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Serum Lipids, Lipoproteins, and Lipid Metabolizing Enzymes in Identical Twins Discordant for Obesity

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ABSTRACT

Obesity is associated with adverse changes in plasma lipoprotein metabolism, but it is not known completely how this association is modified by genetic factors. We assessed the contribution of obesity to serum lipid and lipoprotein levels and lipid metabolizing enzyme activities by examining 23 identical twin pairs (9 male, 14 female) who had, on the average, an 18-kg intrapair difference in BW. Compared with lean co-twins, obese co-twins had approximately 20% higher low-density lipoprotein (LDL) cholesterol ($P < 0.01$), 20% lower high-density lipoprotein$_2$ cholesterol ($P = 0.010$), and 90% (men) or 35% (women) higher ($P \leq 0.06$) total, very-low-density lipoprotein and LDL triglycerides. The pairs were divided into subgroups by the gender-specific median value of abdominal visceral fat (AVF) area in the obese co-twin and by apolipoprotein E 4 phenotype. The intrapair differences in serum cholesterol fractions were similar in twin pairs with high or low AVF, whereas only high AVF pairs showed significant differences in triglyceride fractions. The greatest intrapair differences in total, very-low-density lipoprotein and LDL triglycerides were observed in apolipoprotein E 4-positive pairs expressing high AVF. Compared with lean co-twins, lecithin cholesterol acyltransferase activity was 18% higher ($P < 0.001$) and hepatic lipase activity was 38% higher ($P = 0.016$) in obese co-twins with high AVF. When genetic factors are identical, obesity is associated with an atherogenic lipid profile, especially in subjects with high visceral fat accumulation. (*J Clin Endocrinol Metab* **83:** 2792–2799, 1998)

OBESITY is related to several disturbances in lipid and lipoprotein metabolism. High concentrations of serum triglyceride-rich lipoproteins and a low concentration of high-density lipoprotein (HDL) cholesterol are the most characteristic findings, whereas serum low-density lipoprotein (LDL) cholesterol level is usually much less elevated in obesity (1, 2). Most studies have found no effect of obesity on serum lipoprotein (a) [Lp(a)] levels (3). Altered levels of serum triglycerides and HDL cholesterol in obesity might be mediated by changes in the activities of the key lipoprotein metabolizing enzymes [*i.e.* increased activity of hepatic lipase (4, 5); decreased or unchanged activity of postheparin plasma lipoprotein lipase (LPL) (4–6); high or normal activity of adipose tissue LPL (4, 7–9); high, normal, or low lecithin cholesterol acyltransferase (LCAT) activity (10–12); and high activity of cholesterol ester transfer protein (CETP) (5, 11)].

Because all obese subjects do not exhibit dyslipidemia, two lines of research have emerged in an effort to explain this heterogeneity. One has to do with fat topography; subjects with upper body obesity, particularly visceral obesity are more prone to low HDL cholesterol and high triglycerides than subjects with peripheral obesity (13, 14). The second pertains to genetic variations in apolipoproteins and other relevant genes as potential determinants of a subject susceptibility to dyslipidemia in an obese state. Most of these studies have dealt with the effect of apolipoprotein E (ApoE) polymorphism, but they have not given consistent results (15–18).

Previous cross-sectional studies on the association between serum lipids and obesity have been hampered by the fact that it has not been possible to distinguish between the effects of obesity and those of all genetic factors affecting lipid metabolism. This can be avoided by examining the same obese subjects before and after weight reduction. However, this approach also has its problems, because the metabolism of adipose tissue in the postobese state is known to differ from that of subjects who have constantly been lean (19).

One powerful strategy to study the independent contribution of obesity to lipoprotein metabolism is to examine identical twins who are discordant for obesity. Consequently, we measured serum lipids, lipoproteins, and lipid metabolizing enzymes in 23 pairs of identical twins who had a mean 18-kg intrapair difference in BW. The role of the regional distribution of fat and ApoE polymorphism is emphasized in the present study.

Subjects and Methods

Subjects

The Finnish Twin Cohort includes all pairs (4307 monozygous, 9581 like-sexed dizygous pairs) of adult Finnish twins born before 1958 and

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alive in 1975 (20). Questionnaires to obtain information on subjects' height and weight had been sent to the twins in 1975, 1981, and 1990. Based on the latest questionnaire, identical twin pairs, born between 1932 and 1957, and discordant for obesity were identified. Discordance for obesity was defined as a difference in body mass index (BMI) of at least 4 kg/m^2 and, in addition, the BMI of the obese co-twin had to be more than 27 kg/m^2, and the BMI of the lean co-twin had to be less than 25 kg/m^2. Fifty twin pairs fulfilled the age and BMI criteria. From these pairs, those including subjects with a history of thyroid disorders, psychiatric diseases, diabetes (three pairs), major musculoskeletal problems, and other diseases or taking medications (*e.g.* diuretics or β-blockers) possibly affecting lipid or glucose metabolism were excluded. All eligible twin pairs, based on a response letter, were invited to take part in the present study, provided they still fulfilled the criteria. A total of 28 twin pairs were examined.

