Plasma Cholesteryl Ester Transfer Protein Activity Is Not Linked to Insulin Sensitivity

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Plasma cholesteryl ester transfer protein (CETP) activity has been reported to decline during a hyperinsulinemic-euglycemic clamp. It has been suggested that this suppressive effect of acute hyperinsulinemia is linked to whole body insulin sensitivity, and that the insulin resistance that accompanies obesity leads to high plasma CETP activity found in obese subjects. In the present study, we used 2 experimental approaches to examine the putative link between CETP and insulin action. First, we examined if the clamp-induced suppression of plasma CETP activity is linked to whole body insulin sensitivity. Plasma CETP activity was measured at the beginning and end of a 2-hour hyperinsulinemic-euglycemic clamp in 18 nondiabetic individuals before and after an exercise training regimen that improved insulin sensitivity without weight loss. CETP activity decreased in response to the clamp procedure in 16 of 18 subjects, and on average, by 9% ($P < .001$). While training decreased plasma CETP activity (10%, $P < .05$), the improvement in insulin sensitivity had no statistical effect on the clamp-induced suppression of plasma CETP activity (training*clamp, $P = .26$). Second, we examined if insulin resistance is associated with an elevation in fasting plasma CETP activity when the influence of adiposity and diabetes were negated. Plasma CETP activity was measured in 41 women (12 insulin-sensitive lean; 8 insulin-resistant lean; 10 insulin-sensitive obese; 11 insulin-resistant obese). The level of insulin sensitivity had no significant effect on fasting plasma CETP activity, but CETP levels were 25% higher in obese subjects ($P < .01$). Thus, neither experimental approach provided evidence that plasma CETP levels are linked to insulin and insulin sensitivity. These data suggest that the elevated CETP activity found in obese patients is less associated with hyperinsulinemia and the accompanying insulin resistance, but rather is more related to some other metabolic complication of obesity.

CHOLESTERYL ESTER TRANSFER protein (CETP) is a plasma glycoprotein that is involved in at least 1 arm of reverse cholesterol transport, the process by which cholesterol is collected from the periphery and returned to the liver (for review, see Bruce et al[1]). While this process is generally thought to avert the development of atherosclerosis, elevated CETP expression has also been linked to depressed high-density lipoprotein cholesterol levels. Thus, the atherogenic nature of CETP is the subject of much debate (for review, see Stevenson[2]). Even so, the fact that CETP is capable of modulating the composition and concentration of lipoproteins has led investigators to view it as a potential target in the prevention and treatment of vascular disease. In this light, several studies have focused on understanding the regulation of CETP expression to provide a basis for the development and implementation of better therapeutic regimens for patients with atherosclerosis.

Obesity is consistently accompanied by an elevation in plasma CETP levels,[3-7] and it has been suggested that this perturbation may contribute to the abnormal lipoprotein profiles and the high rate of atherosclerosis in these patients. This observation has led to several investigations concerning what complicating factor(s) of obesity leads to elevated CETP levels. It has been postulated that the insulin resistance that accompanies obesity may be responsible for the altered levels of plasma CETP in these patients. In humans, a hyperinsulinemic-euglycemic clamp has been shown to depress plasma CETP activity,[8,9] suggesting that insulin may either suppress the release of CETP into the plasma or stimulate the clearance of CETP from the plasma. Bruce et al[1] suggested that the suppressive effects of acute hyperinsulinemia may be linked to whole body sensitivity to insulin's actions, and that insulin resistance may be impairing insulin's normal regulation of CETP levels in the plasma. From this perspective, insulin resistance may impair some step in the insulin signaling pathway, preventing its normal suppressive effects on CETP expression. In obese subjects, normally found to be insulin-resistant, this metabolic adjustment could lead to elevated CETP levels in the plasma, despite the chronic hyperinsulinemia found in these patients. However, studies that have addressed the link between insulin resistance and insulin's putative regulatory effects have not been consistent in their findings and have included patients with type 2 diabetes,[8-11] a metabolic syndrome that appears to have independent and opposite effects on CETP expression in obese individuals.[3] Thus, while experts continue to speculate about the role of insulin and insulin resistance in the regulation of CETP expression,[1] there has yet to be clear, convincing evidence that the putative suppressive effects of insulin are linked to peripheral insulin sensitivity, and that insulin resistance results in elevated plasma CETP levels.

