3 were analyzed for protein according to the method of Lowry et al. (14) with fatty acid-free bovine serum albumin as standard. Phospholipid-phosphorus was measured by the method of Bartlett (15). Lipoprotein-triacylglycerols were determined enzymatically with a Triglycerides Test Combination Kit (Sigma Technical Bulletin No. 405) and cholesterol was determined by a standardized AutoAnalyzer II procedure.

For quantitation by GGE, lipoproteins were isolated by ultracentrifugation. One ml of plasma was adjusted to d 1.22 g/ml by the addition of solid KBr (325 mg). Samples were then centrifuged for 20 hr in a Beckman 50.3 Ti rotor at 48,000 rpm, at 10°C. The upper lipoprotein fraction was removed (0.5 ml) and dialyzed against the GGE electrophoresis buffer (0.09 M Tris-HCl, 0.08 M borate, 0.01% EDTA, 0.01% NaN_3, pH 8.35). Lipoproteins were then filtered (0.45 μm Millipore filter) to remove aggregated material. Twenty μl of the sample was mixed with 5 μl of 50% sucrose in the electrophoresis buffer and loaded onto Pharmacia PAA 4/30 gels. Electrophoresis was performed for 18 hr, 125 V, 10°C. After electrophoresis the gels were stained directly with 0.05% Coomassie Brilliant Blue (R 250) in methanol–glacial acetic acid–water 5:1:4 (v/v). The gels were destained for 4 hr with methanol–glacial acetic acid–water 5:1:4 (v/v) and then overnight with methanol–glacial acetic acid–water 1:1:8 (v/v); the gels were scanned with a soft laser scanning densitometer (Helena, Model SL-TRF). The areas corresponding to HDL_{2b} (11) and total HDL were calculated after integration; the % area of each subfraction was assumed to be proportional to HDL_2 and HDL_3 concentration as determined by compositional analysis. Molecular weights were determined using thyroglobulin, apoferritin, lactate dehydrogenase, and bovine serum albumin as standards (High Molecular Weight Electrophoresis Calibration Kit, Pharmacia).

All data were analyzed by the University of Cincinnati CLINFO computer facility. This study was approved by the University of Cincinnati Medical Center Committee on Human Research and all subjects gave informed consent for the studies.

RESULTS

The effect of a high fat versus a high carbohydrate diet on plasma lipids and lipoproteins is shown in **Table 1.** Compared to 2 weeks of a high fat diet (day 21), 4 weeks of a high carbohydrate diet (day 49) was associated with an increase in plasma triglycerides (56 ± 11 to 110 ± 30 mg/dl) and a decrease in both LDL-cholesterol (94 ± 11 vs. 75 ± 8 mg/dl) and HDL-cholesterol (54 ± 2 vs. 43 ± 3 mg/dl).

At days 7, 21, 35, and 49, plasma was fractionated into HDL_2 and HDL_3 by zonal ultracentrifugation. Representative zonal profiles for two of the subjects are shown in **Fig. 1.** Relative to the high fat diet, the high carbohydrate diet was associated with a decrease in the HDL fraction corresponding to HDL_2 and is consistent with other reports (12, 16–18). The mean plasma concentrations of HDL_2 and HDL_3 protein and lipids for the seven subjects are shown in **Fig. 2.** The decrease in plasma HDL-cholesterol in subjects on the high carbohydrate

TABLE 1. Effect of diet on plasma lipids and lipoprotein cholesterol[a]

	Basal	High Fat		High Carbohydrate	
Determination	Day 0	Day 7	Day 21	Day 35	Day 49
Plasma Chol[b]	161 ± 12	164 ± 11	159 ± 13	138 ± 8	140 ± 11
Plasma TG[c]	90 ± 20	77 ± 23	56 ± 11	113 ± 34	110 ± 30
LDL-Chol	91 ± 12	101 ± 9	94 ± 11	73 ± 6	75 ± 8
VLDL-Chol	18 ± 4	15 ± 5	11 ± 2	23 ± 7	22 ± 6
HDL-Chol	53 ± 3	48 ± 2	54 ± 2	42 ± 2	43 ± 3

[a] Values are expressed as mg/dl ± SEM
[b] Chol, total cholesterol
[c] TG, triglycerides

