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Differential expression of cholesteryl ester transfer protein in the liver and plasma of fasted and fed transgenic mice

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*Because cholesteryl ester transfer protein (CETP) is considered a potential target in the treatment of atherosclerosis, several reports have focused on the regulation of this enzyme, and there is evidence that insulin may be a regulatory factor. The present study examines the differential expression of the human CETP gene between physiologic conditions that are accompanied by low (fasted) and high (fed) insulin levels. CETP expression was examined in plasma and tissues of transgenic mice expressing the human CETP minigene after 12 hours of fasting (*n = *20) or ad libitum feeding (*n = *20) with normal mouse chow. Plasma cholesteryl ester transfer activity (CETA) was 20% higher in fed than in fasted mice, reflecting higher levels of CETP (*P < *0.05). This observation was accompanied by higher liver mRNA in fed mice (100%,* P < *0.05), as determined by ribonuclease protection assays, as well as by higher CETA (23%,* P < *0.05) and CETP mass (29%,* P < *0.05) in the particulate fraction of liver homogenates. These parameters of liver CETP expression correlated well with each other, as well as with plasma CETA. CETP in the liver particulate fraction was found as a doublet (approximately 70 and 65 kDa), which resolved to a single band (approximately 60 kDa) upon deglycosylation. No differences in CETP expression were observed in pooled adipose tissue samples from fed and fasted mice. Insulin and glucose were not related to any plasma or tissue parameter of CETP expression. In summary, the concerted, differential expression of CETP in the liver of fed and fasted transgenic mice appears to contribute to higher plasma CETP levels in fed mice, but the precise role of insulin and glucose in regulating CETP expression under fasted and fed conditions needs to be defined.* (J. Nutr. Biochem. 11:318–325, 2000)

Keywords: CETP; fasting; insulin; glucose; liver; SDS-PAGE

Introduction

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that catalyzes the transfer of neutral lipids between the lipoproteins. CETP plays an important role in at least one arm of reverse cholesterol transport (RCT), the beneficial process by which cholesterol is collected from the peripheral tissue and transported to the liver (for review, see Bruce et al.[1]). However, altered CETP expression in certain physiologic states may lead to increased risk and incidence of vascular disease (for review, see Stevenson[2]). Because of the association of CETP with plasma lipoprotein metabolism and its potential as a target in the treatment of atherosclerosis, understanding the regulation of this enzyme has become the focus of several researchers.

A number of studies have shown that CETP expression may be influenced by nutritional and metabolic factors. Increased plasma CETP activity has been reported in rabbits, hamsters, and transgenic mice expressing the human CETP minigene that were fed a high fat, high cholesterol diet,[3–7] a result primarily due to increased levels of CETP mRNA. It is thought that several sterol response elements in the CETP promoter region (between −138 and −570) are responsible for the activation of transcription by cholesterol.[8] Other nutritional and metabolic factors associated with the postprandial state may also be involved in the

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regulation of CETP expression. One possibility is that CETP expression may be influenced by plasma insulin levels.

Several studies have suggested that insulin may regulate CETP expression. Insulin stimulated the secretion of CETP from cultured hamster adipocytes,[7] and plasma CETP activity increased during a hyperinsulinemic euglycemic clamp in rabbits.[9] In humans, the role of insulin in CETP expression regulation is less clear. Consistent with the animal studies, plasma CETP activity is elevated in chronically hyperinsulinemic, obese,[10–13] and insulin-dependent diabetes mellitus patients.[14] However, these observations may have been complicated by accompanying perturbations in insulin sensitivity or glycemic control in the subjects, factors that could influence how insulin affects CETP expression. In contrast to what has been observed in animal studies, plasma CETP activity is decreased during a hyperinsulinemic euglycemic clamp in humans.[15,16] Thus, insulin's effects on CETP expression appear to be species specific, and its regulation of the human CETP gene is not well characterized.

