

# Cholesteryl Ester Transfer Protein Expression Prevents Diet-Induced Atherosclerotic Lesions in Male db/db Mice

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**Objective**—Accompanying more atherogenic lipoprotein profiles and an increased incidence of atherosclerosis, plasma cholesteryl ester transfer protein (CETP) is depressed in diabetic obese patients compared with nondiabetic obese counterparts. The depressed levels of CETP in the plasma of diabetic obese individuals may contribute to the development of an atherogenic lipoprotein profile and atherogenesis. We have examined the effect of CETP expression on vascular health in the db/db model of diabetic obesity.

**Methods and Results**—Transgenic mice expressing the human CETP minigene were crossed with db/db strain, and 3 groups of offspring (CETP, db, and db/CETP) were placed on an atherogenic diet for 16 weeks. The proximal aorta was then excised and examined for the presence of atherosclerotic plaques. In db mice, 9 of 11 had intimal lesions with a mean area of  $26\,098 \pm 7486 \mu\text{m}^2$ . No lesions greater than  $1000 \mu\text{m}^2$  were observed in db/CETP or CETP mice. CETP-expressing mice had lower circulating cholesterol concentrations than db mice. Fractionating plasma lipids by FPLC indicated that the difference in total cholesterol was primarily attributable to differences in VLDL and LDL.

**Conclusions**—The expression of human CETP in db/db mice prevented the formation of diet-induced lesions, suggesting an antiatherogenic effect of CETP in the context of diabetic obesity. (*Arterioscler Thromb Vasc Biol.* 2003;23:1412-1415.)

**Key Words:** cholesterol ■ FPLC ■ VLDL ■ LDL ■ HDL obesity

Cholesteryl ester transfer protein (CETP) is a glycoprotein that catalyzes the transfer of neutral lipids between the plasma lipoproteins. In this respect, this enzyme is involved in at least one arm of reverse cholesterol transport (RCT), an antiatherogenic process by which cholesterol is cleared from peripheral tissues.<sup>1</sup> Even so, the atherogenic nature of CETP has been the subject of much debate because both increased and decreased CETP expression have been linked to elevated risk and incidence of vascular disease.<sup>2</sup> Thus, the atherogenic nature of CETP is thought to depend on the metabolic context in which it influences lipoprotein metabolism.

Obesity is a metabolic condition afflicting more than one third of the population of the United States.<sup>3</sup> Obesity is accompanied by both a mild increase in vascular disease complications<sup>4</sup> as well as elevated plasma CETP activity.<sup>5-9</sup> It is unclear whether this perturbation in CETP activity contributes to altered lipoprotein profiles and elevated vascular disease risk or is a normal consequence of elevated cholesterol levels observed in these patients.<sup>5,10</sup> Interestingly, obese patients with type 2 diabetes have a higher risk of vascular disease complications,<sup>11</sup> higher circulating cholesterol levels,<sup>5,10</sup> and depressed levels of plasma CETP concentra-

tions<sup>5,12</sup> compared with obese nondiabetic controls. This suppressive effect of diabetes on plasma CETP is not apparent in nonobese individuals.<sup>13-16</sup> We have hypothesized that depressed plasma CETP levels in obese patients with diabetes may hinder the clearance of the high levels of peripheral cholesterol that accompany obesity and contribute to elevated atherosclerosis in these patients.

The purpose of this study was to examine the effects of overexpressing CETP on vascular health and lipoprotein profiles in the metabolic context of diabetic obesity. Transgenic mice expressing the human CETP gene were crossed with db/db mice to produce the following 3 groups of experimental offspring: normal mice expressing CETP (CETP), diabetic obese mice not expressing CETP (db), and diabetic obese mice expressing CETP (db/CETP). The 3 groups were fed an atherogenic diet for 16 weeks and examined for atherosclerotic lesion development.

