Increased Serum Cholesteryl Ester Transfer Protein in Syrian Obese Humans

Suriyeli Obez Bireylerde Artmış Serum Kolesterol Ester Transfer Protein Düzeyleri

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ABSTRACT

Objective: To determine whether serum cholesteryl ester transfer protein (CETP), is increased and associated with atherogenic lipoprotein profile in obese subjects.
Methods: Blood was drawn in the morning after an overnight fast from 102 subjects (76 obese subjects and 26 apparently healthy subjects as control group) and, at the same time, anthropometric measurements including height, body weight, waist girth were taken. CETP activity were measured in serum using fluorescence assay, whereas total cholesterol (TC), triglyceride, HDL-cholesterol, LDL-cholesterol and Glucose levels were measured using enzymatic colorimetric assay and insulin was measured using ELISA.
Results: Serum CETP activity was significantly higher (58.15 ± 12.24 vs. 47.75 ± 7.54 pmol/µl/1hr, means ± SEM, $p < 0.0001$) in the obese group than in the control group. There was considering direct relation for CETP activity with BMI, TC, and LDL-C ($p<0.0001$, $p<0.0001$, $p=0.04$ respectively), while this activity correlated negatively with HDL-C ($p<0.0001$) in the obese group.
Conclusion: This study showed a marked increase in serum CETP levels in obese group, and this increase in activity associated with the atherogenic lipoprotein profile in obese subject.

Key Words: Cholesteryl ester transfer protein, obese, lipoproteins, atherosclerosis, insulin, cholesterol

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ÖZET

Amaç: Obez kişilerde serum kolesterol ester transfer proteininin (CETP), artmış ve aterojenik lipoporotein profili ile ilişkili olup olmadığının belirlenmesidir.
Yöntemler: Gece boyu süren açlık sonrası 102 bireyden (76 obez, 26 sağlıklı kontrol) sabah kanı alınmıştır. Aynı zamanda bu bireylerde boy, vücut ağırlığı, bel çevresini içeren antropometrik ölçümler de yapılmıştır. Serumdaki CETP aktivitesi, floresan yöntemle, total kolesterol (TC), trigliserid, HDL-kolesterol, LDL-kolesterol ve glukoz düzeyleri enzimatik kolorimetrik testle ve insülin düzeyi ise ELISA ile ölçülmüştür.
Bulgular: Serum CETP aktivitesi obez grupta kontrol grubuna göre belirgin şekilde yüksek olarak saptandı (58.15 ± 12.24 karşılık 47.75 ± 7.54 pmol/µl/1 saat, ortalama ± standart hata, $p<0.0001$). Obez grupta, CETP aktivitesi ile BMI, TC ve LDL-C arasında direkt bir ilişki saptanırken (sırasıyla $p<0.0001$, $p<0.0001$ ve $p=0.04$), HDL-C ile arasında negatif korelasyon olduğu ($p<0.0001$) gözlenmiştir.
Sonuç: Bu çalışma, obez grubun serum CETP düzeylerinde belirgin bir artış olduğunu ve bu artışın obez bireylerdeki aterojenik lipoprotein profili ile ilişkili olduğunu göstermiştir.

Anahtar Sözcükler: Kolesterol ester transfer proteini, obez, lipoproteinler, ateroskleroz, insulin, kolesterol

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INTRODUCTION

Cholesteryl ester transfer protein (CETP) is a hydrophobic glycoprotein that promotes the redistribution of cholesteryl esters, triglycerides, and, to a lesser extent, phospholipids between plasma lipoproteins. The overall effect of CETP is a net mass transfer of cholesteryl esters from HDLs to TRLs (triglyceride-rich lipoproteins) and LDLs and of triglycerides from TRLs to LDLs and HDLs (1). Although most clinical (2,3) and experimental (4,5) evidence supports the view that CETP is atherogenic in nature, the relation between human CETP deficiencies, either in homozygous or heterozygous form, and the risk of CVD remains a matter of debate (6,7).