Fasting (postabsorptive) and fed (postprandial) states are normal physiologic conditions between which plasma insulin concentrations vary a great deal. Although several studies have examined the effects of high fat and cholesterol consumption on plasma CETP in animals and humans,[3–6,17–20] no study has extensively examined the expression of the human CETP gene in both plasma and tissues with respect to varying endogenous insulin concentrations. Therefore, the purpose of this study was to compare the expression of CETP in fed and fasted states in transgenic mice expressing the human CETP minigene. Because the putative effects of insulin observed by researchers appear to be downstream of transcription,[7,9,15,16] it was important to measure tissue CETP levels with respect to mRNA, protein activity, and protein mass in both liver and adipose tissue. The results of this study not only provide a better understanding of human CETP gene expression in common physiologic states, but also allow the relationship of insulin variations to the changes in CETP expression to be examined.

Methods and materials

Animals treatment

Heterozygote transgenic C57BL/J6 mice for the human CETP allele, obtained from Dr. Jan Breslow (Rockefeller University, New York, NY USA), were housed under a 12-hour light/dark cycle with access to normal mouse chow and water. The mice were bred over three generations to develop a homozygous strain for the human CETP allele. Both plasma CETP activity and Southern slot blot techniques were used to select homozygous animals. In Southern analysis, a cDNA of human CETP was used to make a radiolabeled probe using a random primer kit (Boehringer Mannheim, Indianapolis, IN USA). Genomic DNA was isolated from the tail snips of mice in a buffer containing 50 mM Tris-HCl, 100 mM ethylenediamine-tetraacetic acid (EDTA), 100 mM NaCl, and 1% sodium dodecylsulfate (SDS), pH 7.4. Tail snips were digested in the same buffer containing proteinase K, and RNA was eliminated with RNAse A. Two subsequent generations showing none of the mice lacking the expression of CETP confirmed that the mice were homozygous for the human CETP minigene. This 11-kb minigene expressed by these mice contained 3.2 kb of the 5′ flanking region, the human CETP gene lacking introns 2 to 11, and 2.0 kb of the 3′ flanking region. Tissue distribution and induction of CETP mRNA by cholesterol has been found to be similar to that of humans.[21]

Two groups of male mice were matched for age (approximately 6 months), weight, and initial plasma CETP activity. The fed group ($n = 20$) had free access to water and normal mouse chow for a 12-hour period overnight, and the fasted group ($n = 20$) had access to only water. At the end of the 12-hour period, the mice were euthanized in a carbon dioxide tank. Blood was drawn by cardiac puncture into heparinized tubes, and plasma was isolated by low speed centrifugation (2,500 × g, 30 min, 4°C). Liver and perirenal adipose tissue were excised from the animals, quick frozen in liquid nitrogen, and stored at −80°C until analyzed. Because adipose tissue samples from each animal were not always large enough to examine all aspects of tissue CETP expression, samples were pooled for the various determinations (fed/fasted; $n = 12/12$).

Determination of CETP mRNA

RNA was isolated from the tissues using Trizol Reagent and following the directions of the supplier (Gibco BRL, Rockville, MD USA). Tissues were homogenized in a Polytron homogenizer (Brinkman Instruments, Westbury, NY USA) at a concentration of 100 mg/mL Trizol, and the homogenates were stored at −80°C until further processing. Homogenates were thawed at 4°C with gentle inversion on the day RNA was isolated. Lithium chloride was added to liver preparations to remove glycogen. The isolated RNA samples were then washed with 75% isopropanol and resuspended in diethyl pyrocarbonate (DEPC)-treated water. The RNA was quantified with a spectrophotometer, and the integrity and relative quantity were examined by electrophoresis on an agarose/formaldehyde gel.

The probes for the ribonuclease protection assay (RPA) were prepared using Ambion's Maxiscript T7/T3 In Vivo Transcription Kit (Austin, TX USA). The plasmid used as the DNA template was acquired from Dr. Ruth McPherson (Ottawa Heart Institute, Ottawa, Ontario, Canada). This Bluescript vector contains a 160-bp fragment of the human CETP gene spanning nucleotides 727 to 887. Antisense RNA probes were transcribed in vitro from the T3 promoter in the presence of ^{32}P-labeled UTP (Dupont NEN, Boston, MA USA). The reaction was carried out for 2 hours at 4°C. The template was digested with DNAse 1.