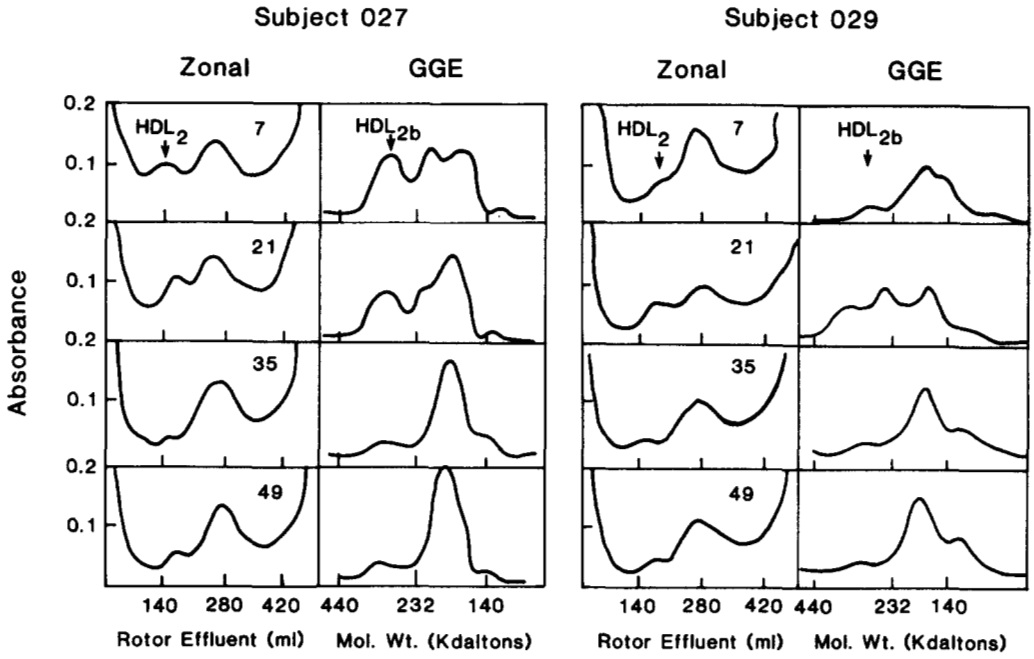

Fig. 1. Representative zonal ultracentrifugal and GGE profiles of plasma HDL. At days 7 (basal diet), 21 (high fat diet), 35, and 49 (high carbohydrate diet), plasma from subjects 027 (female) and 029 (male) was subjected to zonal ultracentrifugation and GGE and described in Methods.

diet was mainly due to a decrease in HDL_2-protein and -lipids; plasma HDL_3 mass was not significantly different on the two study diets.

Total plasma HDL were also fractionated by GGE (Fig. 1). As determined by GGE, the mean ± SEM molecular weight of HDL_{2b} in subjects on the high carbohydrate diet (day 49) was 274 ± 8 × 10^3 versus 288 ± 5 × 10^3 for the high fat diet (day 21); the mean values were not sig-

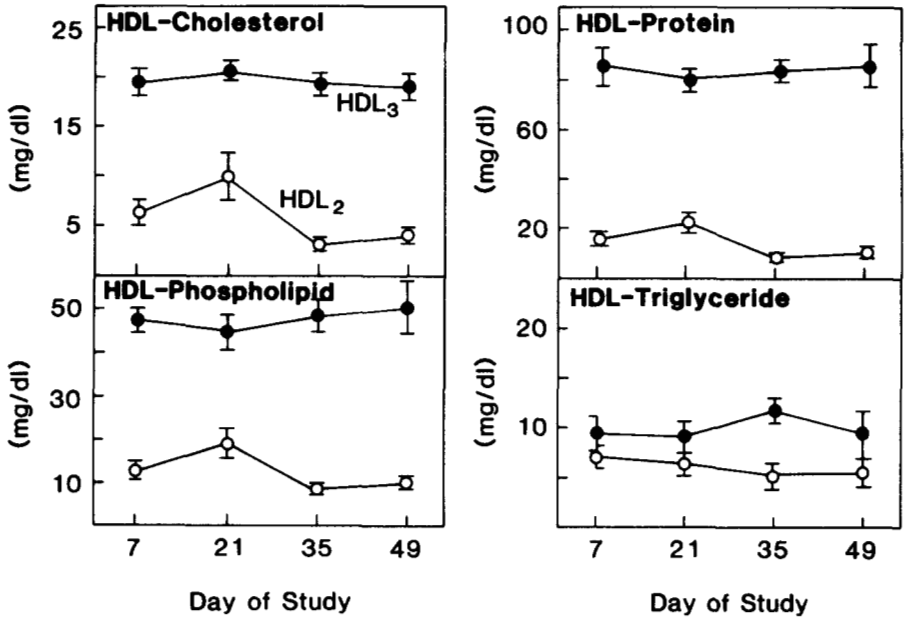

Fig. 2. Effect of diet on HDL-protein and lipids of zonally isolated HDL_2 (○) and HDL_3 (●) mass. The values (mg/dl) represent the mean (± SEM) (n = 7) and are not corrected for losses during ultracentrifugation.

nificantly different. For zonally isolated HDL_2, molecular weights (as determined by GGE of the zonally isolated fractions) were $295 \pm 6 \times 10^3$ in subjects on the high carbohydrate diet versus $282 \pm 6 \times 10^3$ on the high fat diet. Again, there were no significant differences between the two diets or between HDL fractionated by zonal ultracentrifugation or GGE.