The physical examination revealed that two of the pairs had a BMI difference less than 3 kg/m^2, and they were excluded. Three pairs had a BMI intrapair difference between 3 and 4 kg/m^2, and they were included in the final study population. The obese co-twin of one pair was found to have previously undiagnosed overt diabetes mellitus, and this pair was excluded.

The assessment of the zygosity of the twin pairs was originally based on a self-administered questionnaire dealing with similarity during the school ages and validated by 11 blood group markers (21). The monozygosity of the pairs of the present study was confirmed by dermatoglyphic analysis of fingertip prints (22, 23) by a highly experienced expert. All except 6 pairs were confirmed to be monozygotic. DNA samples of the 6 pairs with uncertain zygosity were typed for markers at 6 different polymorphic gene loci (DIS80, APOB, D17S30, COL2A1, VWA, and HUMTH). Four of the pairs were found to be monozygotic, and the 2 other pairs were dizygotic; these 2 pairs were excluded. Thus, the final study sample consisted of 23 identical twin pairs (14 female, 9 male) with more than 3 kg/m^2 intrapair difference in BMI (a mean intrapair discordance in BW of 18 kg) without any diseases or continuous medication. Characteristics of the subjects are shown in Table 1. Three of the 23 pairs had a positive family history for diabetes (diabetes in at least 1 first-degree relative). At the time of the examination, 1 pair was still living in the same household (age, 39 yr), and 22 pairs were living apart. These 22 pairs had lived together, on average, to the age of 19.7 yr (range, 14–28 yr).

The study was approved by the local ethics committee, and informed consent was obtained from all subjects.

Measurement of adiposity and blood pressure and estimation of diet

Adiposity was expressed as BMI (kg/m^2) and as the percentage of body fat. The latter was determined using the four-component method (24). The method is based on the division of body mass into four components with different densities: fat tissue (0.9007 g/cm^3), water (0.994 g/cm^3), minerals (3.042 g/cm^3), and proteins (1.34 g/cm^3). Water mass was estimated by the bioelectric impedance method (BIA-101A/S, RJL System Inc., Clemens, MI) (25). Mineral mass was estimated by dual-energy x-ray absorptiometry (Norland XR26, Norland Corporation, Fort Atkinson, Wisconsin). The density of the whole body was estimated by underwater weighing, corrected for information on body water and mineral mass. The proportion of fat tissue was calculated from the density of the whole body, according to the formula of Siri (26).

The distribution of body fat was measured by magnetic resonance imaging (MRI) (27). Imaging was performed at 0.1 tesla (Mega4, Instrumentarium Co, Imaging Division, Helsinki, Finland). Axial and sagittal localizers were used to obtain a transaxial T1-weighted image (relaxation time/echo time = 155/20; slice thickness, 10 mm) at the level of the fourth lumbar vertebra. Visceral and sc fat areas were measured. MRI was not performed in three pairs (two female, one male), either because of claustrophoby caused by the small space for the subject in the device or because of temporary malfunctioning of the MRI equipment. To classify the twins according to the degree of visceral fat accumulation, the twin pairs were divided according to the median value of abdominal visceral fat (AVF) area in the obese co-twin, as described earlier (13). Because men have a higher proportion of visceral fat than women, separate median values were used for men (109 cm^2) and women (58 cm^2).

Blood pressure was measured with a standard mercury sphygmomanometer in sitting position after a 5-min rest. The fifth phase of the Korotkoff's sounds was taken as diastolic blood pressure.

Food and alcohol consumption were estimated by the 1-yr dietary history method by an experienced nutritionist. Nutrient compositions were analyzed with a Nutrica PC program that uses a data base containing energy and nutrient compositions of more than 650 of the most commonly eaten Finnish foods (28).

Biochemical methods

Venous blood samples were drawn after a 12-h fast. Serum cholesterol concentration was determined using a fully enzymatic method (29) (cholesterol oxidase-p-aminophenazone; Merck, Darmstadt, Germany) in an autoanalyzer (AU 510; Olympus, Hamburg, Germany). For the separation of very-low-density lipoprotein (VLDL) fraction, serum was centrifuged (18 h, 105,000 $\times$ *g*) at a density of 1.006 g/mL (30). After removing VLDL, LDL was precipitated from the infranatant (HDL + LDL) with dextrane sulphate 500,000-magnesium chloride, according to the method of Kostner (31). Cholesterol (29) and triglycerides (fully enzymatic method, Boehringer, Mannheim, Germany) were assayed from the total serum, HDL + LDL and HDL fractions. VLDL and LDL cholesterol and triglycerides were calculated by subtraction. The proportions of HDL subfractions, HDL_2, and HDL_3, were determined by differential precipitation with heparin-manganese and low-molecular-weight dextran sulphate (32). Concentrations of ApoA-I and ApoB were obtained by an immunoprecipitation method (Orion Diagnostica, Es-