Obesity is associated with serious health risks and increased mortality. Several disease states and/or conditions are more prevalent in obese patients. Hypertension, hyperlipidemia, insulin resistance, and glucose intolerance are all known cardiac risk factors that tend to cluster in obese individuals (8). Plasma CETP activity has been reported to be elevated in obese subjects (9-11). Previous studies in obese subjects (9, 12) have indicated that an increase in serum CETP activity is associated with their atherogenic lipoprotein profile.

While increasing HDL-C is a promising strategy for reducing CHD risk, there are currently few treatment options for increasing HDL-C levels, including statins, fibrates and niacin (13,14) . Therefore, there is great interest in developing novel therapies that will raise circulating HDL-C levels in humans for the treatment of cardiovascular disease. CETP has been identified as a novel target for increasing HDL-C levels and decreasing atherosclerosis development.

METHODS

Subjects

A total of people were included in the study was 102 Syrian subjects (76 obese (BMI > 30 kg/m^2), and 26 apparently healthy subjects (BMI < 30 kg/m^2) as control group). Body mass index (BMI) was determined as mass/height^2 (kilograms per square meter).

Serum analyses

Blood samples were collected after an overnight fast in dry tubes. Serum was isolated with centrifugation (5000 x g) and stored at –80°C until the time of analysis. Samples were analyzed enzymatic colorimetric assay for glucose, TC, TG, HDL-C, LDL-C (Roche Diagnostics GmbH, Mannheim), and by enzyme-linked immuno assay (ELISA) for insulin (NOVA TEC Immundiagnostica GmbH, Germany).

CETP activity measurement

CETP activity in serum was measured by fluorescence assay (DRG diagnostics GmbH, Germany), as the rate of the fluorescent neutral lipid transferred from high-density lipoprotein (HDL) (donor) to apoprotein B containing lipoproteins (acceptor), results in an increase in fluorescence. The reaction was incubated at 37°C for 1 h. Calibration curve was prepared by making serial dilutions of the donor molecule in isopropanol and the fluorescence intensity of each dilution was subsequently recorded, by using isopropanol alone as a blank. The fluorescence intensity of the samples, and positive control were measured using a fluorescence plate reader (Ex. = 465 nm; Em. = 535 nm). Then the fluorescence intensity values of the calibration curve were applied directly to the results to express specific activity of the serum sample (pmoles/ µL sample/hr). The activity of the serum sample was calculated as follow:

Y = MX + B

where: Y = Fluorescence Intensity of Sample – Fluorescence Intensity of Blank

M = Slope of the Calibration Curve

X = Concentration of Serum Sample(pmole/ µL sample/1 hr)

B = Intercept of the Calibration Curve

Statistics

Data were analyzed by Microsoft Word Excel. When more than one group was involved, T-student test was used to determine differences between groups. Pearson correlation were calculated to examine the relationships between specified parameters. Statistical significance was assumed when $p< 0.05$.

RESULTS

Patient characteristics

The two groups did not differ in age (Table 1). The obese group was characterized by hyperinsulinemia and euglycemia. Total cholesterol (TC), and triglycerides (TG) concentrations in obese subjects were significantly higher than those in the control subjects. The HDL-C was significantly decreased in the obese subjects, but there was no significant difference in LDL-C concentrations between obese subjects and control subjects.

Table1. Physical characteristics and serum profiles of nonobese, and obese subjects.

	Nonobese	Obese	P (C vs. OB)
N (f/m)	26(18/8)	76(50/26)	-
Age (yr)	40±4	40±4	-
BMI (kg/m^2)	23.9±2.2	34.6±3.9	-
Glucose (mg/dl)	95.5±8	97.18±9.84	0.43
Insulin (µlU/ml)	5.29±2.41	11.1±7.79	<0.0001
TC (mg/dl)	177.69±30.2	192.92±35.46	0.042
TG (mg/dl)	116.42±70.75	154.63±96.58	0.038
LDL-C (mg/dl)	114.6±39.5	118.32±27.93	0.67
HDL-C (mg/dl)	50.46±13.99	42.03±12.75	0.011

Data from fasted subjects are expressed as means ± SEM. F:Female; M:male; C:Control; OB:Obese; TG:Triglyceride; TC:Total cholesterol; HDL-C:HDL cholesterol.