## Methods

Transgenic mice expressing the human CETP minigene were obtained from Dr Jan Breslow (Rockefeller University, New York, NY).<sup>17</sup> Heterozygous (+/db, C57BL/6J strain) male and female mice were obtained from Jackson Laboratories (Bar Harbor, Maine) and

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**Characteristics of CETP, db, and db/CETP Mice After 16 Weeks on a High-Fat Diet**

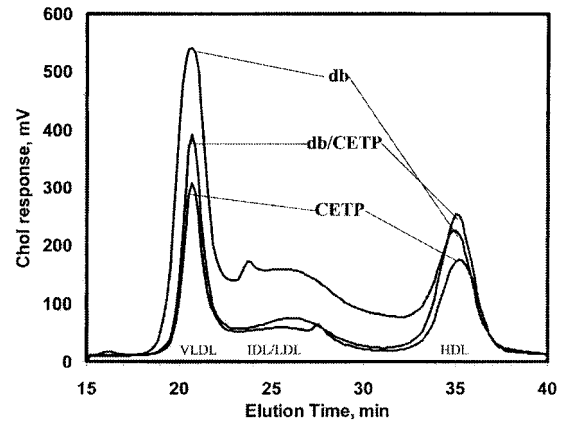
	CETP (Nondiabetic With CETP)	Db (Diabetic Obese Without CETP)	db/CETP (Diabetic Obese With CETP)
Genotype	+/+;C/C	db/db; -/-	db/db;C/C
No. of mice with plaques >1000 $\mu\text{m}^2$ /total	0/15	9/11	0/14
Intramural fat deposition, AU	18 $\pm$ 11	60 $\pm$ 17*	23 $\pm$ 15†
Body mass, g	30 $\pm$ 3	49 $\pm$ 4*	61 $\pm$ 5*†
CETP activity, nmol/mL per h	602 $\pm$ 12	5 $\pm$ 10	610 $\pm$ 22†
TC, mmol/L	4.42 $\pm$ 0.08	7.99 $\pm$ 0.65*	5.36 $\pm$ 0.26*†

\* $P < 0.05$  vs nondiabetic with CETP (CETP).

† $P < 0.05$  vs diabetic without CETP (db).

were crossed with CETP mice over several generations to produce mice for the following 3 experimental groups: CETP (+/+; C/C), db (db/db; -/-), and db/CETP (db/db; C/C). All mice were housed under alternating light/dark conditions (12 hours/12 hours), with access to normal mouse chow and water during the breeding and screening period. At 2 months of age, male mice chosen for the experiment were placed on a 16-week diet containing 15% fat, 1.25% cholesterol, and 0.5% sodium cholate (Dyets Inc) that has previously been shown to promote atherosclerosis in mice.<sup>18</sup> After 16 weeks on the diet, the mice were euthanized in a CO<sub>2</sub> tank, after which blood and tissues were collected. All animal care and handling was monitored by the University Animal Care and Use Committee in accordance with the NIH *Guide for the Care and Use of Laboratory Animals*.

Characterization of atherosclerotic lesions in the proximal aorta was based on the methods of Paigen et al.<sup>19</sup> Frozen serial cross sections of the aorta were cut and stained with oil red O and processed according to Humason.<sup>20</sup> When significant lesions were detected (>1000  $\mu\text{m}^2$ ), the area of the oil red O-positive staining was calculated using NIH Image software. Oil red O staining of arterial cross sections was quantified by image analysis software and is shown to indicate the extent of intramural lipid deposition. Blood samples were measured for CETP activity, glucose, total cholesterol (TC), nonesterified fatty acids (NEFAs), and triglycerides (TG) as previously described.<sup>21,22</sup> Intraperitoneal glucose tolerance tests

