Altered properties of high density lipoprotein subfractions in obese subjects

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Abstract Human HDL are heterogeneous in their metabolism and comprise small, nascent pre-β-HDL and more mature α-HDL. Evidence exists that pre-β_1-HDL is the initial acceptor of cellular free cholesterol, which then transfers sequentially to other pre-β species and then, after esterification, into α-HDL. As HDL particles are themselves transformed during this process, we postulated that in disorders in which HDL-cholesterol is low, such as obesity, the distribution of HDL particles may be disturbed. In this study, we analyzed the HDL profile in 23 obese and 18 lean subjects, and further investigated the effects of dietary change in 15 obese subjects. HDL were separated by two-dimensional nondenaturing electrophoresis and the apoA-I content in each fraction was quantified. α_1-HDL in obese subjects was significantly lower ($P < 0.001$) and α_2-, α_3-, and pre-β_1-HDL were significantly higher ($P < 0.05$ for α_2-HDL, $P < 0.001$ for α_3- and pre-β_1-HDL) than in lean subjects. On stepwise regression analysis, body mass index accounted for 52% (negatively) of the variance in α_1-HDL and for 16% and 33% (positively) for the variances in α_3- and pre-β_1-HDL, respectively. α_1- and pre-β_3-HDL increased significantly after low-fat, oleic acid-rich, or α-linolenic acid-rich diets. The profile of α-HDL particles and also of pre-β-HDL particles therefore shifted to smaller species in obese subjects, and this was influenced by dietary fat. Increased pre-β_1-HDL-apoA-I in obese subjects is likely to derive from increased HDL catabolism but may also reflect diminished transformation of pre-β_1- to pre-β_2-HDL which might reduce capacity for reverse cholesterol transport and partly explain lower HDL-cholesterol levels.—**Sasahara, T., T. Yamashita, D. Sviridov, N. Fidge, and P. Nestel.** Altered properties of high density lipoprotein subfractions in obese subjects. *J. Lipid Res.* 1997. **38:** 600–611.

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It is generally accepted that high density lipoprotein (HDL) is largely responsible for the initial phase of a complex process whereby cellular cholesterol is transported from extrahepatic sites to the liver for partial excretion (1). HDL comprises several populations of particles characterized by its major apolipoproteins (apo) A-I and A-II. Further subfractionation by electrophoresis of apoA-I-containing HDL defines two metabolically interrelated species, the pre-β- and α-HDL. The identification of the specific HDL subclass that might be the primary cholesterol acceptor, and the mechanism(s) responsible for regulating cellular cholesterol release, have been only partially answered (2–6).

Several studies have postulated a specific metabolic role for the pre-β-HDL subfractions. Hara and Yokoyama (7) have proposed that free apolipoproteins (A-I, A-II, and E) react with cellular cholesterol and phospholipid to generate small, disc-shaped pre-β-HDL-like particles. These lipoprotein species had previously been demonstrated to be the initial recipients of effluxed cholesterol in short-term incubations of donor cells with human plasma (2, 4). Kawano et al. (8) demonstrated the presence of two distinct pathways of cholesterol efflux: one is directly proportional to plasma pre-β-HDL concentration and may involve a cell-surface protein and the other is a "nonspecific" efflux process which is protease insensitive. Kinetic and pulse-chase investigations from the laboratories of Castro and Fielding (2), Francone, Gurakar, and Fielding (3), and Huang, von Eckardstein, and Assmann (5), using fibroblasts radiolabeled with cholesterol and fresh human plasma, suggested that cell-derived cholesterol moved along a pathway involving three different pre-β-HDL subclasses. The initial cholesterol acceptor, pre-β_1-HDL, transferred its cholesterol, either by a transfer process or particle fusion, to a larger pre-β_2-HDL, then sequentially to pre-β_3-HDL, and finally to the α-HDL

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; BMI, body mass index; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; HTGL, hepatic triglyceride lipase; LpAI, apolipoprotein A-I-containing lipoprotein; ALA, α-linolenic acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; EDTA, ethylene-diaminetetraacetic acid; apo, apolipoprotein.

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subspecies. Sviridov and Fidge (9) have confirmed the importance of pre-β_1-HDL as the earliest acceptor and have found that over time, the effluxed cholesterol becomes distributed among pre-β- and α-HDL in proportion to the amounts of apoA-I in these particles. This subsequent redistribution is mediated through the esterification of free cholesterol by lecithin:cholesterol acyltransferase (LCAT) and the transfer of cholesteryl esters to other lipoproteins by cholesteryl ester transfer protein (CETP) (10). Thus, not only the concentrations of apoA-I-containing HDL (11, 12) but also the activities of LCAT (13, 14) and CETP (13) and the lipoproteins with which they react combine to affect cholesterol efflux and thus presumably the generation of pre-β-HDL.

Each of these factors is, therefore, likely to determine the amounts of the HDL species in plasma and the amounts of cholesterol that they carry. At present, there is substantial information reported on the ultracentrifugally separated α-HDL species, HDL_2 and HDL_3, but such data are scarce for the pre-β-HDL particles. Thus, it is well known that HDL_2, especially the more cholesterol-rich subspecies HDL_{2b}, is lower in men than in women (15), and in disorders such as obesity (16, 17) and dyslipidemia with coronary artery disease (18–20). By contrast, only Ishida, Frolich, and Fielding (21) have specifically reported on pre-β-HDL levels in hyperlipidemic subjects, finding the average concentration to be above average.

We chose to quantify the distribution of apoA-I among HDL subfractions in obese men and women with several characteristics of the metabolic (insulin-resistance) syndrome in which HDL-cholesterol levels are generally less than normal. Further, we report measurement after dietary changes during which either oleic or α-linolenic acid was substituted for saturated fatty acids, the rationale being the observation by others (22) that increasing the fluidity of discoidal reconstituted HDL phospholipid by raising the content of unsaturated acyl chains promoted cholesterol efflux from cells.

MATERIALS AND METHODS

Subjects

Twenty-three middle-aged obese subjects, 15 men and 8 post-menopausal women, were recruited through advertisement. Inclusion criteria were body mass index (BMI) 25–36 kg/m^2, age 45–65 years, absence of known metabolic disorders other than those resulting from obesity but excluding diabetes, pharmaco-therapy that might affect lipid metabolism, smoking, and alcohol >20 g/day.

Relevant vital data are shown in **Table 1.** Men and women were of similar age. The men were taller and heavier as expected but had average BMI similar to that of the women.