Relative CETP mRNA amounts were determined with the use of Ambion's RPA II Ribonuclease Protection Assay Kit. The probe was hybridized to 30 μg (liver) or 60 μg (adipose tissue) of RNA overnight at 45°C in a hybridization buffer containing 80% deionized formamide, 100 mM sodium citrate, 300 mM sodium acetate, and 1 mM EDTA, pH 6.4. Because the yield from the pooled adipose tissue samples was low, isolated RNA samples were combined 2:1 to provide enough RNA for each assay run (fed/fasted: $n = 6/6$). Unhybridized RNA was then digested with a mixture of RNase A and RNase T1. The protected fragments were run on a 6% polyacrylamaide/7.5 M urea gel. The 160-bp protected fragments on the gel were visualized and quantified by phosphorimaging. RNA concentrations were normalized to total RNA.

Tissue homogenate preparation

For the determination of CETP activity and mass, tissues were homogenized in 10 mM Tris buffer (pH 7.4), 0.25 M sucrose, 1 mM EDTA, 1 mM aprotinin, and 1 mM phenylmethyl sulfonyl fluoride (PMSF). The excess fat in adipose tissue homogenates was removed by centrifugation at 1,000 × g. The homogenates

Table 1 Weight and plasma characteristics of fasted and fed mice

	Fasted	Fed
Weight (g)	23 ± 1	26 ± 1*
Glucose (mM)	7.3 ± 0.6	19.9 ± 1.2*
Insulin (pM)	63 ± 10	223 ± 20*
Plasma CETA (nmol/mL/hr)	242 ± 7	290 ± 10*
Plasma CETP mass (μg/mL)	5.08 ± 0.6	8.35 ± 1.0*

Cholesteryl ester transfer activity (CETA) measured as the rate of ^{3}H-cholesteryl esters transferred from ^{3}H-CE high density lipoprotein to exogenously prepared apoprotein B containing lipoproteins. Cholesteryl ester transfer protein (CETP) mass was determined by Western blot analyses, using the monoclonal antibody TP2 to detect the human CETP protein. Mass determinations were quantified using a semi-purified standard. $n = 40$ for fasted (20) and fed (20) groups with respect to weight, glucose, insulin, and plasma CETA. $n = 10$ for fasted (5) and fed (5) groups with respect to plasma CETP mass. *$P < 0.05$.

were centrifuged at 150,000 × g for 1 hour, and the supernatant (soluble) fraction and the pellet (particulate) were recovered. The supernatant fraction was stored at −80°C until analyzed for CETP activity and mass. The particulate fraction was resuspended in the homogenization buffer containing 1% Triton X-100 and stored at −80°C until analyzed.

Cholesteryl ester transfer activity determination

Cholesteryl ester transfer activity (CETA) in tissues and 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.[22] Both donor and acceptor lipoproteins were collected by ultracentrifugation of density adjusted, pooled plasma, acquired from the blood bank (Red Cross, Greenville, NC USA). In short, 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). The labeled donor (10 μg cholesterol) was incubated with acceptor lipoproteins ($d < 1.063$ g/mL, 250 μg cholesteryl esters), a small volume of sample plasma or tissue homogenate, and a buffer containing 10 mM Tris and 150 mM NaCl (pH 7.4). In plasma analysis, 5 μL of sample were used. For tissues, volumes between 10 and 100 μL were used, depending on the activity present. The reaction was incubated at 37°C between 6 and 16 hours. After the incubation, the acceptor lipoproteins were precipitated with a solution of 1% dextran sulfate (MW 50,000) and 0.5 M magnesium chloride. A portion of the supernatant was then combined with Scinteverse (Fisher Scientific, Pittsburgh, PA USA) and counted. Rates of transfer were calculated by 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 was linear for label transfers of up to 45%, and interassay variation (<5%) was normalized with the use of a standard of pooled plasma. Rates of transfer are expressed per unit time per milligram protein or milliliter plasma. Protein concentration was determined by BCA Protein Assay (Pierce, Rockford, IL USA) using bovine serum albumin as a standard.