TABLE 1. Characteristics of identical twins discordant for obesity

	Men (9 pairs)			Women (14 pairs)		
	Obese	*P*	Nonobese	Obese	*P*	Nonobese
Age (yr)	44 ± 7		44 ± 7	46 ± 8		46 ± 8
Height (cm)	176 ± 9	NS	175 ± 10	163 ± 7	NS	163 ± 6
Weight (kg)	88.9 ± 8.7	<0.001	72.9 ± 9.5	79.1 ± 6.4	<0.001	59.8 ± 6.0
BMI (kg/m^2)	28.8 ± 1.6	<0.001	23.7 ± 1.0	30.0 ± 3.2	<0.001	22.4 ± 1.5
% Body fat	26.0 ± 2.8	<0.001	20.3 ± 3.6	39.7 ± 5.3	<0.001	27.4 ± 5.3
AVF (cm^2)	128 ± 67	0.003	57 ± 25	69 ± 34	<0.001	30 ± 13
ASF (cm^2)	238 ± 61	<0.001	172 ± 55	303 ± 45	<0.001	156 ± 42
Systolic BP (mm Hg)	127 ± 9	0.18	119 ± 12	123 ± 16	0.054	119 ± 13
Diastolic BP (mm Hg)	83 ± 8	0.12	76 ± 9	80 ± 7	<0.001	75 ± 8
Serum gamma GT (U/L)	69 ± 43	0.26	47 ± 41	40 ± 29	0.30	28 ± 29
Alcohol intake (g/day)	9.9 ± 6.7	0.49	14.4 ± 22.3	6.4 ± 10.7	0.16	2.1 ± 2.0
Saturated fat (E%)	16.2 ± 3.8	0.62	17.0 ± 3.4	16.8 ± 3.1	0.61	16.3 ± 3.7
Sucrose (E%)	9.6 ± 2.2	0.13	8.0 ± 2.8	7.6 ± 3.8	0.92	7.5 ± 2.2
No. of smokers	4/9	NS	7/9	3/14	NS	5/14

AVF, Abdominal visceral fat area; ASF, Abdominal subcutaneous fat area; BP, blood pressure; GT, glutamyl transferase; E%, proportion of total daily energy intake; NS, not significant.

poo, Finland), according to Riepponen *et al.* (33). ApoB was determined from total serum and its VLDL fraction. LDL-ApoB was calculated by subtracting VLDL-ApoB from total serum ApoB. Serum Lp(a) was assayed by immunoradiometry (Pharmacia, Uppsala, Sweden) (34).

Serum LCAT activity was measured by a modified method of Alcindor *et al.* (35), according to Rönnemaa *et al.* (36). Postheparin plasma LPL and hepatic lipase activities were measured by the immunochemical method of Huttunen *et al.* (37), as described by Peltonen *et al.* (38). Adipose tissue (biopsy taken from abdominal sc fat) LPL activity was measured from the heparin eluates of the biopsy samples using ^{14}C-triolein (Amersham, Buckinghamshire, England) as substrate (39). Adipose tissue DNA content was determined according to Curtis-Prior *et al.* (40). The activity of adipose tissue LPL was expressed per milligram DNA. Serum CETP activity was determined, as described earlier, by incubating the sample containing CETP with labeled human LDL and unlabeled human HDL and by detecting the transfer of radioactive cholesteryl esters (41).

ApoE phenotyping of serum samples was carried out by isoelectric focusing and immunoblotting after delipidation (42).

Serum γ-glutamyl transferase was measured enzymatically, according to the European Committee for Clinical Laboratory Standards.

Statistical methods

A paired *t* test was used to compare means and the McNemar's test to compare the proportions between obese and lean co-twins. Because of their skewed distributions, triglycerides and their subfractions, and Lp(a) were handled after logarithmic transformation. The data are presented as mean ± SD.

Results

The background characteristics of the study subjects are given in Table 1. The average difference in weight between obese and lean co-twins was approximately 16 kg among men and 19 kg among women. The intrapair difference in body fat percentage was 5.7% in men, and in women 12.3%. Men had a higher intrapair difference in AVF area than women, whereas women had a higher intrapair difference in abdominal sc fat area, compared with men. Systolic and diastolic blood pressure tended to be slightly higher in the obese co-twins, but the intrapair differences were significant only among women. There was no significant difference between obese and lean co-twins in serum γ-glutamyl transferase, alcohol intake, proportion of saturated fat or sucrose from total daily energy intake, or in the proportion of smokers.

Serum total cholesterol was approximately 12% higher, and LDL cholesterol was almost 20% higher, in obese co-twins in both genders (Table 2). Compared with lean co-twins, obese co-twins had approximately 10% lower HDL cholesterol level, and this difference was entirely caused by their lower HDL_2 cholesterol concentration. Thus, the HDL_2/HDL_3 cholesterol ratio was significantly higher in lean co-twins. Compared with lean co-twins, serum total, VLDL, and LDL triglyceride concentrations were significantly higher in obese co-twins (the difference being more marked among male twin pairs). The HDL triglyceride level was higher in male obese co-twins, compared with the lean co-twins.

ApoA-I levels were similar in obese and lean co-twins, whereas the ApoB concentration was 29% higher in male and 22% higher in female obese co-twins. The LDL-ApoB level was higher in obese co-twins in both genders, but the LDL-triglyceride/LDL-ApoB ratio was higher in obese co-twins only among men. Compared with lean co-twins, serum Lp(a) level tended to be slightly higher in obese females, but not in male co-twins. Obese co-twins had significantly higher LCAT activity in both genders, higher postheparin plasma hepatic lipase activity, and a tendency towards lower adipose tissue LPL activity in females, and similar postheparin plasma LPL and plasma CETP activities, compared with lean co-twins, both among men and women.