Healthy lean subjects (BMI <25 kg/m^2), 10 men and 8 post-menopausal women, were selected from other volunteers. Their lipid profiles were normal and they were not on medication known to affect plasma lipids (including hormone replacement therapy). Their gender and age distributions were similar to those of the obese subjects.

Dietary intervention

Fifteen obese subjects, 8 men and 7 post-menopausal women, ate in turn four diets of 4 weeks each: high saturated fat diet, oleic acid low-fat diet (MUFA diet), α-linolenic acid (ALA) low-fat diet (PUFA diet), and again, the high saturated fat diet. As one dietary period (linseed oil) was high in ALA and as the carry-over effects of long-chain n–3 fatty acids extend over many weeks for some biological functions (23), we chose MUFA diet for the first test period. However, there was no significant carry-over effect after 4 weeks saturated fat diet period (data not shown).

The probable account of nutrients eaten during the four periods is shown in **Table 2** (derived from daily diaries of consumed foods and from regular periods when foods were weighed). Fat intake during the two high saturated fat periods was 35% of energy (high fat), about the Australian average, as were the proportions of saturated, polyunsaturated (mainly linoleic acid), and monounsaturated fatty acids. During the two low-fat intervention periods, fat intake fell to 26% energy and dietary cholesterol more modestly to about 210 mg daily. Supplemental foods were provided during the test periods, comprising soft margarine and biscuits and muffins baked with the margarine. Purified deodorized linseed oil was the basic oil in the PUFA period (percent fatty acid composition of the margarine: palmitic 8.7, stearic 3.7, oleic 15, linoleic 10.5, α-linolenic 36.7, and *trans* 7.4). ALA provided 9.5% energy in the whole diet. Sunola oil, an oleic acid-rich variant of sunflower oil, provided the basic oil for the other test period (MUFA) (percent fatty acid composition of the margarine: palmitic 9.2, stearic 4.4, oleic 52, linoleic 11, α-linolenic 0.3 and *trans* 4.6). Oleic acid provided 13.3% of the whole diet. α-Tocopherol was added to equalize its concentration in both margarines and to provide antioxidant to the linseed products (final α-tocopherol was 463 mg/kg in both margarines).

Blood was obtained on 2 consecutive days at the end of all dietary periods for determination of fasting state lipids.

The subjects maintained their normal activities and were encouraged to exercise constantly and similarly

TABLE 1. Clinical characteristics and plasma parameters in control and obese subjects

	Age	Height	Weight	BMI	Cholesterol	Triglyceride	HDL-C	LDL-C	ApoA-I
	yr	*cm*	*kg*	*kg/m²*	*mmol/L*		*mmol/L*		*mg/dl*
Male									
Control (n = 10)	54.1 ± 8.3	178.7 ± 5.3	75.5 ± 4.1	23.7 ± 1.8	5.0 ± 1.0	1.0 ± 0.3	1.2 ± 0.2	3.5 ± 1.0	125.0 ± 14.1
Obese (n = 15)	54.0 ± 7.8	174.9 ± 5.1[c]	94.2 ± 9.1[d]	30.9 ± 3.5[d]	5.5 ± 1.0	1.8 ± 1.0[c]	1.0 ± 0.2[d]	4.2 ± 0.9	115.5 ± 14.0
Obese (diet study)									
High saturated (n = 8)	54.5 ± 5.7	175.7 ± 3.8	96.8 ± 8.3	31.4 ± 2.9	5.4 ± 1.0	2.1 ± 1.0	0.9 ± 0.2	4.0 ± 1.0	113.9 ± 17.5
MUFA (n = 8)			95.0 ± 9.4	30.8 ± 3.1	4.7 ± 0.9	1.8 ± 0.7	0.9 ± 0.2	3.4 ± 0.8	103.5 ± 14.1
PUFA (n = 8)			95.8 ± 9.6	31.0 ± 3.2	4.9 ± 0.7	1.8 ± 0.9	1.0 ± 0.3	3.6 ± 0.7	99.4 ± 5.1
Female									
Control (n = 8)	52.9 ± 3.4	162.9 ± 10.9[b]	59.0 ± 6.3[b]	22.3 ± 1.5	5.5 ± 1.2	1.2 ± 0.4	1.7 ± 0.3[b]	3.6 ± 1.1	138.2 ± 11.5[a]
Obese (n = 8)	52.0 ± 7.1	163.2 ± 9.3[b]	77.0 ± 6.8[bd]	30.0 ± 2.5[d]	5.2 ± 0.7	1.9 ± 2.2	1.2 ± 0.3[bd]	3.7 ± 0.8	127.5 ± 16.5
Obese (diet study)									
High saturated (n = 7)	53.0 ± 7.0	162.2 ± 9.5[b]	77.2 ± 7.4[b]	29.4 ± 2.4	5.2 ± 0.8	2.0 ± 2.3	1.2 ± 0.3[a]	3.6 ± 0.8	125.4 ± 16.6
MUFA (n = 7)			77.2 ± 7.4[b]	29.4 ± 2.4	5.0 ± 0.7	1.5 ± 1.1	1.2 ± 0.3[a]	3.4 ± 0.6	121.8 ± 19.8
PUFA (n = 7)			77.2 ± 8.1[b]	29.4 ± 2.7	5.0 ± 0.7	1.7 ± 1.3	1.2 ± 0.3[a]	3.5 ± 0.7	115.7 ± 13.6[b]
Total									
Control (n = 18)	53.6 ± 6.2	172.2 ± 11.8	68.2 ± 9.8	22.9 ± 1.8	5.2 ± 1.1	1.1 ± 0.3	1.4 ± 0.3	3.6 ± 1.0	130.9 ± 14.3
Obese (n = 23)	53.3 ± 7.4	170.8 ± 8.7	88.2 ± 11.7[c]	30.2 ± 3.3[c]	5.4 ± 0.9	1.9 ± 1.5[c]	1.0 ± 0.3[c]	4.0 ± 0.9	119.7 ± 15.7[c]
Obese (diet study)									
High saturated (n = 15)	53.8 ± 6.1	169.4 ± 9.7	87.6 ± 12.6	30.4 ± 2.8	5.3 ± 0.9	2.1 ± 1.7	1.1 ± 0.3	3.8 ± 0.9	119.2 ± 17.5
MUFA (n = 15)			86.7 ± 12.3	30.1 ± 2.8	4.8 ± 0.8[f]	1.7 ± 0.9	1.0 ± 0.3	3.4 ± 0.7	112.0 ± 18.9[f]
PUFA (n = 15)			87.1 ± 12.9	30.3 ± 3.0	4.9 ± 0.7	1.8 ± 0.9	1.0 ± 0.3	3.6 ± 0.7	107.0 ± 12.7[f]

High saturated, high saturated fat diet; MUFA, monounsaturated fatty acid-rich low-fat diet; PUFA, polyunsaturated fatty acid (α-linolenic, n−3 + linoleic, n−6, acids)-rich low-fat diet; BMI, body mass index; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; apoA-I, apolipoprotein A-I. Values are mean ± SD.