CETP mass determinations

CETP mass was determined by Western blot analysis, using partially purified CETP with a known amount of CETP protein as a standard. Samples were diluted with Laemmli sample buffer (Sigma Chemical Co., St. Louis, MO USA), which contains 4% SDS, 20% glycerol, 10% mercaptoethanol, 0.004% bromophenol blue, and 0.125 M Tris, pH 6.8. Diluted samples were heated to 100°C for 3 min and loaded on an acrylamide gel (5% stacking, 12% running). The minigels (BioRad Laboratories, Hercules, CA USA) were run with constant voltage (100 V, stacker; 200 V running) for approximately 1 hour in a buffer consisting of 25 mM Tris and 192 mM glycine, pH 8.3. The proteins were then transferred to a nitrocellulose membrane, and probed with the primary antibody TP2, a monoclonal antibody against CETP (obtained from Dr. Ruth McPherson). The membranes were incubated in 5% milk containing the secondary antibody that is conjugated to horseradish peroxidase. Bound proteins were then incubated in a 1:1 mixture of Renaissance Enhanced Luminol Reagent and Oxidizing Reagent (Dupont NEN), visualized on X-ray film and quantified with a densiometer. Multiple exposures were used in the quantification process to protect from the loss of linearity. CETP concentration in the tissues was calculated relative to the known concentration of the semi-purified CETP that was applied to the same gel. Deglycosylation experiments were done on homogenate subfractions with a commercially available kit (BioRad), using NANase II, O-Glycosidase, and PNGase F. Most reagents and apparati were acquired from BioRad. All other materials not specified were obtained from Sigma or other reputable sources.

Plasma insulin and glucose determinations

Blood samples were analyzed spectrophotometrically for glucose (Sigma 16-UV) and by a radioimmunoassay (Linco Research, Inc.,

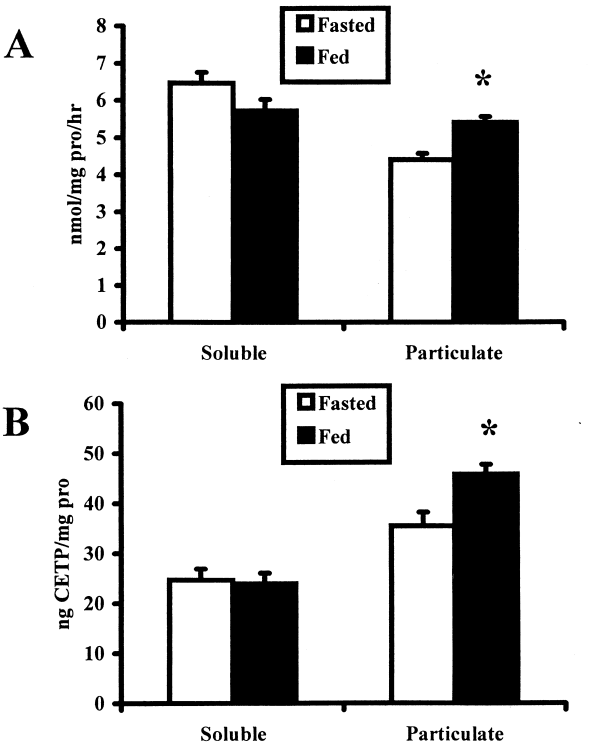

Figure 1 Liver cholesteryl ester transfer activity (CETA) and cholesteryl ester transfer protein (CETP) mass. (A) CETA is shown for the supernatant and particulate subfractions of the liver excised from fasted and fed mice. CETA was measured as the rate of ^{3}H-cholesteryl esters transferred from ^{3}H-CE high density lipoprotein to exogenously prepared apoprotein B containing lipoproteins. (B) CETP mass was determined by Western blot analyses using the monoclonal antibody TP2 to detect the human CETP protein. Mass determinations were quantified using a semi-purified standard. $n = 40$ for fasted (20) and fed (20) groups. *$P < 0.001$.