The purpose of this study was to investigate what role, if any, insulin action plays in: (1) the suppressive effects of acute hyperinsulinemia on plasma CETP activity; and (2) determining fasting plasma CETP levels. To achieve our first objective, we examined the suppressive effects of acute hyperinsulinemia during a hyperinsulinemic-euglycemic clamp on plasma CETP activity both before and after an exercise training regimen that improves insulin sensitivity without weight loss. To achieve

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Submitted July 31, 2000; accepted January 11, 2001.

Supported by a grant from the National Institutes of Health (Department of Health and Human Services-Public Health Service DK45029).

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0026-0495/01/5007-0013$35.00/0

doi:10.1053/meta.2001.24205

our second objective, we examined plasma CETP activity in nondiabetic subjects that were divided into insulin-resistant and insulin-sensitive groups, with and without the presence of obesity. The results of this study shed light on the putative link between insulin action and the suppressive effects of acute hyperinsulinemia on plasma CETP activity, as well as the role of chronic insulin resistance in influencing plasma CETP levels.

MATERIALS AND METHODS

Patient Characteristics and Treatment

Subjects. Subjects included in the hyperinsulinemic-euglycemic clamp were sedentary volunteers who were recruited from patients examined at the Human Performance Laboratory at East Carolina University. The subjects were nonsmokers who were free from cardiovascular disease, diabetes, and orthopedic problems. None of the subjects were taking any drugs known to affect lipid metabolism or plasma CETP activity. Patient characteristics in the study of the effects of insulin resistance and obesity on plasma CETP levels are reported elsewhere.[12] Written consent was obtained from the patients after they were informed of the nature and potential risk of the study. The Institutional Review Board for human subject research approved protocols used in this study.

Hyperinsulinemic-euglycemic clamp. Hyperinsulinemic-euglycemic clamps were performed as we have previously reported[13] according to the modification of Defronzo et al.[14] In short, following an overnight (12 hour) fast, an intravenous catheter was placed in an antecubital vein for infusion of glucose and insulin. Another catheter was placed retrograde in a dorsal hand vein for blood sampling. This hand was kept in a warming box at 60°C for the collection of arterialized blood. Before infusion, samples were collected for the determination of fasting glucose, insulin, and CETP activity. A primed continuous infusion of insulin (Humulin; Eli Lilly, Indiannapolis, IN) at a submaximal dosage of 100 $\mu U \cdot m^{-2} \cdot min^{-1}$ was then initiated. Four minutes after the infusion of insulin began, a variable 20% glucose infusion was begun. Plasma glucose levels were monitored at 5-minute intervals, and the infusion of glucose was adjusted to maintain euglycemia. Plasma was collected at 10-minute intervals for the determination of insulin concentrations. Glucose infusion rate (GIR) was calculated from the final 30 minutes of the clamp. At the end of the clamp procedure, plasma was collected for the final determination of plasma CETP activity.

Exercise training regimen. Before the training regimen was implemented, VO_{2max} was measured during incremental exercise on an electrically braked cycle ergometer (Lode; Diversified, Brea, CA) as previously described.[15] Subjects in the hyperinsulinemic-euglycemic clamp study underwent the procedure before and after a 7-day exercise training regimen, supervised and monitored by investigators in the Human Performance Laboratory. The exercise was performed on a cycle ergometer for 7 consecutive days (60 minutes/day), and the workload was adjusted to achieve approximately 75% of VO_{2max}. Heart rate during exercise training was monitored continuously by telemetry (Polar XL, Stamford, CT). This training protocol has been shown to increase insulin sensitivity without significant changes in body mass or body composition.[16-20]