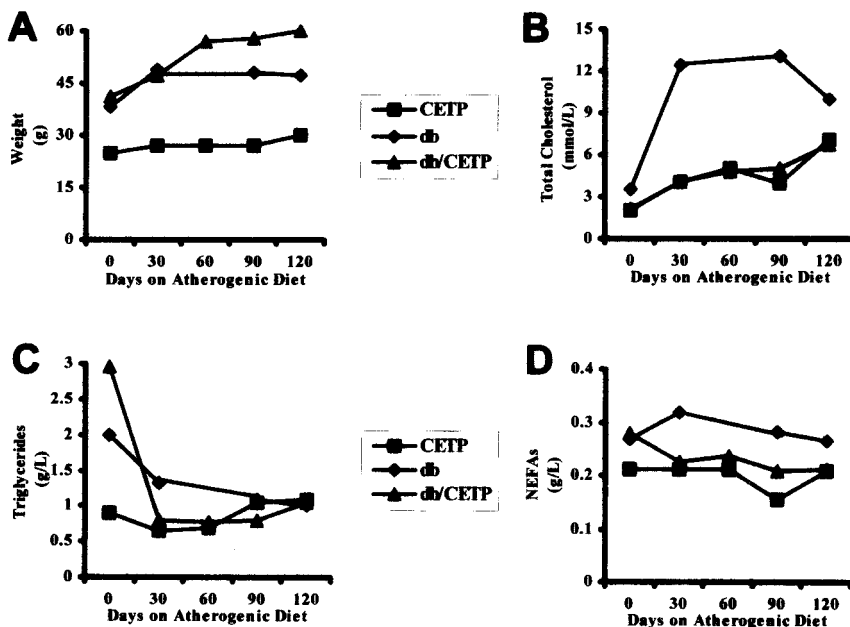


**Figure 1.** Cholesterol distribution among the plasma lipoproteins. Plasma samples were collected 90 days into the dietary regimen, pooled, and characterized by FPLC to obtain the relative concentration of cholesterol in VLDL, IDL/LDL, and HDL.

were performed as described by others,<sup>23</sup> and results are expressed as area under the glucose curve. Lipoproteins were separated with FPLC, and cholesterol was quantified with an in-line detection system based on that described by Kieft et al.<sup>24</sup> Data were analyzed by one-way ANOVA with Fisher's least-significant difference post-hoc test (Systat, SPSS Inc).

**Results**

Regardless of the expression of the CETP transgene, mice that were heterozygous for the db mutation were severely obese (CETP, 24 $\pm$ 3; db, 41 $\pm$ 4; db/CETP, 38 $\pm$ 4 g), hyperglycemic (CETP, 72 $\pm$ 4; db, 139 $\pm$ 13; db/CETP, 152 $\pm$ 13 mg/dL), and less tolerant of a glucose challenge (CETP, 8.8 $\pm$ 0.9; db, 23.2 $\pm$ 3.1; db/CETP, 20.2 $\pm$ 2.6 g/dL per min). Fasting plasma CETP activity was approximately 3 times higher ( $\approx$ 250 nmol/mL per h) in CETP and db/CETP mice than is normally found in humans,<sup>5,25</sup> whereas the activity in db mice was negligible. After being fed the atherogenic diet for 16 weeks, the animals were killed and examined for oil



**Figure 2.** Weight and plasma lipid concentrations in response to 16 weeks on an atherogenic diet. The 3 groups of mice were placed on a 16-week atherogenic diet, during which weight (A), plasma total cholesterol (B), plasma total TG (C), and nonesterified fatty acids (D) were determined at 30-day intervals.

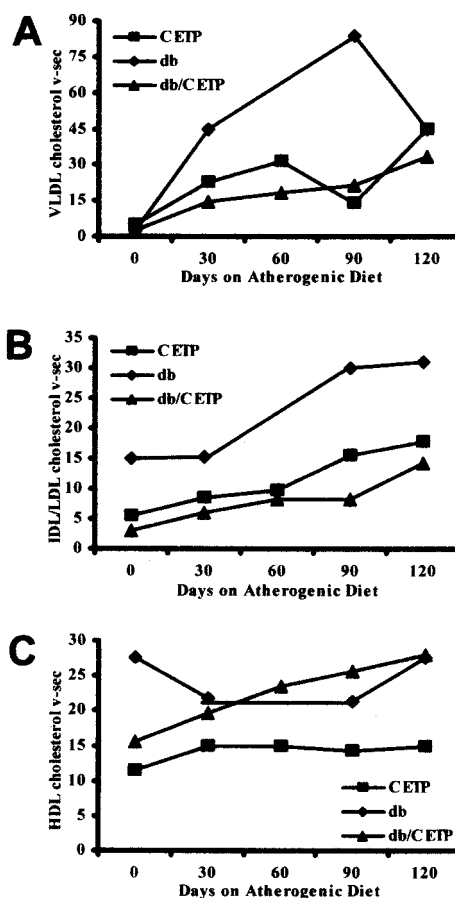
red O-positive intimal plaque lesions and intramural fat deposition (Table). Substantial lesions ( $>1000 \mu\text{m}^2$ ) were only detected in db mice. These lesions consisted of cells filled with oil red O droplets, previously referred to as foam cells,<sup>19</sup> and penetrated deep within the endothelial lining of the ascending aorta. There were 1 to 3 lesions per section examined, and the area of individual lesions varied between 1067 and 96 887  $\mu\text{m}^2$ , with an average of  $26\,098 \pm 7486 \mu\text{m}^2$ . Mice expressing CETP had lower levels of circulating cholesterol than db mice (Table). Blood samples that were collected and pooled 90 days into the dietary regimen and fractionated by FPLC indicated that the higher TC observed in db mice was reflected primarily in a greater amount of VLDL-C and IDL/LDL-C subfractions (Figure 1).