To determine whether the association between obesity and lipid and lipoprotein concentrations is modified by visceral

TABLE 2. Serum lipids, lipoproteins, and lipid metabolizing enzymes in identical twins discordant for obesity

Variable	Men (9 pairs)			Women (14 pairs)		
	Obese	*P*	Nonobese	Obese	*P*	Nonobese
Cholesterol						
Total, mmol/L	6.28 ± 0.95	0.017	5.59 ± 1.33	6.41 ± 1.07	0.021	5.79 ± 0.94
VLDL, mmol/L	0.49 ± 0.23	0.61	0.43 ± 0.14	0.46 ± 0.25	0.30	0.39 ± 0.14
LDL, mmol/L	4.93 ± 0.84	0.004	4.18 ± 1.18	4.86 ± 0.91	0.008	4.21 ± 0.94
HDL, mmol/L	0.85 ± 0.10	0.082	0.95 ± 0.16	1.09 ± 0.23	0.093	1.19 ± 0.30
HDL_2, mmol/L	0.26 ± 0.05	0.010	0.34 ± 0.07	0.43 ± 0.17	0.011	0.54 ± 0.21
HDL_3, mmol/L	0.59 ± 0.07	0.47	0.61 ± 0.11	0.65 ± 0.11	0.73	0.65 ± 0.11
HDL_2/HDL_3	0.45 ± 0.08	0.004	0.55 ± 0.11	0.64 ± 0.23	0.005	0.80 ± 0.24
Triglycerides						
Total, mmol/L	1.92 ± 0.72	0.004	1.14 ± 0.29	1.54 ± 0.64	0.056	1.14 ± 0.61
VLDL, mmol/L	0.72 ± 0.29	0.014	0.41 ± 0.12	0.53 ± 0.29	0.057	0.39 ± 0.28
LDL, mmol/L	1.07 ± 0.51	0.001	0.63 ± 0.23	0.84 ± 0.36	0.051	0.61 ± 0.32
HDL, mmol/L	0.13 ± 0.03	0.019	0.10 ± 0.03	0.17 ± 0.05	0.15	0.15 ± 0.06
ApoA-I, g/L	1.23 ± 0.10	0.66	1.26 ± 0.18	1.33 ± 0.17	0.25	1.40 ± 0.26
ApoB, g/L	1.26 ± 0.27	0.003	0.98 ± 0.25	1.11 ± 0.25	0.006	0.91 ± 0.27
LDL-ApoB, g/L	1.05 ± 0.22	<0.001	0.83 ± 0.24	0.97 ± 0.20	0.009	0.79 ± 0.23
LDL-TG/LDL-ApoB	1.00 ± 0.31	0.022	0.77 ± 0.18	0.83 ± 0.28	0.45	0.74 ± 0.32
Lp(a), mg/L	135 ± 216	0.17	152 ± 199	224 ± 184	0.052	187 ± 141
LPL, U/L	270 ± 56	0.48	252 ± 78	242 ± 29	0.75	238 ± 50
Hepatic lipase, U/L	265 ± 140	0.25	226 ± 144	179 ± 87	0.002	133 ± 61
CETP, μmol/L/h	80.8 ± 21.4	0.38	77.9 ± 24.4	97.7 ± 23.1	0.77	99.1 ± 23.2
LCAT, % chol est/h	27.3 ± 3.1	0.002	23.3 ± 4.4	24.6 ± 3.1	0.013	22.1 ± 2.9
Adipose tissue LPL, μmol FFA/mgDNA/h	26 ± 17	0.66	33 ± 45	30 ± 14	0.061	47 ± 27

FFA, Free fatty acids.

fat level, we compared twin pairs with AVF area above and below gender-specific median value in the obese co-twin. Genders were combined in these analyses (Tables 3 and 4). In addition to a greater intrapair difference in AVF area, also the intrapair differences in BMI, percent body fat, and sc fat area were higher in the subgroup with high AVF area, compared with the subgroup with low AVF area. However, the average intrapair differences in abdominal sc fat area were 134 cm^2 and 95 cm^2 in the high and low AVF groups, respectively (*i.e.* only a 1.4-fold difference), whereas the intrapair differences in AVF area were 78 cm^2 and 26 cm^2 in the high and low AVF groups, respectively (*i.e.* a 3-fold difference).

With respect to serum cholesterol subfractions, the intrapair differences were essentially similar in high and low AVF subgroups, except that the difference in the HDL_2/HDL_3 cholesterol ratio was greater in the subgroup with high AVF area compared with the subgroup with less visceral fat (Table 4). On the other hand, serum total, VLDL, LDL, and HDL triglyceride concentrations were higher in the obese (compared with the lean) co-twins only in the subgroup with high visceral fat accumulation. The LDL-ApoB level was higher in obese co-twins, independent of AVF subgroup, but the LDL-triglyceride/LDL-ApoB ratio was higher in obese co-twins only in the high AVF subgroup. Compared with the lean co-twins, postheparin plasma hepatic lipase and plasma LCAT activities were significantly higher, and adipose tissue LPL tended to be lower, in the obese co-twins only in the subgroup with high AVF level.