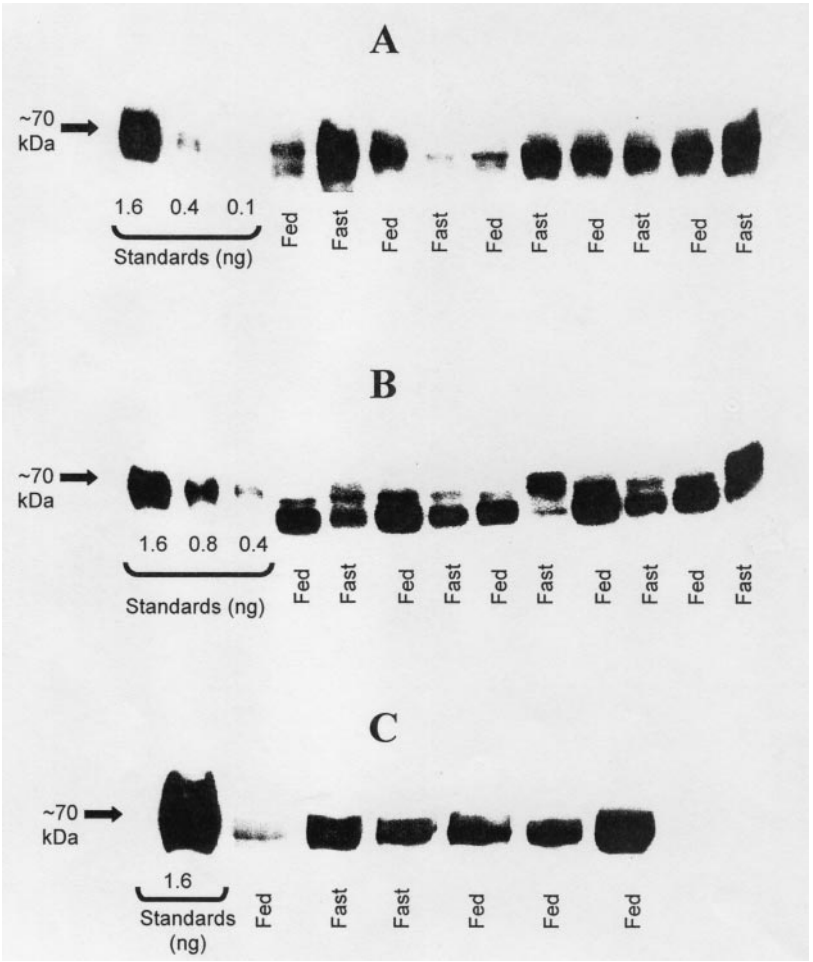

Figure 2 Detection of cholesteryl ester transfer protein (CETP) by Western blot in liver homogenates. Representative Western blots of (A) soluble fraction of liver homogenates, (B) particulate fraction of liver homogenates, and (C) particulate fraction of pooled adipose tissue homogenates. CETP in 40 μg of total protein was visualized after the sequential binding of a monoclonal antibody specific to human CETP (TP2) and a horseradish-peroxidase conjugated secondary antibody, followed by chemiluminescence. The relative mobility of molecular weight standards is shown to the left of each blot. Standards were purified from human plasma and represent serial dilutions of an 80 ng/μL stock.

St. Louis, MO USA) for insulin concentrations. This technique uses a rat insulin antibody to bind and precipitate sample insulin and ^{125}I-insulin in a competitive manner. The rat antibody used has been shown to bind with mouse and rat insulin with a similar affinity.