[a] $P < 0.05$, [b] $P < 0.01$ versus male subjects (unpaired *t*-test).

[c] $P < 0.05$, [d] $P < 0.01$, [e] $P < 0.001$ versus control subject (unpaired *t*-test).

[f] $P < 0.05$ versus baseline of obese subjects. (ANOVA and pairwise multiple comparison procedure (Student-Newman-Keuls method)).

during each period. Supervision was maintained by at least two weekly visits to the unit, supplemented by frequent telephone calls and occasional home spot-checks.

Food records were analyzed by a program based on English food tables supplemented with relevant Australian food data (24).

Blood sampling

Blood samples were taken after the subjects had fasted overnight and were immediately placed on ice. Ethylene-diaminetetraacetic acid (EDTA) was used as the anticoagulant at a final concentration of 1.0 mg/ml blood. Plasma samples were obtained by centrifugation at 4°C (2000 *g*, 15 min) and frozen at −80°C. Freezing and thawing of plasma did not affect the distribution of apoA-I-containing lipoprotein subclasses. Frozen plasma was thawed at 37°C for about 1 min and used immediately in the two-dimensional electrophoresis.

Two-dimensional nondenaturing electrophoresis

The distribution of apoA-I in HDL subfractions was determined by two-dimensional nondenaturing electro-

TABLE 2. Calculated dietary intakes obtained from food records

	Saturated Fat Diet	MUFA Low-Fat Diet	PUFA Low-Fat Diet	Saturated Fat Diet
Energy (kcal)	2605 ± 496	2588 ± 426	2732 ± 533	2496 ± 493
Fat[a] (% energy)	35.9 ± 9.4	26.3 ± 4.2	26.4 ± 4.2	34.7 ± 6.8
CHO[a] (% energy)	45.2 ± 8.5	57.9 ± 5.0	58.4 ± 4.5	46.6 ± 6.7
Protein[a] (% energy)	17.2 ± 3.4	15.0 ± 2.3	14.2 ± 2.4	18.0 ± 3.0
SFA[a] (% total fat)	50.7 ± 4.4	25.8 ± 2.7	23.4 ± 2.1	46.9 ± 7.3
MUFA[a] (% total fat)	35.3 ± 3.0	58.1 ± 1.8	27.5 ± 1.5	36.2 ± 6.5
PUFA[a] (% total fat)	14.3 ± 4.8	16.1 ± 1.7	49.1 ± 2.1	16.4 ± 6.2
Cholesterol[a] (mg)	295 ± 105	206 ± 65	210 ± 83	287 ± 104
Fiber (g)	31 ± 6	33 ± 8	34 ± 9	37 ± 13
Sodium (mg)	3584 ± 1420	3017 ± 799	3070 ± 1045	3353 ± 992

CHO, carbohydrate; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. Values are mean ± SD.

[a] $P < 0.001$ by repeated-measures analysis of variance.

phoresis as described previously (2, 6, 9). In brief, in the first dimension, 20 µl of plasma was separated by electrophoresis on a 0.75% agarose gel equilibrated in a 50 mM barbital buffer (pH 8.6) at 4°C. Agarose gel strips containing the preseparated lipoproteins were then transferred to 3–15% polyacrylamide gradient gel (Gradiopore, North Ride, NSW, Australia) electrophoresis. Separation in the second dimension was performed at 300 V for 1.5 h at 4°C in the second dimension running buffer (200 mM glycine, 25 mM Tris, pH 8.3). After electrophoresis, the proteins were transferred by electroblotting to 0.1 µm nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) in the transfer buffer (25 mM Tris-glycine buffer, pH 8.3, containing 20% (v/v) methanol) at 20 V overnight at 4°C.

Immunoblotting

Nitrocellulose blots were incubated for 2 h in buffer A (10 mM Tris-HCl and 50 mM NaCl (pH 7.5)) containing 3% (w/v) skimmed milk. After incubation for 1 h with rabbit anti-human apoA-I polyclonal antibody (9), membranes were washed five times with washing buffer (PBS (pH 7.5) containing 0.5% Tween-20), and subsequently incubated for 1 h with goat ^{125}I-labeled anti-rabbit IgG (Amersham, England). Membranes were washed five times with washing buffer. All the procedures were performed at room temperature. Membranes were then exposed to imaging plates (Fuji Photo Film, Japan) for 24 h, and the distribution of ^{125}I radioactivities among lipoprotein subfractions was analyzed by using a bioimage analyzer BAS-1000 (Fuji Photo Film, Japan). ApoA-I concentration in each HDL subfraction was calculated by percent distribution of apoA-I in each HDL subfraction and total plasma apoA-I concentration.

Linearity standards

To demonstrate linearity of the measurement of apoA-I concentrations, the following experiments were performed. Plasma samples containing 0, 1, 2, 5, 10, 15, and 20 µg of apoA-I were spotted on nitrocellulose membranes and incubated with the above antibodies. Membranes were then exposed to imaging plates for 24 h, and the ^{125}I radioactivities in spots were analyzed by using a bioimage analyzer BAS-1000 (25). Similar linear experiments were performed with plasma samples of obese subjects following the high saturated fat diet, MUFA low-fat diet, and PUFA low-fat diet to check for possible perturbations in affinity of HDL-apoA-I for the antibodies.

Analytical methods

Plasma cholesterol and triglycerides were determined using enzymatic kits on a Cobas-Bio automated analyzer (Roche, Basel). HDL-cholesterol was measured after precipitation of apoB-containing lipoproteins with heparin/$MnCl_2$. Levels of plasma apoA-I were measured by immunoturbidimetric analysis on a Cobas-Bio automated analyzer (Boehringer Mannheim, Germany).

Statistical analysis

The data were calculated as means ± SD. Differences between apoA-I concentrations in HDL subfractions, clinical characteristics, and plasma parameters for lean and obese subjects were carried out by unpaired Student's *t*-test.