To clarify the possible role of ApoE phenotype in the association between obesity and lipid and lipoprotein values, we divided the twin pairs into two subgroups: ApoE4+ group, consisting of pairs with either E4/3 (7 pairs) or E4/4 (4 pairs) phenotype; and ApoE4- group, consisting of pairs with either E3/3 (10 pairs) or E3/2 (2 pairs) phenotype (Table 5). There were no pairs with phenotype E2/2 or E4/2. In ApoE4+ pairs, mean ± SD percent body fat was 34.4 ± 8.6 and 25.3 ± 5.3% ($P < 0.001$); and in ApoE4− pairs, 34.0 ± 7.8 and 23.8 ± 6.4% ($P < 0.001$) in obese and lean co-twins, respectively. The intrapair differences in serum cholesterol and its subfractions, as well as differences in serum triglycerides and their subfractions, were essentially similar in ApoE4+ and ApoE4− subgroups. However, both obese and nonobese ApoE4+ subjects had higher total and LDL cholesterol; lower HDL_2 cholesterol and HDL_2/HDL_3 cholesterol ratio;, and higher total, VLDL, and LDL triglyceride concentrations (compared with ApoE4− subjects of each body mass category).

The possible interaction between excess adiposity, high AVF, and ApoE phenotype, with respect to serum triglycerides and their subfractions, was studied by further subgrouping of the pairs (Fig. 1). Compared with lean co-twins, the average serum total, VLDL, and LDL triglyceride levels were approximately 2.5-fold higher in obese co-twins in the high AVF, ApoE4+ subgroup and approximately 1.5-fold higher in the high AVF, ApoE4− subgroup. On the contrary, in the low AVF, ApoE4− subgroup, there were no differences in serum triglycerides or their subfractions between obese and lean co-twins.

Discussion

Our results showed that an average overweight amount of 18 kg was associated with a 20% increase in LDL cholesterol; a 22% decrease in HDL_2 cholesterol (but no change in HDL_3 cholesterol); and a striking 90% increase in total, VLDL, and LDL triglycerides in men (but only a 35% increase in women). Moreover, HDL-triglycerides were higher in obese male co-twins, compared with lean co-twins. Our results are in accordance with the results from earlier studies, in large populations, on the association between obesity and the main CHD lipid and lipoprotein risk factors (1, 2). However, because our study was performed in identical twins, differences in serum lipids and lipoproteins between obese and lean co-twins are entirely caused by obesity or obesity-related factors and are not confounded by genetic factors like those in studies comparing unrelated obese and lean subjects. In two previous twin studies with a design resembling ours, much smaller intrapair differences were found in serum cholesterol, HDL cholesterol, and triglyceride concentrations (43, 44). However, in these studies, the obese twins were only approximately 5–7 kg heavier than the lean co-twins, and lipid subfractions were not examined. Thus, compared with previous studies, our results provide stronger evidence for the independent role for obesity in the pathophysiology of lipid disorders.

Because obese co-twins, especially men, had a greater increase in LDL-triglyceride level than in LDL-Apo B level, our data indicate that obesity was associated with compositional changes in LDL. This is in accordance with studies showing that subjects with a high BMI more often have small dense LDL particles than lean subjects (45).

When the twin pairs were divided into subgroups according to the level of visceral fat, markedly higher intrapair differences were observed in serum triglycerides and their subfractions among pairs with high AVF area, compared with pairs with low AVF area. Our pairs with high AVF area also had a greater intrapair difference in percent body fat,

TABLE 3. Adiposity variables in identical twins stratified by gender-specific median value of abdominal visceral fat area in the obese cotwin; genders combined

Variable	AVF greater than median (10 pairs)			AVF less than median (10 pairs)		
	Obese	*P*	Nonobese	Obese	*P*	Nonobese
BMI, kg/m^2	30.2 ± 2.2	<0.001	22.5 ± 1.9	27.9 ± 1.2	0.001	23.2 ± 0.5
% Body fat	35.7 ± 7.6	<0.001	23.5 ± 5.6	31.4 ± 5.6	<0.001	25.3 ± 4.2
AVF, cm^2	127 ± 60	<0.001	49 ± 28	58 ± 24	0.001	32 ± 12
ASF, cm^2	275 ± 79	<0.001	141 ± 47	279 ± 37	<0.001	184 ± 39

AVF, Abdominal visceral fat area; ASF, Abdominal subcutaneous fat area.

TABLE 4. Serum lipids, lipoproteins, and lipid metabolizing enzymes in identical twins stratified by gender-specific median value of abdominal visceral fat area in the obese cotwin; genders combined