Anthropometric analyses. Body mass and height were recorded to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI) was determined as mass/height^2 (kg/m^2). In the study examining the effects of insulin resistance and obesity, umbilicus, minimal waist, and maximal hip girths were obtained in duplicate as previously described,[21] and body density was determined by hydrostatic weighing following expiration to residual volume, as determined by oxygen dilution.[22] Body density was used to calculate percent body fat using the Siri equation.[23]

Plasma Analyses

Blood samples used for CETP assays, insulin assays, and all other measurements of glucose were collected and treated with the addition of sodium azide (10 KIU/mL) and aprotinin (0.1 mg/mL). Plasma was separated by low-speed centrifugation (2,500 $\times$ *g*) for 30 minutes at 4°C and stored at −80°C until analyzed. CETP activity in the plasma was measured as the rate of ^{3}H-cholesteryl esters transferred from ^{3}H-CE high-density lipoprotein (HDL) to apoprotein B containing lipoproteins as described by Tollefson and Albers.[24] Both donor and acceptor lipoproteins were collected by ultracentrifugation of density adjusted, pooled plasma, acquired from the blood bank (Red Cross, Norfolk, VA). Human HDL_3 was labeled with ^{3}H-CE by endogenous lecithin: cholesterol acyltransferase (LCAT) during an incubation at 37°C in the presence of ^{3}H-cholesterol (Dupont NEN, Boston, MA). The labeled donor (10 μg cholesterol) was incubated with acceptor lipoproteins (d $<$ 1.063 g/mL, 250 μg cholesteryl esters), 10 μL of plasma, and a buffer containing 10 mmol/L Tris and 150 mmol/L NaCl (pH 7.4). The reaction was incubated at 37°C for 16 hours. After the incubation, the acceptor lipoproteins were precipitated with a solution of 1% dextran sulfate (molecular weight [MW] 50,000) and 0.5 mol/L magnesium chloride. A portion of the supernatant was then combined with Scinteverse (Fisher Scientific, Pittsburgh, PA) and counted. Activity was calculated from a percentage of the total cholesteryl ester transferred (subtracting the counts in the sample from the counts in a blank tube and then dividing by the counts in the blank tube). The reaction is linear for label transfers of up to 45%. Samples with relevant comparisons were run in duplicate in the same assay. Within-assay variation was less than 2%. In a number of samples, CETP-specific cholesteryl ester tranfer in the plasma was measured by running the assay in the presence and absence of the monoclonal antibody known to inhibit human CETP activity, TP2. TP2 inhibited 92% of the transfer activity in the plasma, and the 2 measures were strongly associated (r = .98, $P < .001$). We have also confirmed that this assay is a good estimate of CETP protein concentrations by comparing CETP activity with CETP mass determined by Western blot analysis (data not shown). Total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), HDL-cholesterol (HDL-C), total triglycerides (TG), and very–low-density lipoprotein-triglycerides (VLDL-TG) in the plasma were determined commercially (Lipoprofiling; Lipomed, Raleigh, NC) by nuclear magnetic resonance (NMR) spectroscopy as described by others.[25] Plasma samples were analyzed spectrophotometrically for glucose (Beckman II glucose analyzer, Fullerton, CA) and by microparticle enzyme immunoassay for insulin (IMx; Abbott Labs, Abbott Park, IL).

Statistics

Data from the exercise training study were analyzed with paired *t* tests, unless pre- and postclamp measurements were included, in which case a repeated measures analysis of variance (ANOVA) model was used to examine the effect of exercise, the effect of the clamp, and their interaction. In the examination of insulin-resistant and insulin-sensitive subjects, the data was analyzed with a 2-way ANOVA model examining the effects of obesity (obese *v* lean), insulin resistance (sensitive *v* resistant), and their interaction. Pearson (single comparison) or Bonferroni's (multiple comparisons) correlation coefficients were calculated to examine the relationships between certain variables. Statistical significance was inferred when $P < .05$ (Systat; SPSS Inc, Chicago, IL).

