



## Impact of Type 2 Diabetes on the Serum CETP Levels

Soumaia Sayed Rammadan\*<sup>1</sup>, Cemal Cevik<sup>2</sup>, Faizeh Al-Quobaili<sup>3</sup>

<sup>1</sup> Master degree in clinical biochemistry, faculty of pharmacy, Damascus University, Damascus, Syria.

<sup>2</sup> Prof. Dr. Department of clinical biochemistry, faculty of Medicine, Gazi University, Ankara, Turkey.

<sup>3</sup> Prof. Dr. Department of clinical biochemistry, faculty of pharmacy, Damascus University, Damascus, Syria.

\*Corresponding author's E-mail: [somramadan@yahoo.com](mailto:somramadan@yahoo.com)

Accepted on: 12-02-2014; Finalized on: 30-04-2014.

### 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, HDL 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 group. This study showed a considering relation between serum CETP levels with TC 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.

**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<sup>1,2</sup>. 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)<sup>3</sup>. 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<sup>4</sup>. 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)<sup>5</sup>.

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<sup>6</sup>. 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<sup>1</sup>.

Many of the studies Have shown that the CETP deficiency is associated with high levels of HDL<sup>6,7</sup>.

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<sup>8,9</sup>. 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/m<sup>2</sup>). Body mass index (BMI) was determined as mass/height<sup>2</sup> (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/  $\mu$ L 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/  $\mu$ L 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 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.

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

Data from fasted subjects are expressed as means  $\pm$  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

	r	p
BMI	0.252	0.03
GLU	0.01	0.8
TG	0.38	0.001
TC	0.52	<0.001
HDL	- 0.52	<0.001

Our study, shown that plasma CETP activity is similar in diabetic individuals when compared with normal obese individuals. This finding previously reported<sup>10</sup>. whereas MacLean P et al, study has shown that diabetes type 2 suppresses the expression of the hepatic CETP in obese individuals,<sup>11</sup> 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 /m<sup>2</sup>, while the average of BMI in diabetes individuals in our study was 35.42 kg/m<sup>2</sup>.

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<sup>11-14</sup>. 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 studies<sup>12,15</sup>. And it has been reported that most individuals with type 2 diabetes exhibit dyslipidemia (high triglyceride levels and low HDL-cholesterol levels),<sup>16</sup> this finding previously reported in Arai T et al, study which shown that CETP correlated negatively with HDL cholesterol<sup>15</sup>.

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<sup>17</sup>. But it can also be argued that high CETP is the result rather than the cause of dyslipidemia<sup>18</sup>. 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<sup>19,20</sup>. 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

- Guerin M, Le Goff W, Lassel T, Van Tol A, Steiner G, Chapman M, Proatherogenic role of elevated cholesteryl ester transfer from HDL to VLDL1 and dense LDL in type 2 diabetes: impact of the degree of triglyceridemia, *Arterioscler Thromb Vasc Biol*, 21, 2001, 282-288.
- van Venrooij F et al, Common cholesteryl ester transfer protein gene polymorphisms and the effect of atorvastatin therapy in type 2 diabetes, *Diabetes Care*, 26(4), 2003, 1216-1223.
- Luc G et al, Value of HDL cholesterol, apolipoprotein A-I, lipoprotein A-I, and lipoprotein A-I/A-II in prediction of coronary heart disease: The PRIME Study, *Arterioscler. Thromb. Vasc. Biol*, 22, 2002, 1155-1161.
- Boden W, High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the Veterans Affairs High Density Lipoprotein Intervention Trial, *Am. J. Cardiol*, 86, 2000, 19L-22L.
- Masson D, Cheng Jiang X, Lagrost L and Tall A, The role of plasma lipid transfer proteins in lipoprotein metabolism and atherogenesis, *J. Lipid Res*, 50, 2009, S201-S206.
- Barter P, Brewer Jr H, Chapman M, Hennekens C, Rader D, Tall A, Cholesteryl Ester Transfer Protein, A Novel Target for Raising HDL and Inhibiting Atherosclerosis, *Arterioscler Thromb Vasc Biol*, 23, 2003, 160-167.
- Zhong S, Sharp D, Grove J, Bruce C, Yano K, Curb J, and Tall A, Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels, *J. Clin. Invest*, 97, 1996, 2917-2923.
- Birjmohun R, Hutten B, Kastelein J and Stroes E, Efficacy and safety of high-density lipoprotein cholesterol-increasing compounds: a meta-analysis of randomized controlled trials, *J. Am. Coll. Cardiol*, 45, 2005, 185-197.
- Goldberg A, Alagona P, Capuzzi D, Guyton J, Morgan J, Rodgers J, Sachson R and Samuel P, Multiple-dose efficacy and safety of an extended-release form of niacin in the management of hyperlipidemia, *Am. J. Cardiol*, 85, 2000, 1100-1105.
- Elchebly M, Porokhov B, Pulcini T, Berthezene F and Ponsin G, Alterations in composition and concentration of lipoproteins and elevated cholesteryl ester transfer in non-insulin-dependent diabetes mellitus (NIDDM), *Atherosclerosis*, 123, 1996, 93-101.
- MacLean P, Vadlamudi S, MacDonald K, Pories W and Barakat H, Suppression of hepatic cholesteryl ester transfer protein expression in obese humans with the development of type 2 diabetes mellitus, *J. Clin. Endocrinol. Metab*, 90, 2005, 2250-2258.
- MacLean P, Bower J, Vadlamudi S, Green T, Barakat H, Lipoprotein subpopulation distributions in lean, obese, and type 2 diabetic women: a comparison of African and white Americans, *Obes Res*, 8, 2000, 62-70.
- Riemens SC, van Tol A, Sluiter WJ, Dullaart RP, Plasma phospholipid transfer protein activity is related to insulin resistance: impaired acute lowering by insulin in obese type II diabetic patients, *Diabetologia*, 41, 1998, 934-929.
- Asayama K, Hayashibe H, Dobashi K, Uchida N, Nakane T, Kodera K, Increased serum cholesteryl ester transfer protein in obese children, *Obes Res*, 10, 2002, 446-439.
- Arai T, Yamashita S, Hirano K, Sakai N, Kotani K, Fujioka S, Increased plasma cholesteryl ester transfer protein in obese subjects. A possible mechanism for the reduction of serum HDL cholesterol levels in obesity, *Arterioscler Thromb*, 14, 1994, 1136-1129.
- Triplitt C, Reasner C and Isley W. Endocrinologic disorders. In: Dipiro J, Talbert R, Yee G, Matzke G, Wells B and Michael Posey L, eds. *Pharmacotherapy*. The McGraw-Hill Companies, 2008, 1238-1245.
- de Grooth G, Smilde T, van Wissen S, Klerkx A, Zwinderman A, Fruchart J, Kastelein J, Stalenhoef A, and Kuivenhoven J, The relationship between cholesteryl ester transfer protein levels and risk factors in patients with familial hypercholesterolemia, *Atherosclerosis*, 173, 2004, 267-261.
- Van Tol A, CETP-catalysed transfer of cholesteryl esters from HDL to apo B-containing lipoproteins in plasma from diabetic patients, *Eur J Clin Invest*, 23, 1993, 856.
- Luo Y, and Tall A, Sterol up regulation of human CETP expression in vitro and in transgenic mice by an LXR element, *J Clin Invest*, 105, 2000, 520-513.
- Richardson MA, Berg DT, Johnston PA, McClure D, Grinnell BW, Human liposarcoma cell line, SW872, secretes cholesteryl ester transfer protein in response to cholesterol, *J Lipid Res*, 37, 1996, 1166-1162.

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