The amount of HDL_2 (HDL_{2b}), as percent of total HDL, determined by the zonal ultracentrifugation method versus GGE is shown in **Fig. 3** and **Fig. 4.** By the zonal method, the amount of HDL_2, as % of total HDL mass, was 27 ± 4 (mean ± SEM) on day 21 (high fat) as compared to 16 ± 2% on day 49 (high carbohydrate); by GGE, the values of HDL_2 were 27 ± 4% and 14 ± 1% on days 21 and 49, respectively. Values obtained with both methods were closely correlated with changes in plasma HDL-cholesterol. The coefficient of correlation for the zonal method versus GGE was 0.894 ($P < 0.001$) (Fig. 4).

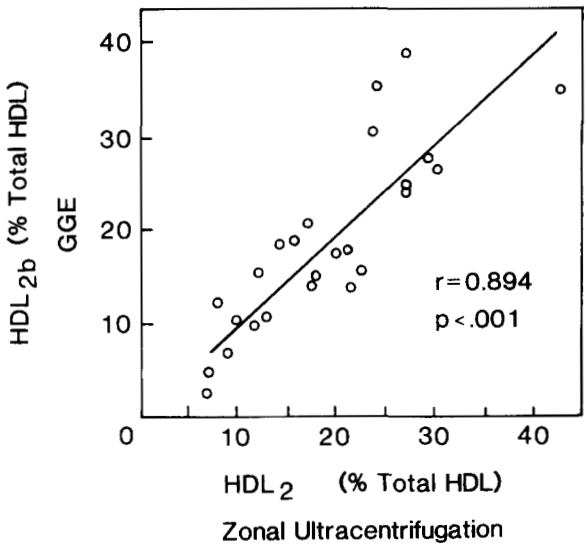

Fig. 4. The amount of HDL_{2b} (HDL_2) as percent of total HDL as determined by zonal ultracentrifugation and GGE (n = 25).

DISCUSSION

Patsch, Kostner, and Patsch (19) were the first to show, in a single subject, that zonally isolated HDL_2 migrated on GGE with a molecular weight corresponding to HDL_{2b} in the nomenclature of Nichols et al. (20). However, Patsch et al. (19) did not compare the two methods for quantitation of HDL_2. The results of the present study show that the decrease in plasma HDL-cholesterol associated with a high carbohydrate diet is more reflected by changes in zonally isolated HDL_2 and the GGE fraction corresponding to HDL_{2b}. HDL_2-cholesterol, -phospholipid, -protein, and -triglycerides all decreased in subjects on the high carbohydrate diet, suggesting that the reduction in plasma HDL-cholesterol is due to fewer circulating particles and not to less sterol per particle. By zonal ultracentrifugation or GGE (Fig. 3), HDL_{2b} accounted for approximately 27% of total HDL in subjects on the high fat diet as compared to 15% in subjects on the high carbohydrate diet; the coefficient of correlation for the two methods of quantitation was excellent.

Since the GGE method provides for processing of large numbers of samples, the quantitative information provided in this report should prove of interest to investigators interested in measuring the amount of HDL subfractions.

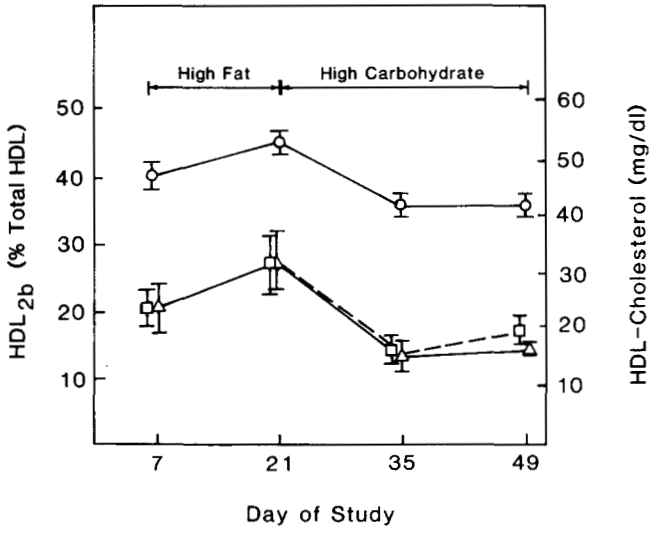

Fig. 3. Effect of diet on HDL_{2b} as percent of total HDL as determined by zonal ultracentrifugation (□) and GGE (△). The values represent the mean ± SEM (n = 7). Plasma HDL-cholesterol is represented as (○).

This work was supported by Public Health Service Grants HL-22618 and HL-24744 and General Clinical Research Center and CLINFO Grant NIH RR00068. We gratefully acknowledge the assistance of Ms. Robin Wright in preparing the manuscript for publication and Ms. Gwen Kraft for the figures.

Manuscript received 11 June 1985.

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