RESULTS

Table 1 shows the physical characteristics of 18 male subjects who underwent a hyperinsulinemic-euglycemic clamp. This cohort of subjects reflected a wide range of ages (19 to 64 years), BMI (22 to 37 kg/m^2), and percent body fat (9% to

Table 1. Characteristics of Subjects Before and After a 7-Day Exercise Training Regimen

	Pretraining	Posttraining
Age (yr)	37 ± 5	—
Body mass (kg)	86 ± 3	86 ± 3
BMI (kg/m^2)	27.7 ± 1.0	27.7 ± 1.0
Insulin (μU/mL)	5.6 ± 1.1	5.5 ± 1.1
Glucose (mg/dL)	93 ± 4	92 ± 3
GIR (mg · kg^{-1} · min^{-1})	7.14 ± 0.39	9.11 ± 0.58*
TC (mg/dL)	153 ± 10	149 ± 10
LDL-C (mg/dL)	100 ± 8	98 ± 9
HDL-C (mg/dL)	34 ± 2	34 ± 2
TG (mg/dL)	120 ± 17	100 ± 11
VLDL-TG (mg/dL)	90 ± 15	71 ± 10

NOTE. Characteristics are shown for 18 male subjects who underwent a hyperinsulinemic euglycemic clamp (18 men) before and after an exercise training regimen designed to improve insulin sensitivity without weight loss. Fasting plasma lipid concentrations were determined commercially by NMR spectroscopy. Data were analyzed by with a paired *t* test and are expressed as mean ± SEM.

* Significantly different from pretraining value, $P < .05$.

32%) and were normoinsulinemic, normoglycemic, and normolipidemic. During the clamp procedure, plasma insulin levels were elevated to 172 ± 8 μU/mL, while euglycemia was maintained at 87 ± 4 mg/dL, similar to what we have previously reported with this procedure.[13] Plasma CETP activities at the beginning and end of the 2-hour procedure are shown in Fig 1A. In response to the hyperinsulinemic-euglycemic clamp, plasma CETP activity decreased in 16 of 18 patients. On average, plasma CETP activity decreased 9% from 59.9 ± 3.6 to 54.1 ± 3.0 nmol/mL/hr ($P < .001$). The decrease in plasma CETP activity appeared to begin within the first 15 minutes of the clamp procedure and was statistically significant by 45 minutes (data not shown).

To investigate if an improvement in insulin sensitivity would influence insulin's suppressive effects on plasma CETP activity, all 18 subjects were again examined after a week-long exercise training regimen designed to improve insulin action. GIR increased from 7.14 ± 0.39 to 9.11 ± 0.58 mg · kg^{-1} · min^{-1} ($P < .001$), while plasma insulin, plasma glucose, and BMI were not significantly altered (Table 1). While percent body fat was not determined after training, several studies have shown that this particular training regimen does not result in significant changes in body mass or body composition.[16-20] In a 2-way repeated measures ANOVA testing for the effects of exercise, hyperinsulinemia, and their interaction, both exercise and acute hyperinsulinemia significantly depressed plasma CETP activity (Fig 1B). The suppressive effects of the hyperinsulinemic-euglycemic clamp were not significantly affected by the exercise training regimen (interaction, hyperinsulinemia*exercise, $P = .26$). Changes in GIR were not significantly related to changes in fasting plasma CETP activity (data not shown). Furthermore, age, when added to the model as a covariate or as a separate factor (separating the subjects into 2 groups: 19 to 35 years *v* 50 to 66 years) did not have a significant effect on the clamp-induced suppression of CETP levels before or after the improvement in insulin sensitivity.

In a previous study, we examined the effects of insulin resistance on lipoprotein subpopulation distribution in 20 lean