Differences among apoA-I concentrations in HDL subfractions and other parameters measured during dietary treatments were determined by repeated-measures analysis of variance (ANOVA), and if significantly different, contrasts in dietary periods were compared by the paired Student-Newman-Keuls method.

RESULTS

Table 1 shows the clinical characteristics and plasma concentrations of lipids of the subjects. Differences between lean men and lean women for body height and body weight were significant ($P < 0.01$), but mean BMI values were not significantly different.

Differences between lean subjects and obese subjects for body weight and BMI were, as expected, significant ($P < 0.01$ both in men and in women, and $P < 0.001$ in all subjects). Whereas plasma total and low density lipoprotein (LDL) cholesterol levels were on average similar in the obese and lean subjects (for men and for women), plasma triglycerides were significantly higher in the obese men and in obese men and women together ($P < 0.05$), while HDL-cholesterol and apoA-I levels were significantly lower for all obese subjects ($P < 0.05$).

Characterization of HDL subfractions by two-dimensional nondenaturing electrophoresis

Figure 1 presents the distribution of HDL subfractions after separation of normal plasma by two-dimensional nondenaturing electrophoresis. Three major particles appeared with α-mobility: α_1, α_2 and α_3 and three minor particles appeared with pre-β-mobility: pre-β_1, pre-β_2, and pre-β_3 (6).

Linearity standards

Figure 2 demonstrates linearity in the quantification of HDL-apoA-I. The concentrations of responses were linear with HDL-apoA-I concentrations up to 20 µg and

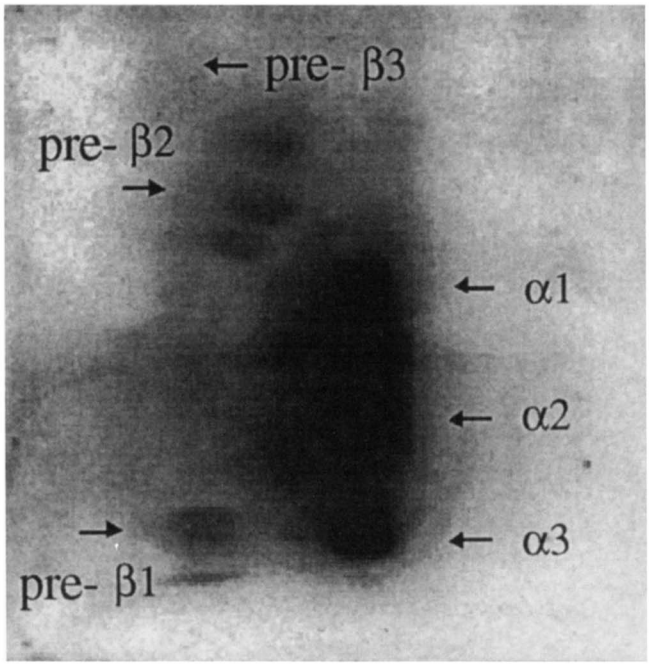

Fig. 1. Distribution of apoA-I in normal plasma. The distribution of apoA-I in plasma was determined by two-dimensional nondenaturing electrophoresis. After electrophoresis, the gels were transferred to nitrocellulose membranes, and apoA-I-containing lipoproteins were detected using a rabbit anti-human apoA-I polyclonal antibody, goat ^{125}I-labeled anti-rabbit IgG, and bioimage analyzer BAS-1000.

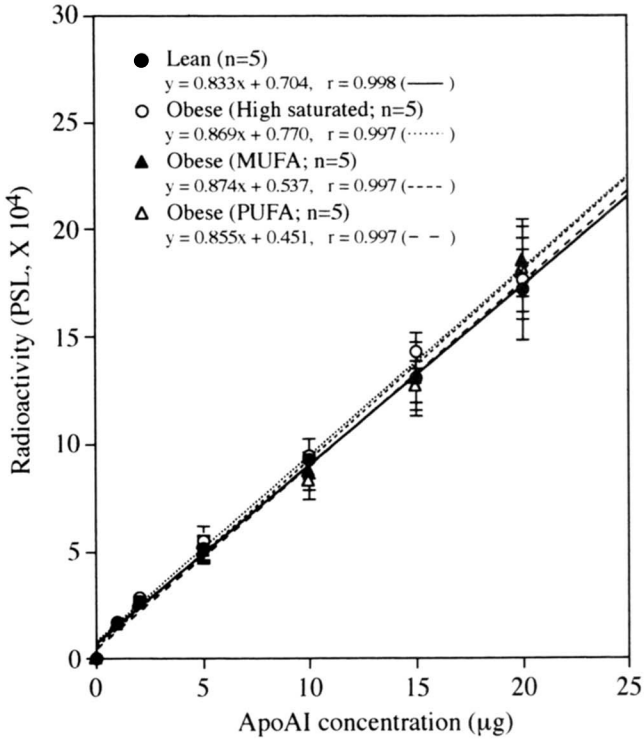

Fig. 2. Comparison of dose-responses of ^{125}I-radioactivity using the quantification of HDL-apoA-I. Radioactivities after immunoblotting were measured by BAS 1000 in plasma in lean subjects, obese subjects with high-saturated fat diet, those with MUFA low-fat diet, and those with PUFA low-fat diet. PSL, photostimulated luminescence; Lean, lean subjects; High saturated, high saturated fat diet; MUFA, monounsaturated low-fat diet; PUFA, polyunsaturated low-fat diet. The error bars represent standard error.

were similar for all subjects tested (lean and obese, and eating different diets). These results indicate that the affinity of the antibody for apoA-I does not change in HDL in which the fatty acid composition had been altered

Characterization of HDL subfractions in lean and obese subjects

Figure 3A shows the combined data (for men and women) of the distribution of apoA-I in HDL subfractions. ApoA-I percentage in α_1-HDL was significantly lower in obese subjects than that in lean subjects. Values for α_1-HDL in lean and obese subjects were 22.40 ± 4.05 and 12.77 ± 3.78 % of total apoA-I, respectively ($P <$ 0.001). However, the proportions of apoA-I in α_2-, α_3-, and pre-β_1-HDL in obese subjects were significantly higher than those in lean subjects. Percent distribution of α_2-, α_3-, and pre-β_1-HDL was 43.30 ± 6.42, 16.23 ± 4.61, and 9.18 ± 3.13 in lean subjects, and 46.74 ± 4.92, 20.98 ± 4.26, and 12.51 ± 3.57% in obese subjects, respectively ($P < 0.05$ for α_2- and $P < 0.001$ for α_3- and pre-β_1-HDL). The percentage of apoA-I in pre-β_2-HDL in obese subjects tended to be lower than that in lean subjects, though the difference was not significant.