Variable	AVF greater than median (10 pairs)			AVF less than median (10 pairs)		
	Obese	*P*	Nonobese	Obese	*P*	Nonobese
Cholesterol						
Total, mmol/L	6.51 ± 0.82	0.012	5.70 ± 0.89	6.09 ± 1.13	0.072	5.62 ± 1.24
VLDL, mmol/L	0.50 ± 0.24	0.22	0.38 ± 0.10	0.37 ± 0.22	0.81	0.39 ± 0.14
LDL, mmol/L	5.01 ± 0.74	0.004	4.25 ± 0.91	4.72 ± 0.98	0.029	4.08 ± 1.14
HDL, mmol/L	1.00 ± 0.23	0.074	1.07 ± 0.27	1.01 ± 0.25	0.12	1.13 ± 0.32
HDL_2, mmol/L	0.33 ± 0.13	0.017	0.43 ± 0.21	0.41 ± 0.19	0.041	0.49 ± 0.20
HDL_3, mmol/L	0.67 ± 0.11	0.28	0.64 ± 0.09	0.60 ± 0.07	0.34	0.64 ± 0.15
HDL_2/HDL_3	0.49 ± 0.15	0.010	0.66 ± 0.25	0.67 ± 0.24	0.038	0.75 ± 0.24
Triglycerides						
Total, mmol/L	1.79 ± 0.66	0.004	0.98 ± 0.29	1.54 ± 0.80	0.17	1.19 ± 0.42
VLDL, mmol/L	0.64 ± 0.29	0.004	0.34 ± 0.14	0.49 ± 0.27	0.29	0.39 ± 0.16
LDL, mmol/L	1.00 ± 0.41	0.003	0.53 ± 0.17	0.90 ± 0.53	0.11	0.66 ± 0.28
HDL, mmol/L	0.15 ± 0.03	0.010	0.11 ± 0.04	0.15 ± 0.06	0.56	0.14 ± 0.07
ApoA-I, g/L	1.29 ± 0.16	0.98	1.29 ± 0.22	1.30 ± 0.18	0.17	1.40 ± 0.27
ApoB, g/L	1.19 ± 0.27	0.002	0.90 ± 0.24	1.11 ± 0.28	0.022	0.93 ± 0.26
LDL-ApoB, g/L	1.05 ± 0.19	0.003	0.79 ± 0.22	0.95 ± 0.23	0.021	0.80 ± 0.24
LDL-TG/LDL-ApoB	0.92 ± 0.29	0.035	0.65 ± 0.15	0.92 ± 0.35	0.53	0.84 ± 0.31
Lp(a), mg/L	158 ± 189	0.44	141 ± 108	237 ± 233	0.63	225 ± 215
LPL, U/L	263 ± 37	0.77	268 ± 71	242 ± 51	0.19	220 ± 49
Hepatic lipase, U/L	261 ± 114	0.016	189 ± 67	193 ± 120	0.25	168 ± 144
CETP, μmol/L/h	89.9 ± 27.0	0.75	88.6 ± 23.9	87.8 ± 20.6	0.97	88.0 ± 29.8
LCAT, % chol est/h	27.0 ± 3.7	<0.001	22.9 ± 3.4	24.3 ± 3.1	0.061	21.6 ± 3.9
Adipose tissue LPL, μmol FFA/mgDNA/h	26 ± 12	0.063	51 ± 38	31 ± 19	0.85	33 ± 26

FFA, Free fatty acids.

TABLE 5. Serum lipids, lipoproteins, and lipid metabolizing enzymes in identical twins discordant for obesity stratified by ApoE4 phenotype; genders combined

Variable	ApoE4 + (11 pairs)			ApoE4 − (12 pairs)		
	Obese	*P*	Nonobese	Obese	*P*	Nonobese
Cholesterol						
Total, mmol/L	6.76 ± 0.96	0.073	6.25 ± 0.91	5.98 ± 0.93	0.005	5.21 ± 1.01
VLDL, mmol/L	0.52 ± 0.27	0.38	0.43 ± 0.17	0.42 ± 0.20	0.50	0.38 ± 0.10
LDL, mmol/L	5.29 ± 0.74	0.022	4.72 ± 0.86	4.52 ± 0.83	0.002	3.72 ± 0.94
HDL, mmol/L	0.96 ± 0.20	0.065	1.09 ± 0.29	1.04 ± 0.24	0.15	1.10 ± 0.28
HDL_2, mmol/L	0.33 ± 0.12	0.007	0.43 ± 0.17	0.39 ± 0.18	0.036	0.48 ± 0.22
HDL_3, mmol/L	0.62 ± 0.11	0.42	0.66 ± 0.14	0.65 ± 0.09	0.080	0.62 ± 0.09
HDL_2/HDL_3	0.54 ± 0.16	0.004	0.65 ± 0.18	0.59 ± 0.24	0.013	0.75 ± 0.27
Triglycerides						
Total, mmol/L	1.96 ± 0.75	0.047	1.30 ± 0.59	1.44 ± 0.53	0.011	0.99 ± 0.37
VLDL, mmol/L	0.67 ± 0.29	0.082	0.45 ± 0.27	0.55 ± 0.30	0.011	0.35 ± 0.16
LDL, mmol/L	1.14 ± 0.49	0.030	0.72 ± 0.31	0.74 ± 0.27	0.014	0.51 ± 0.23
HDL, mmol/L	0.15 ± 0.06	0.14	0.13 ± 0.07	0.15 ± 0.03	0.022	0.13 ± 0.04
ApoA-I, g/L	1.30 ± 0.15	0.42	1.36 ± 0.25	1.28 ± 0.16	0.34	1.33 ± 0.23
ApoB, g/L	1.29 ± 0.26	0.018	1.07 ± 0.23	1.05 ± 0.22	<0.001	0.81 ± 0.23
LDL-ApoB, g/L	1.13 ± 0.16	0.015	0.94 ± 0.20	0.90 ± 0.18	0.001	0.70 ± 0.20
LDL-TG/LDL-ApoB	1.00 ± 0.36	0.078	0.74 ± 0.21	0.82 ± 0.22	0.54	0.76 ± 0.31
Lp(a), g/L	210 ± 208	0.02	187 ± 197	170 ± 195	0.52	161 ± 132
LPL, U/L	254 ± 49	0.80	251 ± 78	251 ± 38	0.45	237 ± 42
Hepatic lipase, U/L	243 ± 148	0.068	189 ± 146	184 ± 71	0.014	149 ± 57
CETP, μmol/L/h	90.2 ± 27.3	0.86	89.2 ± 29.5	91.9 ± 20.7	0.91	92.4 ± 22.3
LCAT, % chol est/h	26.7 ± 3.0	0.052	24.1 ± 2.9	24.7 ± 3.4	<0.001	21.2 ± 3.6
Adipose tissue LPL, μmol FFA/mgDNA/h	27 ± 15	0.15	42 ± 31	30 ± 15	0.36	43 ± 38

