Impact of Type 2 Diabetes on the Serum CETP Levels

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ABSTRACT

We aimed to study the effect of Diabetes Mellitus type 2 on CETP activity and to evaluate the relationship of CETP activity levels with Chol, TG, and HDL. Blood was drawn in the morning after an overnight fast from 136 subjects (70 Diabetic obese patients, 66 obese subjects as control group) CETP activity were measured in Serum using Fluorescence assay, whereas TC, TG, and Glu were measured using Enzymatic colorimetric assay. There was no significant difference between CETP activity in diabetic obese patients and obese subjects (60.38, 58.15 pmol/μl/1hr, respectively; P=0.28). There was considering direct relation for CETP activity with BMI, TC, and TG (P=0.03, P<0.0001, P= 0.001, respectively) while this activity correlated negatively with HDL-C in Diabetic obese patients. This study showed a considering relation between serum CETP levels with TC and HDL-C in diabetic patients and obese groups. This suggested that CETP may affect the dyslipidemia noted in type 2 diabetes particularly obese one.

Keywords: Cholesteryl ester transfer protein; diabetes type 2; obese, lipoproteins; cholesterol.

INTRODUCTION

The lipoprotein profile in type 2 diabetes is characterized by the presence of increased TG-rich lipoprotein remnant particles, small dense LDL particles, and decreased levels of HDL cholesterol. Numerous epidemiological studies over the past decades have demonstrated that high density lipoprotein cholesterol (HDL-C) is inversely correlated with the incidence of coronary heart disease (CHD). An increase of 1 mg/dl of HDL-C is associated with a 2-3% decrease in the risk of death from CHD, independent of low-density lipoprotein cholesterol (LDL-C) levels. Therefore, it is important to understand the causes of the dyslipidemia noted in type 2 diabetes, to find effective ways to improve lipid metabolism and reducing CHD risk.

CETP is a 476 amino acid protein (74 kDa) with a highly hydrophobic amino acid content and four N-linked glycosylation sites (N88, N240, N341, N396).

It promotes the redistribution of cholesteryl esters, triglycerides, and, to a lesser extent, phospholipids between plasma lipoproteins. CETP transfers lipids from 1 lipoprotein particle to another in a process that results in equilibration of lipids between lipoprotein fractions. The overall effect of CETP is a net mass transfer of cholesteryl esters from HDLs to TRLs and LDLs and of triglycerides from TRLs to LDLs and HDLs. Thus, CETP-mediated transfers from HDL to VLDL and LDL provide a potential indirect pathway by which HDL cholesteryl esters can be delivered to the liver. When the concentration of VLDLs is increased, as in patients with type 2 diabetes, HDL cholesteryl esters are preferentially transferred by CETP to larger VLDL particles that become cholesterol rich and thus, potentially more atherogenic.

Many of the studies have shown that the CETP deficiency is associated with high levels of HDL-C.

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. Therefore, there is great interest in developing novel therapies that will raise circulating HDL-C levels in humans for the treatment of cardiovascular disease.

MATERIALS AND METHODS

Subjects

A total of people were included in the study was 136 Syrian subjects (70 Diabetic obese patients, 66 obese subjects as control group; BMI >30 kg/m2). Body mass index (BMI) was determined as mass/height2 (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 (Roche Diagnostics GmbH, Mannheim).

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:

\[ Y = \text{Fluorescence Intensity of Sample} - \text{Fluorescence Intensity of Blank} \]
\[ M = \text{Slope of the Calibration Curve} \]
\[ X = \text{Concentration of Serum Sample (pmole/µL sample/1 hr)} \]
\[ B = \text{Intercept of the Calibration Curve} \]

**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 AND DISCUSSION**

**Patient characteristics**

Total cholesterol (TC), and triglycerides (TG) concentrations in diabetes subjects were significantly higher than those in the obese subjects (Table 1). Whereas the HDL-C concentrations was significantly decreased in the diabetes subjects.

**Table 1:** Physical characteristics and serum profiles of diabetes, and obese subjects.

<table>
<thead>
<tr>
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<th>Dia</th>
<th>Ob</th>
<th>( P ) (Dia vs. Ob)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N(f/m)</td>
<td>70(41/29)</td>
<td>66(40/26)</td>
<td>-</td>
</tr>
<tr>
<td>Age(yr)</td>
<td>53±10</td>
<td>40±10</td>
<td>-</td>
</tr>
<tr>
<td>BMI</td>
<td>35.42±4.4</td>
<td>34.69±3.9</td>
<td>-</td>
</tr>
<tr>
<td>GLU (mg/dl)</td>
<td>194.54±78.48</td>
<td>97.18±9.84</td>
<td>( P = 0.0001 )</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>228.45±156.2</td>
<td>154.63±96.58</td>
<td>( P = 0.001 )</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>206.28±44.18</td>
<td>192.92±35.46</td>
<td>( P = 0.048 )</td>
</tr>
<tr>
<td>HDL-C</td>
<td>37.01±0.62</td>
<td>42.03±12.75</td>
<td>( P = 0.01 )</td>
</tr>
</tbody>
</table>

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

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 concentrations were not related to serum CETP activity. However, serum HDL-C was negatively related, whereas BMI, TC, and TG 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 diabetes subjects

<table>
<thead>
<tr>
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<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>0.252</td>
<td>0.03</td>
</tr>
<tr>
<td>GLU</td>
<td>0.01</td>
<td>0.8</td>
</tr>
<tr>
<td>TG</td>
<td>0.38</td>
<td>0.001</td>
</tr>
<tr>
<td>TC</td>
<td>0.52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.52</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Our study, shown that plasma CETP activity is similar in diabetic individuals when compared with normal obese individuals. This finding previously reported10, whereas MacLean P et al, study has shown that diabetes type 2 suppresses the expression of the hepatic CETP in obese individuals,11 may be due to the difference in the average of BMI in individuals between the two studies, the average of BMI in diabetes individuals at MacLean’s study was 52 kg/m², while the average of BMI in diabetes individuals in our study was 35.42 kg/m².

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 reported11-14. Thus obesity, is a risk factor for the development of diabetes type 2, and is associated with dyslipidemia.

We also noted that CETP activity was positively correlated with TG whereas negatively correlated with HDL-C, these results indicate that the CETP activity may be contributes to an atherogenic lipid phenotype in obese diabetic subjects, and this result previously reported in some studies12,15. And it has been reported that most individuals with type 2 diabetes exhibit dyslipidemia (high triglyceride levels and low HDL-cholesterol levels),16 this finding previously reported in Arai T et al, study which shown that CETP correlated negatively with HDL cholesterol15.

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 hypercholesterolemia17. But it can also be argued that high CETP is the result rather than the cause of dyslipidemia18. 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

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transcription factors bound to the proximal promoter of the human CETP gene\textsuperscript{19,20}. 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 humans with and without type 2 diabetes.

**CONCLUSION**

This study showed a considering relation between serum CETP levels with Chol and HDL-c in diabetic patients and obese groups. This suggests that CETP may affect the dyslipidemia noted in type 2 diabetes particularly obese one.

**REFERENCES**


**Source of Support:** Nil, **Conflict of Interest:** None.