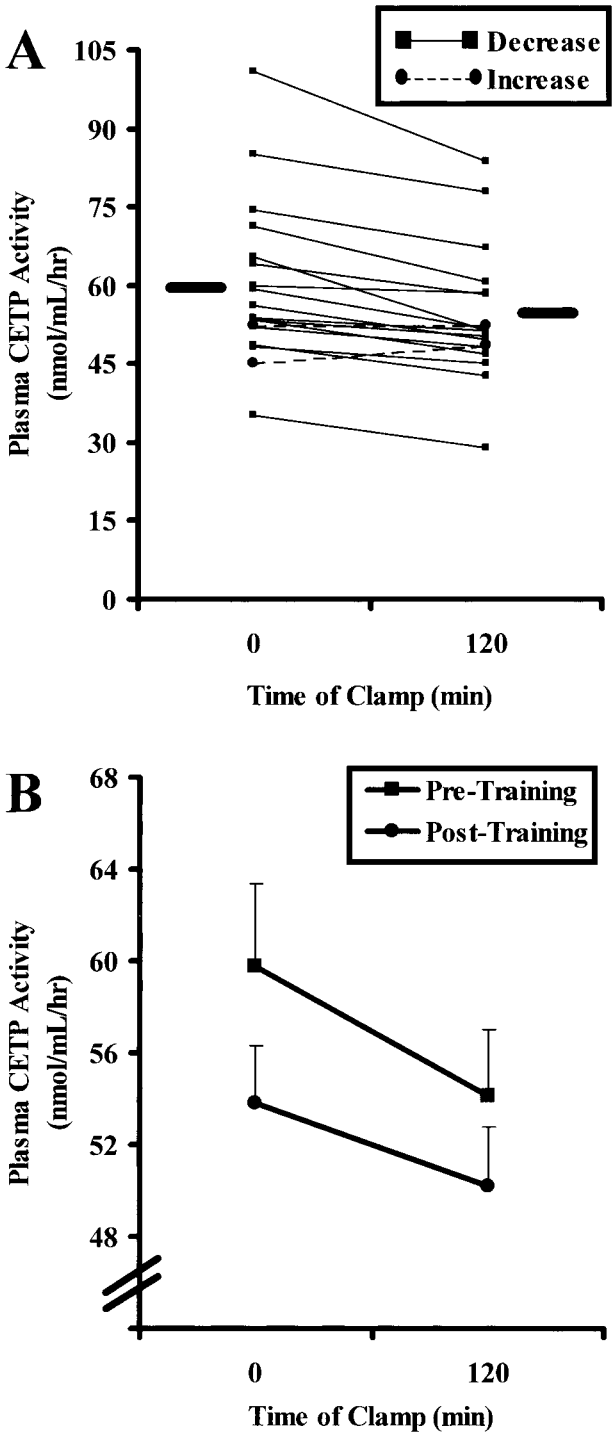

Fig 1. Plasma CETP activity in response to a hyperinsulinemic-euglycemic clamp. (A) Individual plasma CETP activity values before (t = 0 minutes) and after (t = 120 minutes) a hyperinsulinemic-euglycemic clamp in 18 men are diagrammed (means are represented by a bold line). In a paired *t* test, plasma CETP activity was significantly lower after the 2-hour hyperinsulinemic-euglycemic clamp, $P < .05$). (B) Average plasma CETP activity values before (t = 0 minutes) and after (t = 120 minutes) a hyperinsulinemic-euglycemic clamp in 18 men before and after 1 week of exercise training are diagrammed. Data are expressed as mean and SEM (error bars) and were analyzed by 2-way, repeated measures ANOVA for the effects of exercise ($P < .01$), clamp ($P < .001$), and the interaction between the 2 effects ($P = .26$).

and 21 morbidly obese, nondiabetic women.[12] Because that study design is relevant to the issues of this study, we measured plasma CETP activity in this same cohort of patients and report the values here. The full complement of patient characteristics are published in that report. Briefly, plasma CETP activity was measured in insulin-sensitive lean, insulin-resistant lean, insulin-sensitive obese, and insulin-resistant obese women. Plasma CETP activity in these subjects was analyzed in a 2-way ANOVA model, testing for the effects of insulin resistance, obesity, and their interaction. Only the effect of obesity was statistically significant (Fig 2). Consistent with this observation, plasma CETP activity was not related to plasma glucose, plasma insulin, or S_I, but was related to body mass, BMI, waist circumference, percent body fat, and fat mass (Table 2).