Figure 3B shows apoA-I distribution in HDL subfractions in men alone. ApoA-I percentage in α_1-HDL in obese men was significantly lower in obese than that in lean men ($P < 0.001$), while those in α_2- and α_3-HDL were significantly higher in obese men ($P < 0.001$ for α_2- and $P < 0.05$ for α_3-HDL). The percentage of apoA-I in pre-β_1-HDL in obese men tended to be higher than that in lean men, and those in pre-β_2- and pre-β_3-HDL tended to be lower, though the differences were not significant.

Figure 3C shows the distribution of apoA-I in HDL subfractions in women alone. Mean apoA-I percentage in α_2-HDL in lean women was significantly higher, and that in pre-β_1-HDL was significantly lower than that in lean men ($P < 0.05$ for both). ApoA-I percentage in α_1-HDL in obese women was significantly lower and those in α_3- and pre-β_1-HDL were significantly higher than in lean women ($P < 0.001$ for α_1- and pre-β_1-HDL and $P < 0.05$ for α_3-HDL). ApoA-I in pre-β_2- and pre-β_3-HDL

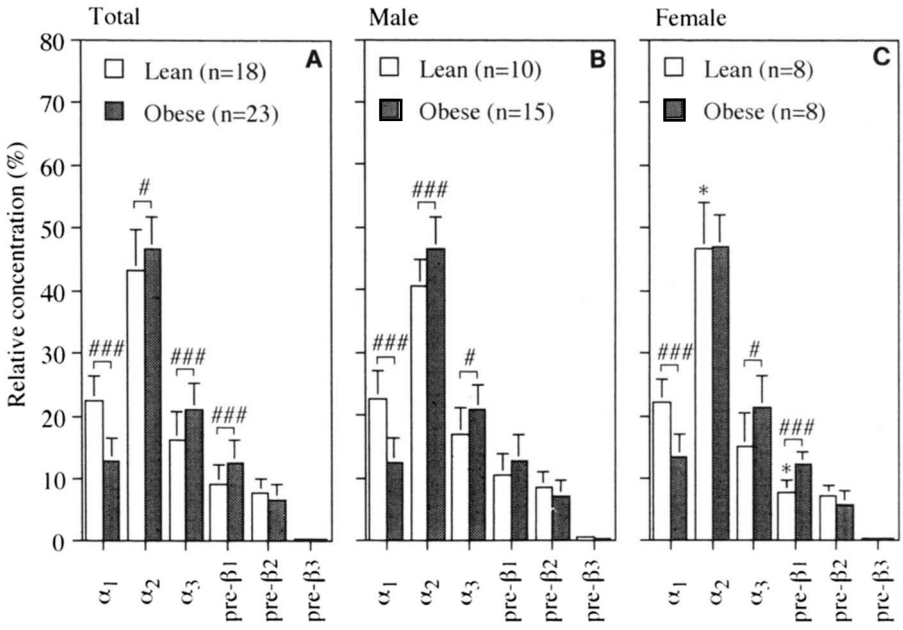

Fig. 3. Relative concentrations of apoA-I in HDL subfractions in lean and obese subjects. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. lean subjects (Student's *t*-test). $^{*}P < 0.05$ vs. lean male subjects (Student's *t*-test). The error bars represent standard deviation.

tended to be lower than that in lean women, but the differences were not significant.

The apparent anomaly in the obese subjects having higher pre-β_1-HDL but if anything, lower pre-β_2-HDL is emphasized in the ratios (**Fig. 4**). Pre-β_2/pre-β_1 ratio in obese subjects was significantly lower than that in lean subjects. Values for pre-β_2/pre-β_1 ratios in lean and obese subjects were 1.06 ± 0.62 and 0.47 ± 0.20 ($P <$ 0.05 in women) and 1.02 ± 0.62 and 0.60 ± 0.35 ($P <$ 0.01 in men and women), respectively. That in obese men tended to be lower than that in lean men, but the difference was not significant. The α_1/α_2 ratio in obese subjects was significantly lower than that in lean subjects. Values for α_1/α_2 in lean and obese subjects were 0.56 ± 0.14 and 0.27 ± 0.10 ($P < 0.001$ in men), 0.49 ± 0.13 and 0.29 ± 0.10 ($P < 0.001$ in women) and 0.53 ± 0.14 and 0.28 ± 0.10 ($P < 0.001$ in men and women), respectively.

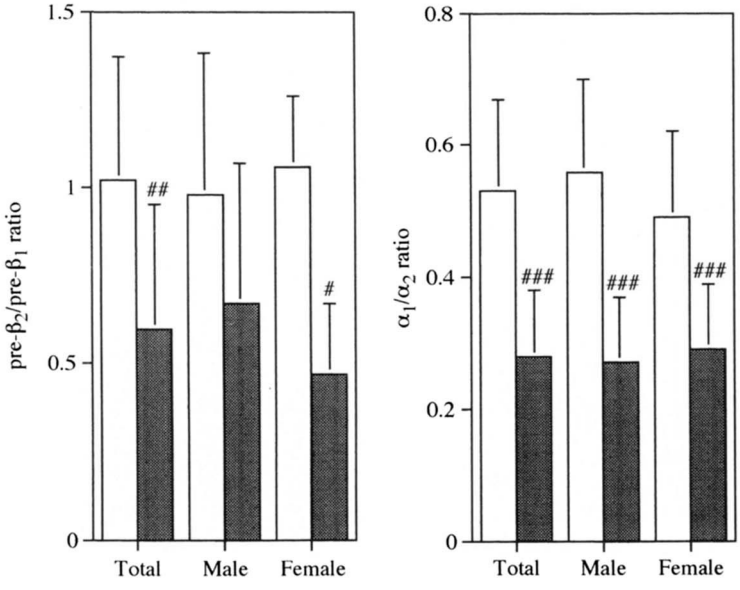

Fig. 4. Pre-β_2/pre-β_1 and α_1/α_2 ratios in lean and obese subjects. Lean subjects (male n = 10, female n = 8, all n = 18), open column; obese subjects (male n = 15, female n = 8, all n = 23); shaded column. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. control subjects, respectively (Student's *t*-test). The error bars represent standard deviation.