FFA, Free fatty acids.

compared with low AVF pairs. This might have affected our findings concerning the association between visceral obesity and high levels of triglyceride-rich lipoproteins. However, the average intrapair differences in abdominal sc fat area were 134 cm^2 and 95 cm^2 in the high and low AVF groups, respectively (*i.e.* only a 1.4-fold difference), whereas the intrapair differences in AVF area were 78 cm^2 and 26 cm^2 in the high and low AVF groups, respectively (*i.e.* a 3-fold difference). Thus, our obese co-twins in the high AVF subgroup were specifically characterized by visceral fat accumulation and, to a much lesser extent, by sc fat accumulation. Therefore, our results are in accordance with findings from studies

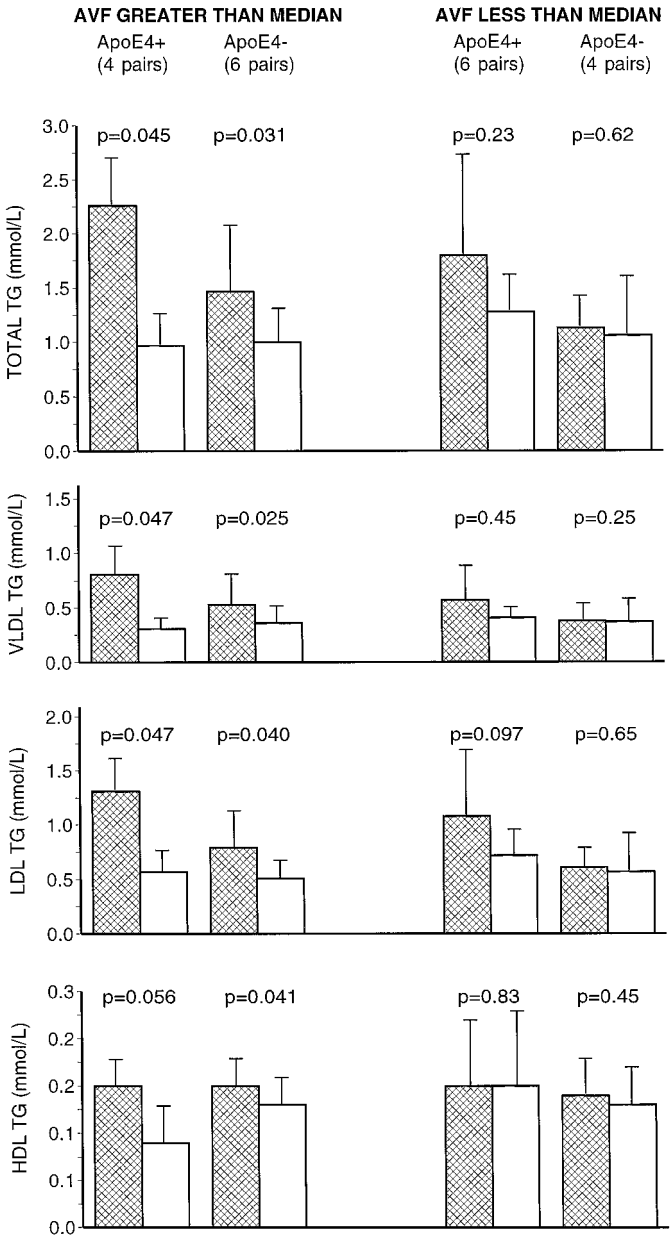

FIG. 1. Serum triglycerides and their subfractions in identical twins discordant for obesity, stratified by gender-specific median value of AVF area in the obese co-twin and by ApoE4 phenotype. Genders were combined. *Hatched bars*, Obese co-twins; *open bars*, lean co-twins; TG, triglycerides. .

in unrelated subjects indicating the importance of visceral fat accumulation in the development of increased triglyceride concentrations (13). Hypertriglyceridemia in visceral obesity might be explained by a higher flux of free fatty acids to the liver, followed by increased VLDL production, in subjects with visceral obesity (46). On the other hand, the intrapair differences in serum LDL-cholesterol level were similar in high and low AVF subgroups. In a previous study, obese women with visceral fat or sc fat accumulation had an almost similar increase in serum LDL cholesterol, compared with unrelated lean women (13). These data together suggest that fat mass, rather than its distribution, is important in the regulation of plasma LDL cholesterol levels in obesity.