Table 2. Relationship of Plasma CETP Activity With Several Characteristics of Insulin-Resistant and Insulin-Sensitive Lean and Obese Women

	Plasma CETP Activity
S_I	−0.27
Plasma insulin	0.32
Plasma glucose	0.29
Body mass	0.61*
BMI	0.66†
Waist circumference	0.65†
Percent body fat	0.69†
Fat mass	0.67†

NOTE. Relationships are indicated by Bonferonni's correlation coefficient. S_I, Bergman's minimal model estimation of insulin sensitivity (n = 41).

*$P < .01$.

†$P < .005$.

DISCUSSION

Several studies have observed perturbations in the plasma CETP activity of obese and diabetic subjects.[3-7] Over the past several years, there have been many reports investigating the role of insulin in regulating CETP expression with much speculation about the possible role of insulin resistance in modifying plasma CETP levels. Two studies reported that plasma CETP activity decreased during a hyperinsulinemic-euglycemic clamp,[8,9] concluding that the hyperinsulinemia was responsible for the effect. In a recent review, it was suggested that the suppressive effects of acute hyperinsulinemia on plasma CETP activity levels were linked to peripheral insulin sensitivity, implying that insulin resistance could influence the normal suppressive effects and lead to perturbed plasma CETP activity.[1] In the present study, we could not provide any evidence that the clamp-induced suppression of plasma CETP activity was linked to insulin action. Furthermore, when the confounding variables of adiposity, body fat distribution, diabetes, and physical conditioning were controlled for in our cross-sectional examination, the level of plasma CETP activity was similar for insulin-resistant and insulin-sensitive subjects. Obesity, on the other hand, was accompanied by an elevation in plasma CETP activity irrespective of the level of insulin sensitivity.

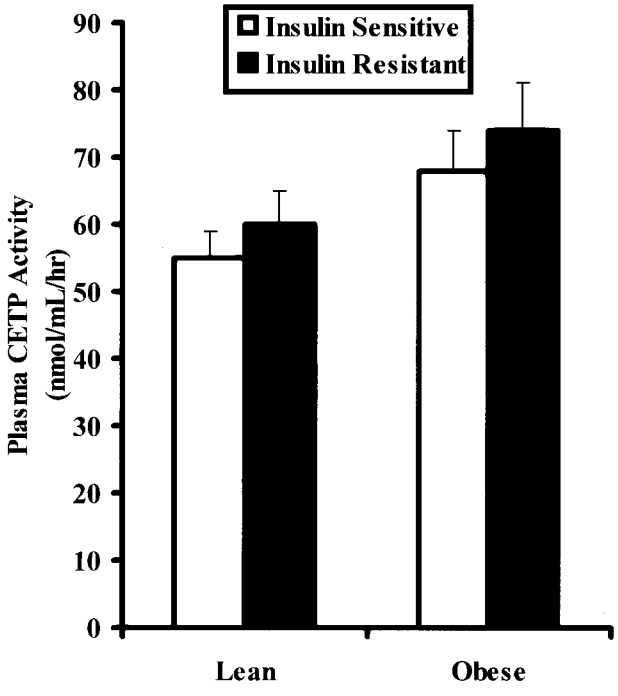

Fig 2. Plasma CETP activity of insulin-sensitive and insulin-resistant, lean and obese women. Plasma CETP activity was measured in 41 nondiabetic women (12 insulin-sensitive, lean; 8 insulin-resistant, lean; 10 insulin-sensitive, obese; 11 insulin-resistant, obese). Data were analyzed by 2-way ANOVA for the effects of obesity ($P < .01$), insulin resistance ($P = .34$), and their interaction ($P = .82$). Thus, obese subjects had higher CETP activity than their lean counterparts, regardless of the level of insulin sensitivity.

There has been some controversy concerning the suppressive effect of the hyperinsulinemic-euglycemic clamp on plasma CETP activity. Arii et al[9] observed a significant (≈9%) drop in both diabetic and nondiabetic subjects in response to the clamp. In smaller cohorts of subjects, Sutherland et al[8] observed a decrease in plasma CETP activity in diabetic subjects, but not in normal subjects, while Van Tol et al[10] reported no statistical change in plasma CETP activity in either group. Similar to Arii et al, the present study reports a 9% drop in plasma CETP activity in response to the clamp (Fig 1). Taken together with the present study, it is likely that the statistically insignificant decreases reported by some were a result of the small number of subjects examined. Thus, we conclude that plasma CETP activity is decreased in response to a hyperinsulinemic-euglycemic clamp.