TABLE 3. Matrix of univariate correlations reaching or approaching significance, or where $r > 0.4$

	α_1		α_2		α_3		Pre-β_1		Pre-β_2		Pre-β_3	
Variable	r	P	r	P	r	P	r	P	r	P	r	P
BW (kg)	−0.51	<0.001					0.52	<0.001				
BMI (kg/m^2)	−0.72	<0.001			0.40	0.009	0.57	<0.001				
TC (mmol/L)												
TG (mmol/L)												
HDL-C (mmol/L)	0.58	<0.001			−0.40	0.011	−0.42	0.007				
LDL-C (mmol/L)												
ApoA-I (mg/dl)	0.44	0.004										
Sex			—	<0.05			—	<0.05				

BW, body weight; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein-cholesterol; LDL-C, low density lipoprotein-cholesterol; apoA-I, total apolipoprotein A-I.

Correlations

Univariate correlations for all obese and lean subjects, between various parameters and HDL subfractions are shown in **Table 3.** There was a significant inverse correlation between BMI and the proportion of apoA-I in α_1-HDL ($r = -0.72$, $P < 0.001$), also shown in **Fig. 5.** The percentage of apoA-I in pre-β_1-HDL was also significantly and positively correlated with BMI, also shown in **Fig. 6.** HDL-cholesterol was significantly correlated with both α_1- and α_3-HDL-apoA-I and also with pre-β_1-HDL-apoA-I. There was no significant relationship between either total cholesterol, triglycerides, or LDL-cholesterol and the distribution of apoA-I among HDL subfractions. ApoA-I concentrations among HDL subfractions differed between men and women within α_2- and pre-β_1-HDL ($P < 0.05$ for both).

Stepwise regression analysis

Stepwise regressions were carried out with those univariate correlations that had reached or approached a 1% level of significance or where $r > 0.4$, as shown in Table 3. **Table 4** shows those correlations that have contributed to the changes in apoA-I relative concentrations in HDL subfractions which in several analyses represent the combined contributions from each set of variables to changes in apoA-I relative concentrations in HDL subfractions (R^2). Decreasing BMI maintained independent associations with increasing apoA-I in α_1-HDL which accounted for 52% of the variance. BMI maintained significant independent association with apoA-I in pre-β_1-HDL (positive) which accounted for 33% and in α_3-HDL (negative), but the contribution to the variance was less (16%).

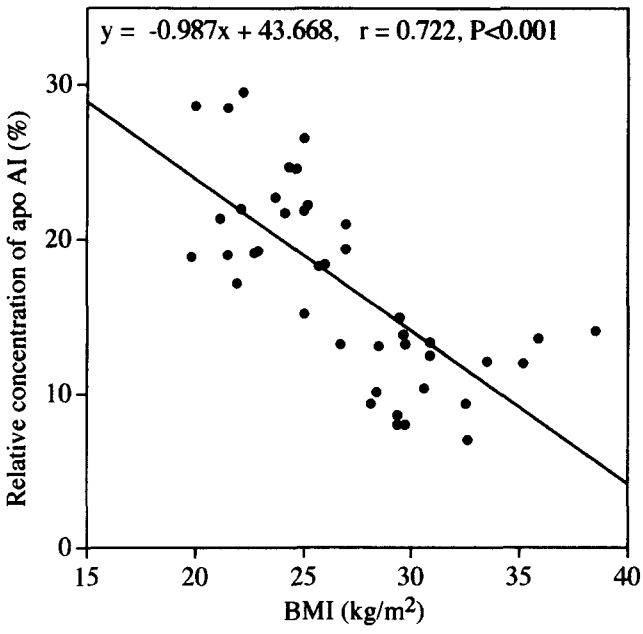

Fig. 5. Relationship between apoA-I in α_1-HDL (% of total apoA-I) and BMI (n = 41).

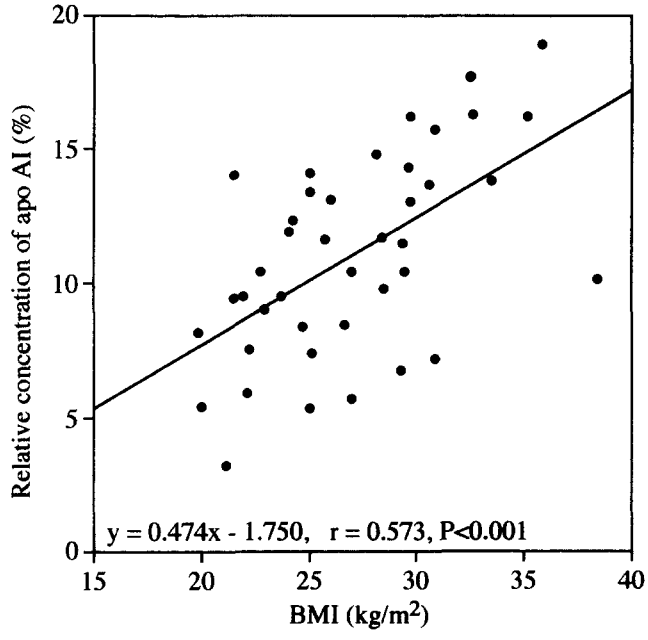

Fig. 6. Relationship between apoA-I in pre-β_1-HDL (% of total apoA-I) and BMI (n = 41).

TABLE 4. Matrix of stepwise regression analysis of univariate correlations

	Step	Variable	R^2	P
α_1	1	−BMI	0.52	<0.0001
α_2				
α_3	1	+BMI	0.16	0.0088
Pre-β_1	1	+BMI	0.33	0.0001
Pre-β_2				
Pre-β_3				

BMI, body mass index.

Effects of dietary intervention

Effects of oleic rich (MUFA) and α-linolenic acid rich (n−3 + n−6; PUFA) low-fat diets on plasma lipids and plasma apoA-I concentrations in obese subjects are also shown in Table 1. Total cholesterol level fell after MUFA diet only ($P < 0.05$), and total apoA-I level fell with both MUFA and PUFA diets ($P < 0.05$). Changes in apoA-I relative concentrations in HDL subfractions after diets are shown in **Fig. 7.** After MUFA diet apoA-I in α_1- and pre-β_3-HDL increased from 11.61 ± 2.87 and 0.34 ± 0.30 to 16.64 ± 2.91 and 0.57 ± 0.23%, respectively ($P < 0.05$ for both), and that in α_3-HDL decreased from 21.10 ± 4.90 to 15.91 ± 3.64% ($P < 0.05$). The percentage of apoA-I in α_2-HDL decreased and those in pre-β_1 and pre-β_2-HDL increased after the diet, but the differences were not significant. After PUFA diet, apoA-I relative concentrations in α_1- and pre-β_3-HDL increased to 15.87 ± 3.76 and 0.60 ± 0.15% respectively ($P < 0.05$ for both), and that in α_3-HDL decreased to 17.84 ± 4.45% ($P < 0.05$). The tendencies in other fractions were similar to those with the MUFA diet. Men and women showed similar responses to the diets with respect to α_1-, α_3-, and pre-β_3-HDL, but responses for α_2-, pre-β_1-, and pre-β_2-HDL were different. ApoA-I percentages in α_2-, pre-β_1-, and pre-β_2-HDL did not change in men, but α_2-HDL decreased and pre-β_1- and pre-β_2-HDL increased in women, though the differences were not significant.