Figure 2 displays body weight and pooled plasma determinations of TC, TG, and NEFAs throughout the course of the dietary regimen. Interestingly, after 60 days, there was a distinct difference in body weight between db and db/CETP mice. Plasma cholesterol was higher in db mice than in db/CETP or CETP mice throughout the entire dietary regimen (Figure 2B). Plasma TGs were higher in mice homozygous for the db mutation (Figure 2C), but these differences were eliminated by the end of the dietary regimen. NEFAs were elevated in db mice throughout the diet (Figure 2D). The peak area for VLDL, IDL/LDL, and HDL was obtained from FPLC chromatograms derived from the 3 groups. VLDL-C was higher in the db mice than in the other groups in all but the final time point (Figure 3A). IDL/LDL-C gradually increased during the dietary regimen in all 3 groups of mice but was higher in the db mice than in the mice expressing CETP (Figure 3B). At the start of the dietary regimen, HDL-C was 3-fold higher in db mice than in those expressing the CETP transgene (Figure 3C). HDL-C in db/CETP mice gradually increased during the diet to a level similar to that found in db mice.

### Discussion

The expression of CETP in db/db mice prevented the development of diet-induced intimal plaque lesions. The vascular health of diabetic obese mice that expressed CETP was similar to the nondiabetic transgenic mice that we report here and to nondiabetic male wild-type mice (+/+; -/-) reported elsewhere<sup>18</sup> in that lesion size, if any was less than  $1000 \mu\text{m}^2$ . Consistent with these observations, the lipid and lipoprotein profile seemed to be more atherogenic in db mice than in either of the other groups expressing CETP with respect to their higher levels of VLDL-C and LDL-C. These data suggest that in the metabolic context of diabetic obesity, CETP may play an important role in prevention of atherogenic lipoprotein profiles and atherosclerosis.

The effect of CETP on vascular health has been the subject of much research and debate,<sup>2</sup> pitting the suppressive effects of CETP on HDL versus the putative role of CETP in reverse cholesterol transport. Although the introduction of CETP into db mice seemed to lower HDL-C levels, it also prevented significant lesion development in response to the diet. Our studies are consistent with the finding that the introduction of the human CETP gene decreased atherosclerosis in hypertriglyceridemic mice<sup>26</sup> and those that overexpress lecithin:cho-



**Figure 3.** Relative amount of cholesterol in VLDL, IDL/LDL/C, and HDL in response to 16 weeks on an atherogenic diet. The 3 groups of mice were placed on a 16-week atherogenic diet, during which plasma samples were collected and pooled at 30-day intervals. A, VLDL; B, IDL/LDL; and C, HDL in the pooled samples were separated with FPLC and cholesterol quantified with an in-line detection system.

lesterol acyltransferase.<sup>27</sup> From a mechanistic perspective, CETP has been shown to promote cholesterol efflux from cultured smooth muscle cells<sup>28</sup> and from lipid-loaded foam cells.<sup>29</sup> It is unclear whether these observed effects are the result of the effects of CETP on HDL's clearance of cholesterol from the periphery or on apolipoprotein B-containing lipoprotein metabolism. Although additional studies are required to identify the mechanisms behind this protective effect in this model, these results suggest that in this metabolic context of diabetic obesity, CETP may have beneficial effects on vascular health.

In summary, the introduction of CETP into the diabetic mouse model, db/db, resulted in less atherosclerosis, lower TC, lower VLDL-C, and lower IDL/LDL-C. In this particular model, CETP is clearly antiatherogenic, suggesting that in the context of diabetic obesity, CETP may be antiatherogenic. Even so, there are certain conditions in which CETP may promote atherosclerosis, as in the case when LDL receptor-mediated LDL uptake or alternative RCT pathways are impaired. Thus, clinical treatments of vascular disease in humans that target CETP may need to be directed according to the metabolic context of the patient and may need to

include the concomitant targeting of other steps in the reverse cholesterol transport pathway.

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