What, then, is responsible for the decrease in plasma CETP activity during the clamp? One possibility is that hyperinsulinemia suppresses CETP expression and/or secretion. However, there is little evidence to implicate a particular mechanism for CETP expression regulation. In a recent study, we were unable to establish a link between insulin and CETP promoter activity,[26] suggesting that the effects, if any, were downstream of transcription. Even so, studies in animal and tissue culture models report an increase, if any effect at all, in CETP secretion in response to insulin treatment.[27-29] Another explanation is that CETP clearance is enhanced during the clamp procedure. It has been suggested by others that hyperinsulinemia would lead to increased TG clearance from the lipoproteins by stim-

ulating lipoprotein lipase.[8] This, in turn, would lead to increased hepatic clearance of the lipoproteins themselves, to which CETP binds.[30] Interestingly, VLDL-TG tended to decrease during the hyperinsulinemic-euglycemic clamp (by 20%, $P = .07$, data not shown) in our subjects. However, the observations by others that insulin stimulates adipose tissue lipoprotein lipase while inhibiting the muscle isoform suggests that this hypothesis, while seemingly plausible, needs further substantiation.

One other possibility to explain the suppressive effects of the clamp on plasma CETP activity is hemodilution. Even though 2 hours of infusion with equivalent volumes of saline does not significantly effect plasma CETP activity,[8,10] this does not take into account the possible dilutition that may occur in response to hyperinsulinemia. Interestingly, when normalized to plasma protein levels, we still observed a decrease in plasma CETP activity in response to the clamp (data not shown). Yet, this approach also has its limitations given insulin's effects on protein metabolism. Thus, we cannot be sure of the mechanism by which CETP decreases in response to the hyperinsulinemic-euglycemic clamp. However, the findings of the present study make the mechanistic issue irrelevant, as we observed the suppressive effect is not linked to insulin action. Without this link, the physiologic relevance of the clamp-induced suppression of plasma CETP activity remains in question.

Chronic perturbations in plasma insulin levels, as well as alterations in plasma CETP levels, are common in patients who display characteristics of the insulin resistance syndrome. Chronic hyperinsulinemia, obesity, diabetes (poor glucose control), and insulin resistance are all closely related metabolic disorders of the insulin resistance syndrome.[31-33] As a consequence of the close association between these parameters, it has been difficult for investigators in the scientific community to distinguish which parameter, if any, was responsible for the perturbations in plasma CETP activity. Plasma CETP activity is consistently higher in nondiabetic obese subjects when compared with normoinsulinemic, lean counterparts.[3-7] In these studies, both the degree of adiposity and insulin levels have been related to the alterations in plasma CETP activity. A long-term ($\approx$1 year) exercise training regimen that improved insulin sensitivity has been shown to decrease plasma CETP activity, even when weight loss was minimal.[34] Consistent with this report, the present study shows that 1 week of training that improved insulin sensitivity without weight loss lowered plasma CETP activity (Fig 1B). While exercise-induced plasma dilution may account for some of the reduction in CETP levels, it was suggested that insulin resistance results in the elevation of plasma CETP activity through the impairment of insulin's normal suppressive effects, and that improvements in insulin action normalize these perturbations. However, we did not observe a significant alteration in the suppression of plasma CETP during the hyperinsulinemic-euglycemic clamp after an improvement in insulin sensitivity. Furthermore, the changes in GIR did not correlate with the changes in plasma CETP activity, suggesting that the exercise training-induced decrease in plasma CETP activity in our patients is due to some effect of training besides the accompanying improvement in insulin sensitivity. Thus, we were unable to establish a link between the suppressive effects of acute hyperinsulinemia and whole body sensitivity to insulin's glucoregulatory effects. Without this link, there is little evidence to support the idea that the development of insulin resistance leads to perturbations in CETP activity by impairing insulin's suppressive effects.