DISCUSSION

Identification of a subpopulation of HDL particles, named pre-β-HDL because of their electrophoretic mobility, and their apparent importance in cholesterol transport has stimulated interest in their role in lipid metabolism. Yet little is known of the concentrations of these lipoproteins in the circulation of either normal subjects or in subjects with disorders that involve reduced HDL-cholesterol concentrations. Certain forms of dyslipidemia appear to favor paradoxically raised pre-

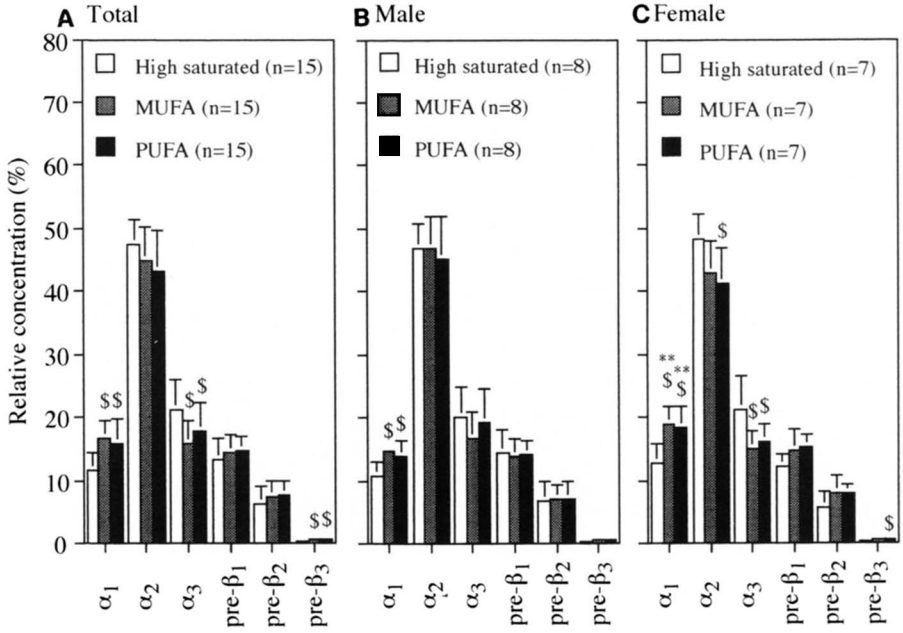

Fig. 7. Effects of mono- and polyunsaturated fatty acid-rich (MUFA and PUFA) low-fat diets on the distribution of apoA-I in HDL subfractions in obese subjects. High saturated, high saturated fat diet; MUFA, monounsaturated fatty acid-rich low-fat diet; PUFA, polyunsaturated fatty acid-rich low-fat diet $^{\$}P < 0.05$ vs. High saturated (ANOVA and paired Student-Newman-Keuls methods). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. male subjects, respectively (Student's *t*-test). The error bars represent standard deviation.

β-HDL, despite a low total HDL (21). In this paper, we report the concentrations of six species of apoA-I-containing HDL (quantified by their apoA-I content) in obese men and women, most of whom showed less than average HDL-cholesterol levels. As this group was gender-matched with a lean group, differences in the HDL distribution between men and women were also noted. The profile of HDL subfractions differed between obese and lean subjects (Fig. 3); α_1-HDL was significantly reduced in the obese, which was expected, as it is largely identical to apoA-I-containing lipoproteins (LpAI) in the largest HDL, HDL_{2b} (by the ultracentrifugal nomenclature), which is known to be low in obesity (16, 26, 27). The finding of raised pre-β_1-HDL is novel, and together with the raised α_3-HDL fraction (largely HDL_3 LpAI), it raises questions about the metabolic interrelationships of these HDL particles.

The correlations also supported the differences found between obese and lean people. BMI was directly correlated with pre-β_1-HDL and α_3-HDL and inversely with α_1-HDL, on both univariate and stepwise regression analysis (Tables 3 and 4).

Within the lean group of women the apoA-I concentration was significantly higher in α_2-HDL and lower in pre-β_1-HDL than in men. This is also consistent with the well-known gender differences in LpAI concentrations in larger HDL (15). Therefore, in this group of subjects, higher α_1-HDL (as apoA-I particles) were associated with higher total HDL-apoA-I levels (in lean people and in lean women) while higher pre-β_1- and α_3-HDL were linked to lower HDL-apoA-I levels (in obese people and in lean men).

To gain further insight into the relationship between subclass distribution and total apoA-I concentrations, HDL levels were further reduced in 15 obese subjects by dietary fat reduction (and possibly also by fatty acid modification) (Fig. 7). With both interventions (high oleic or high α-linolenic acids), α_1-HDL, which had been low in subjects on the high-fat, high saturated fatty acid diet, rose significantly despite lower apoA-I levels. The total reduction in apoA-I was reflected mainly in the α_2 (or LpAI in HDL_{2a}) fraction and α_3 (HDL_3-LpAI) fraction, although not all differences were significant. Changes in pre-β fractions were minor: pre-β_3 rose but is quantitatively a very small fraction.

In considering the implications for HDL metabolism, the origins and metabolism of pre-β-HDL particles are of particular interest. As pre-β-HDL are discoidal particles comprising protein, phospholipid, and free but minimal esterified cholesterol (2), they are regarded as possibly the earliest nascent HDL. Their origin may be multiple and their metabolic cycle relates to the key lipases and transfer proteins involved in the modeling of the HDL species from nascent to mature and back to nascent particles (10, 13). During the initial anabolic cycle, apoA-I in plasma and probably lymph associates with lipids (free cholesterol and phospholipid) to generate pre-β-HDL. The source of that lipid is from the surface of cells as part of the cholesterol efflux process or from the surface of catabolized mature HDL_2 (7, 10, 13) and other lipoproteins, and even as free fatty acid as shown recently by Clay and Barter (28). As HDL_2 transfers cholesteryl esters to apoB-containing lipoproteins under the influence of CETP, or has its core reduced through the action of hepatic triglyceride lipase (HTGL), excess surface lipid and apoA-I become dissociated (10). Thus, pre-β-HDL may also be generated from the catabolism of the most mature HDL.