Statistics

Data were analyzed by independent *t*-tests to examine differences between fed and fasted groups. Pearson correlation coefficients (single comparison) or Bonferroni correlation coefficients (multiple comparisons) were calculated to examine relationships between variables of interest. Statistical significance was assumed when the *P*-value was less than 0.05.

Results

The weight and plasma characteristics of the mice are shown in *Table 1*. Although the groups were matched on weight before the experiment, fed mice weighed more than fasted mice after the treatment period. Both glucose and insulin were elevated in the fed animals. Plasma CETA was 20% ($P < 0.05$) higher in fed mice than in fasted mice. In a small number of randomly selected animals ($n = 10$), plasma CETP mass as estimated by Western blot was also higher in the fed state. Plasma CETP appeared as a single band at approximately 70 kDa (data not shown), consistent with the reports of secreted CETP observed between 65 and 74 kDa.[23–26] Plasma CETA and CETP mass were significantly related in these animals ($r = 0.68$, $P = 0.03$), reflecting a slightly weaker relationship to what we have observed in humans (unpublished observations).

CETA and CETP mass in liver soluble and particulate subfractions are shown in *Figures 1A* and *1B*, respectively. No significant differences between fasted and fed mice were observed in CETP activity or mass in the soluble fraction of liver homogenates. In contrast, CETA was 23% higher ($P < 0.05$) in the particulate fraction of fed mice than in fasted mice. Likewise, fed mice had 29% higher ($P < 0.05$) CETP mass in the particulate fraction than did those that were fasted. CETP activity correlated with CETP mass in both the soluble ($r = 0.41$, $P = 0.03$) and particulate ($r = 0.55$, $P = 0.01$) fractions of liver homogenates. The differential expression of CETP in fed and fasted mice can be observed in representative Western blots of liver soluble (*Figure 2A*) and particulate samples (*Figure 2B*). The doublet observed in the Western blot of liver particulate samples represents two CETP species with varying degrees of glycosylation, as deglycosylation with NANase II, O-Glycosidase, and PNGase F resulted in a single band (*Figure 3*). CETP mRNA was twofold higher ($P < 0.05$)

in the livers of fed mice than in those of fasted mice (*Figure 4*).

No differences in CETP expression parameters were observed in pooled perirenal adipose samples from fed (n = 12) and fasted (n = 12) groups. Relative to the amount of tissue, subcellular levels of CETP protein were over 60 times higher in the liver than in adipose tissue (5.3 ± 0.3 μg/g tissue vs. 80 ± 16 ng/g tissue). CETP was not detectable with activity or mass determinations in the soluble fraction of adipose tissue homogenates. In the particulate fraction of pooled adipose homogenates, no significant differences were observed between fasted and fed mice in CETA (112 ± 13 vs. 88 ± 12 nmol/mg protein/hr) or in CETP mass (21.2 ± 2.4 vs. 24.5 ± 2.7 ng/mg protein). CETP in the particulate fraction appeared as a single band with a similar mobility to that found with the plasma standard (*Figure 2*). As was found with CETP mass and activity, the relative amounts of mRNA did not differ between fasted (n = 6) and fed (n = 6) animals (100 ± 16 vs. 111 ± 18 AU).

Plasma CETA correlated with liver particulate CETP mass and mRNA (*Table 2*), and particulate mass, but not soluble mass, was related to mRNA levels. These relationships remained significant when the data from fed and fasted groups were analyzed separately. Plasma insulin and glucose concentrations were not associated with any parameter of CETP expression (data not shown).