Consistent with these findings, we have reported the first examination of the relationship of plasma CETP activity to insulin action that carefully controlled for the confounding variables of adiposity, body fat distribution, and diabetes. The subjects represented in Fig 2 are the same cohort of nondiabetic subjects published in a previous report.[12] Because this particular study design was relevant to the issues of this study, we have reported our findings in these subjects here. This design consisted of 4 groups of nondiabetic women: 2 groups of lean (BMI, $\approx$24 kg/m^2) women who were either insulin-sensitive (S_I, 6.5 $\pm$ 0.6 min^{-1}/μU/mL) or insulin-resistant (S_I, 1.9 $\pm$ 0.3 min^{-1}/μU/mL) and 2 groups of obese (BMI, $\approx$38 kg/m^2) women who were either insulin-sensitive (S_I, 5.6 $\pm$ 0.6 min^{-1}/μU/mL) or insulin-resistant (S_I, 1.9 $\pm$ 0.3 min^{-1}/μU/mL). Insulin-resistant women had higher plasma glucose (5.2 $\pm$ 0.1 *v* 4.8 $\pm$ 0.1 mmol/L) and insulin (66 $\pm$ 7 *v* 30 $\pm$ 3 pmol/L) concentrations when compared with insulin-sensitive women. Furthermore, in both lean and obese comparisons, insulin-sensitive subjects were similar in body weight, percent body fat, and waist circumference when compared with their insulin-resistant counterparts. Insulin resistance and the accompanying hyperinsulinemia had little effect on plasma CETP activity (Fig 2). In contrast, the degree of adiposity (obesity) had a significant and substantial effect. Furthermore, the indices of adiposity (body mass, BMI, percent body fat, waist circumference) were closely associated with plasma CETP activity (Table 2). The 25% increase in CETP activity with obesity in these subjects, regardless of the level of insulin sensitivity, is comparable to what has been previously reported by us and others when lean and nondiabetic obese patients are compared.[3-7] Thus, the elevation in CETP expression often found in nondiabetic, obese patients is more likely due to some complicating factor(s) of obesity other than insulin resistance and hyperinsulinemia. What other factor(s) of obesity is involved, as well as what influence diabetes may have, are issues that should be addressed in future studies. It is interesting to note in these patients that neither obesity nor insulin resistance alone resulted in atherogenic lipoprotein profiles.[12] A larger average VLDL size and smaller average LDL and HDL sizes were only observed when both obesity and insulin resistance were present. This observation is consistent with the concept that there are several factors that influence the metabolism of lipoproteins, only 1 of which is CETP.

In summary, neither experimental approach used in this study could link plasma CETP levels to insulin and/or insulin action, and these observations suggest that some other factor associated with obesity is responsible for the perturbations in plasma CETP that frequently accompany the insulin resistance syndrome. While short-term exercise training lowered plasma CETP activity, the contribution of the accompanying improvement in insulin action is unclear in that the suppressive effects of acute hyperinsulinemia during the clamp procedure were not significantly altered. Furthermore, the changes in insulin action were not related to the changes in fasting plasma CETP activity. Thus, the suppressive effect of acute hyperinsulinemia on plasma CETP does not appear

to be linked to whole body insulin sensitivity. These data lend little credence to the idea that insulin resistance leads to elevated CETP levels by chronically impairing the suppressive effects of insulin. Moreover, in the cross-sectional examination, insulin resistance and the accompanying chronic hyperinsulinemia were observed to have little effect on plasma CETP activity, while the effects of adiposity were significant and substantial. Taken together, these data suggest that the alterations in plasma CETP activity found in patients who display characteristics of the insulin resistance syndrome are related to some complicating factor of obesity other than insulin resistance and the accompanying hyperinsulinemia.

ACKNOWLEDGMENT

We thank Dr Jim Otvos, Department of Biochemistry, North Carolina State University, for assistance in the processing and interpretation of NMR data and Dr Lynis Dohm and Joseph Bower for their review and editing of the manuscript. We appreciate the excellent technical assistance provided by Brian Roberts.

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