Small discoidal HDL are primary acceptors of cellular cholesterol that is then partially esterified by LCAT, for which such discoidal particles are the preferred substrate (3, 29, 30). During this continuing cycle of lipid uptake into nascent HDL and the esterification of its free cholesterol, pre-β_1 particles are transformed into larger pre-β-HDL and eventually into α-HDL (5). The particles become progressively enriched in cholesteryl esters partly through the action of CETP, which is a requirement for the continuing activity of LCAT upon which the HDL metabolic cycle depends (31). LCAT directly esterifies a minor proportion of cell-derived cholesterol during its passage through pre-β-HDL, whereas most of the cholesterol is esterified in α-HDL, some after retransfer from LDL (5). Two lipid transfer proteins contribute to further HDL remodeling (32).

In this context, are there known effects of excess body weight on the preceding regulatory steps? To what extent may the different HDL profiles between men and women be explained on such a basis? The profile of HDL subfractions in obese subjects differed from that in lean subjects in that apoA-I concentration in α_1-HDL in obese subjects was significantly lower whereas those in α_2-, α_3-, and pre-β_1-HDL were significantly higher in obese subjects than those in lean subjects. Further, BMI correlated significantly with apoA-I concentration in each of the above HDL fractions except α_2-HDL and, on stepwise regression analysis, BMI accounted for 52% of the variance in α_1, 33% of the variance in pre-β_1-HDL, and for 16% of the variance in α_3-HDL.

Possible reasons for these findings include the demonstration of raised HTGL activities in male obese subjects (33) and higher plasma CETP activities in obese subjects of both sexes compared with lean subjects (33). The fractional catabolic rate of LpAI has been reported to be strongly correlated with intraabdominal fat (34). These regulatory steps might lead to higher pre-β_1 production. Increased HTGL activity would also lead to reduced α_1 and increased α_3-HDL as others have documented (35). Obese subjects also show an increased

turnover of body cholesterol including that from fat stores (36), and this increased flux of cholesterol through plasma might be expected to lead to more pre-β_1 formation. The low pre-β_2/pre-β_1 ratio (Fig. 4) in the obese suggests, in addition, possible impairment in the transformation of the smaller to the larger pre-β species and then to α-HDL. However, cholesterol esterification, measured kinetically in vivo, is increased in fat subjects (37). As the esterification of cholesterol by LCAT takes place on HDL, CETP may be expected to relieve product inhibition of this reaction by transferring cholesteryl esters out of HDL. Transgenic mice expressing human CETP and apoA-I show higher pre-β-HDL levels and heightened LCAT-mediated cholesterol esterification of effluxed cholesterol (38). CETP activity may therefore be expected to maintain the discoidal particles such as pre-β-HDL by continually removing cholesteryl ester as soon as produced by LCAT. These factors may underlie the higher levels of pre-β_1-HDL but they do not offer a reason for the trend to reduced pre-β_2-HDL.

Hypertriglyceridemia has been reported to lead to an increase in plasma pre-β-HDL of up to 40% of total plasma apoA-I compared with normolipidemic subjects (21). In the present study, plasma triglycerides were significantly higher than those in lean subjects ($P < 0.001$ for all subjects, $P < 0.01$ for either men or women), although there was no significant relationship between triglycerides and pre-β-HDL–apoA-I levels. HTGL activity is also raised in hypertriglyceridemia associated with low HDL (39), which together with increased cholesteryl ester transport (40) offer a likely explanation for increased pre-β-HDL concentrations.

With respect to the differences between men and women, plasma CETP levels have been shown to be about 25% higher in women than in men (41). Shoji et al. (42) reported that there was no significant difference in LCAT activities between genders, but men had 1.7- to 2.5-fold higher HTGL activities than women. The latter is consistent with reports that men have significantly higher HDL_{3b} and significantly lower HDL_{2a} (α_2) and HDL_{2b} (α_1) levels than women (15), and also consistent with our results that men have significantly lower α_2-HDL and significantly higher pre-β_1-HDL levels than women. The HDL apoA-I fractional catabolic rate in women is lower than in men accounting partly for their higher apoA-I concentrations (43).

Although there was only a trend to lower pre-β_2-HDL, the absence of a rise to match the level in pre-β_1 raises the possibility of some inhibition of pre-β_1 to pre-β_2 transformation.

In the current study, the low-fat (26% energy fat) diet led to lower total apoA-I concentrations as anticipated. This has been reported to be due to increased catabolism of HDL (44, 45), but we did not observe any increase in pre-β_1-HDL. There was, however, a rise in α_1-HDL that had previously been depressed and this may reflect increased cholesterol transport which has been reported to occur with increasing unsaturation of HDL phospholipid acyl chains (22). However, the number of subjects in the diet study was only 15, and therefore the results should be viewed as tentative. Nevertheless, both unsaturated fatty acids led to a rise in α_1-HDL.

Finally, what are the implications of these findings for cholesterol efflux? Despite the presumptive initial role for pre-β_1-HDL in this process, other HDL species, especially those containing apoA-I rather than apoA-II, have the capacity to accept cellular cholesterol (11, 46) and in this respect, HDL_3 or α_3-HDL have been shown to be particularly potent (47). Even in severe inherited abnormalities of apoA-I and HDL in general, in vitro efflux of cholesterol, though reduced, is not abolished (48, 49). An apoE-containing particle, although a very minor component of HDL, has been shown to be capable of accepting surface cholesterol (50) as can apoB-containing lipoproteins (5).

We have found that both pre-β- and α-HDL particles, measured by their apoA-I content, are affected by obesity. Further, within both the pre-β- and the α-groups, there is a dissociation in their distribution, so that apoA-I concentrations in pre-β_1- and α_3-HDL rise as BMI increases whereas those in α_1-HDL fall. Although the responsible mechanisms are uncertain, it is possible that the reduced HDL-cholesterol levels often observed in obese subjects represent, in part, disturbance in reverse cholesterol transport that may require a sequential transfer of cholesterol along a chain of their HDL particles.

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