Serum CETP activity was significantly higher (58.15 ± 12.24 vs. 47.75 ± 7.54 pmol/µl/1hr, means ± SEM, $p<0.0001$) in the obese group than in the control group. In an effort to understand what factors may be influencing or affected by the levels of CETP in the serum, the relationship of serum CETP activity to several patient characteristics is shown in Table 2. Serum glucose, insulin, and TG concentrations were not related to serum CETP activity. However, serum HDL-C was negatively related, whereas BMI, TC, and LDL-C were positively related to plasma CETP activity. Interestingly, serum TC, HDL-C concentrations showed the strongest relationship to serum CETP activity.

Table 2. Relationships between CETP and other blood biochemistry data for obese subjects.

	r	P
BMI	0.45	<0.0001
Glucose	-0.06	0.59
Insulin	0.15	0.17
TC	0.58	<0.0001
TG	0.06	0.6
HDL	-0.59	<0.0001
LDL	0.23	0.04

TC:Total cholesterol, TG:Triglyceride, HDL:High-density lipoprotein, LDL:Low-density lipoprotein.

In this study, the serum CETP activity was significantly correlated with BMI; this relationship indicate that the obesity-induced elevation in serum CETP activity previously reported (9-11,15,16).

It has been suggested by several researchers that insulin may be an important modulator of CETP expression in humans and that insulin resistance may lead to perturbations in plasma CETP levels (16-18). This has been a controversial issue in the literature, because reports are not clear as to whether insulin might be a positive or negative regulator of CETP expression (19-23), and no clear effect of insulin resistance has been observed (24,25) .

In our study, we observed that serum insulin and glucose concentrations were not related to serum CETP activity, supporting the notion that neither insulin nor insulin resistance directly influences serum CETP concentrations. However, insulin and/or insulin resistance may be indirectly affecting the total cholesteryl ester transfer rates in vivo. Cholesteryl ester transfer between lipoproteins is dependent on both CETP and the substrate lipoproteins, and the substrate lipoproteins are altered in insulin-resistant states (26).

We also noted that CETP activity was positively correlated with LDL-C, whereas negatively correlated with HDL-C, these results indicate that the CETP activity may be contributes to an atherogenic lipid phenotype in obese subjects, and this result previously reported in some studies (9,12).

The strong relationship between serum TC concentration and serum CETP activity, might imply that CETP is deleterious through its role in enhancement of atherosclerotic lesions development, this enhancement by CETP was secondary to a redistribution of cholesterol from HDLs to the VLDL/LDL fraction. De Grooth G et al, study has also shown that plasma CETP was found to be increased in individuals with hypercholesterolemia (27). But it can also be argued that high CETP is the result rather than the cause of dyslipidemia (28). As it has been shown that CETP gene expression is stimulated by dietary cholesterol and endogenous hypercholesterolemia as a result of activation of liver X receptor /retinoid X receptor transcription factors bound to the proximal promoter of the human CETP gene (29, 30). Therefore, positive correlation between CETP and TC levels in our study suggests that plasma cholesterol may be an important determinant of plasma CETP activity in obese subjects.

CONCLUSION

This study showed a marked increase in serum CETP levels in obese group, and this increase in activity associated with a more atherogenic lipid profile and increased progression of atherosclerosis in obese subjects. In addition the fact that CETP is capable of modulating the composition and the concentrations of lipoproteins in the plasma makes it an attractive therapeutic and preventative target in the treatment of vascular disease.

Conflict of Interest

No conflict of interest was declared by the authors.

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