The intrapair differences in serum lipid and lipoprotein values were of the same magnitude in twin pairs with and without ApoE4 phenotype. However, in twin pairs expressing both ApoE4 phenotype and a high AVF level, the intrapair differences in serum triglycerides and their subfractions were particularly high. Previously, Fumeron and coworkers (15) reported that obese subjects, carrying the apolipoprotein epsilon 4 allele, express hypertriglyceridemia more often than obese subjects with epsilon 2 or 3 alleles. This and our results are in contrast to the findings by Pouliot and co-workers (17), who reported that visceral fat was not associated with hypertriglyceridemia in ApoE4-positive subjects. It is possible that differences in other genetic factors, such as LPL *Hin*dIII polymorphism (47) or ApoB-100 *Eco*RI polymorphism (48), also reported to influence the association between triglycerides and visceral obesity, may explain these discrepancies. Although the number of ApoE4-positive pairs in the high AVF area subgroup was small in our study, the identical genetic background of these pairs enables us to suggest that being ApoE4 positive, as defined here, may aggravate the disadvantageous effect of visceral fat on serum triglyceride levels. Serum total and LDL cholesterol levels were markedly higher in ApoE4-positive subjects than in ApoE4-negative subjects, independently of obesity, confirming earlier findings on the role of ApoE phenotype in determining plasma LDL cholesterol levels (49–51).

Postheparin plasma hepatic lipase activity tended to be higher in obese co-twins than in lean co-twins, especially in the subgroup with high AVF level. Arai *et al.* (5) have also shown that obese men and women have higher hepatic lipase activity than lean controls. Despres and co-workers have shown that, in obese women, the percentage of body fat and waist-to-hip ratio correlated positively to hepatic lipase activity (4). They also showed that this lipase activity was inversely correlated with plasma HDL, and especially HDL_2, cholesterol level. These observations and our data strongly suggest that increased activity of hepatic lipase may be crucial for the adverse effects of obesity on HDL metabolism.

Plasma LCAT activity in the present study was significantly increased in the obese co-twins. In agreement with this finding, LCAT activity was decreased after weight reduction, after gastroplasty (10). Previous studies have revealed a positive correlation between LCAT activity and total and LDL cholesterol and triglyceride levels (36, 52). Increased activity of LCAT in obesity may reflect a physiologic response to an altered demand for cholesterol esterification in plasma.

Plasma CETP activities were almost identical in obese and lean co-twins, independent of AVF level. Arai *et al.* (5) previously reported that obese subjects weighing, on average, 30 kg more than the controls, had approximately a 20% increased CETP activity and that the activity of CETP was inversely related to HDL and HDL_2 cholesterol levels in the obese population. It is difficult to argue that the smaller difference in body mass between obese and lean twins in our study could have hampered the detection of a potential obesity-related increase in CETP activity as observed by Arai *et al.* (5). CETP activity, as measured in our study of identical twins discordant for obesity, may not be responsible for the

changes in triglyceride and HDL metabolism because of obesity.

The physiologic function of adipose tissue LPL is to facilitate lipid clearance after food ingestion and to promote adipose tissue storage. Many studies have found that adipose tissue LPL is increased in obesity (7, 8), but others have not observed a correlation between body fat and adipose tissue LPL (4, 6). On the other hand, adipose tissue LPL is known to be decreased in primary hypertriglyceridemia in nonobese subjects but not in obese subjects (53). We found that adipose tissue LPL was either similar (men) or tended to be decreased (women) in obese twins. We cannot exclude the possibility that a low LPL activity, leading to a decreased capacity of triglyceride removal from plasma also in the fasting state, could explain the intrapair difference in serum triglyceride values, at least in some of our twin pairs.

Postheparin plasma LPL represents mainly LPL released into the circulation from adipose and muscle tissues. We found no difference in postheparin plasma LPL between obese and lean co-twins. Previous studies have either found no association between obesity and postheparin plasma LPL activity (4, 6) or have observed a decreased activity in obesity (5). On the other hand, studies on the association between postheparin plasma LPL activity and plasma triglyceride and HDL concentrations in obesity have not given consistent results (5, 6). Skeletal muscle LPL activity was reduced in obese hyperinsulinemic subjects and was inversely related to serum total and VLDL triglyceride concentrations in a male population (6). Unfortunately, we did not measure skeletal muscle LPL activity in our twin study.

Serum Lp(a) levels were slightly higher in obese female twins, compared with lean co-twins, but no such difference was observed in male pairs. This is consistent with previous studies showing either no association between BMI and Lp(a) level or a tendency towards lower Lp(a) values after weight reduction in female obese subjects (3, 54).

In conclusion, in identical twins discordant for obesity, the obese co-twins had higher LDL cholesterol levels, independent of visceral fat level, whereas the differences in serum triglyceride-rich lipoprotein levels between obese and lean twins were greater in those pairs with more visceral fat. The ApoE4 phenotype may aggravate the risk for high triglyceride values in visceral obesity. A high hepatic lipase activity may be causally related to (and an increased LCAT activity may reflect a physiologic response to) the dyslipidemia of obesity.

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