Discussion

This study presents the first examination of CETP expression with regard to mRNA, tissue CETA and CETP mass, and plasma CETA and CETP mass in fasted and fed transgenic mice expressing the human CETP gene. Plasma CETP activity and mass were higher in the fed state than in the fasted state, reflecting higher levels of CETP mRNA, protein, and activity observed in the liver. In the limited number of animals in which plasma CETP mass was estimated, CETA appeared to be a good estimate of plasma CETP mass. These observations are in contrast to a previous study in hamsters that reported higher plasma CETA after a 24-hour fast, which was associated with increases in adipose tissue CETA.[7] However, this effect appeared to be species specific, because the same researchers observed that plasma CETA was not altered in fasted rats. Consistent with the findings in the present study, plasma CETA has been shown to be elevated in humans postprandially,[17–19,27] particularly after a meal high in fat and cholesterol. Interestingly, this effect is inconsistently observed in nondiabetic subjects.[17–20] Although these researchers discussed this postprandial increase in relation to the well-established stimulation of CETP gene transcription by cholesterol, Durlach et al.[17] reiterated the contention that CETP levels in the plasma may be dependent on plasma insulin and/or insulin status.

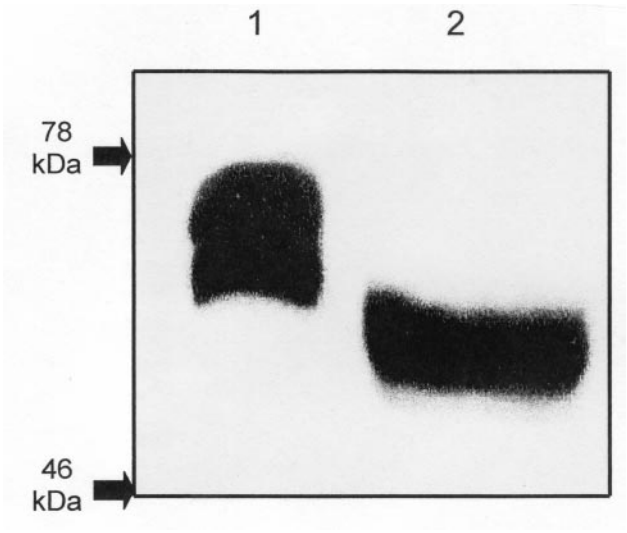

Figure 3 Deglycosylation of liver pellet cholesteryl ester transfer protein (CETP). CETP in 40 μg of total protein was visualized after the sequential binding of a monoclonal antibody specific to human CETP (TP2) and a horseradish-peroxidase conjugated secondary antibody, followed by chemiluminescence, both before (lane 1) and after (lane 2) N-linked and O-linked deglycosylation with NANase II, O-Glycosidase, and PNGase F.

The results of this study are relevant to this issue, relating a wide range of physiologic insulin concentrations to various parameters of CETP expression. Although insulin and glucose were related to plasma CETA, they did not relate to CETP mRNA, CETP mass, or CETA in the liver. Thus, it is unlikely that variations in plasma insulin and glucose levels were responsible for the altered expression of CETP in the liver. It is possible, however, that CETP expression from other tissues may be contributing to the plasma CETP pool or that rates of degradation and clearance may be altered. Insulin and/or glucose could be involved in regulating these steps. However, there are several metabolic differences between fed and fasted states other than plasma glucose and insulin concentrations that could be responsible for the differential CETP expression. In the liver CETP transcription is stimulated by cholesterol[21] and may be increasing expression in the fed mice. Alternatively, corticosteroids, which have been shown to decrease CETP mRNA and plasma activity,[28,29] are usually elevated during fasting and may have contributed to the lower levels of CETP expression in fasted mice. Both of these mechanisms of regulation may be contributing to the differences in CETP expression in the livers of fed and fasted mice. Unfortunately, sample availability did not allow the determination of cholesterol and corticosteroid levels in the plasma of the mice.

In the liver, the increased expression of CETP in fed animals can be explained by an increase in transcription, which led to an increase in the amount of translated protein. CETP mass and CETA were determined in both soluble and particulate subfractions of tissue homogenates. The relationship between CETA and CETP was relatively weak, suggesting that other factors in the liver may influence CETA estimations of CETP mass. The majority of soluble subfraction samples were found to contain a single band, consistent with the approximately 70-kDa band found with mature, fully glycosylated CETP found in the plasma that we observed in a limited number of mice as well as in humans.[23,30] It is unclear why CETP levels in this fraction of tissue homogenates were not related to the other param-

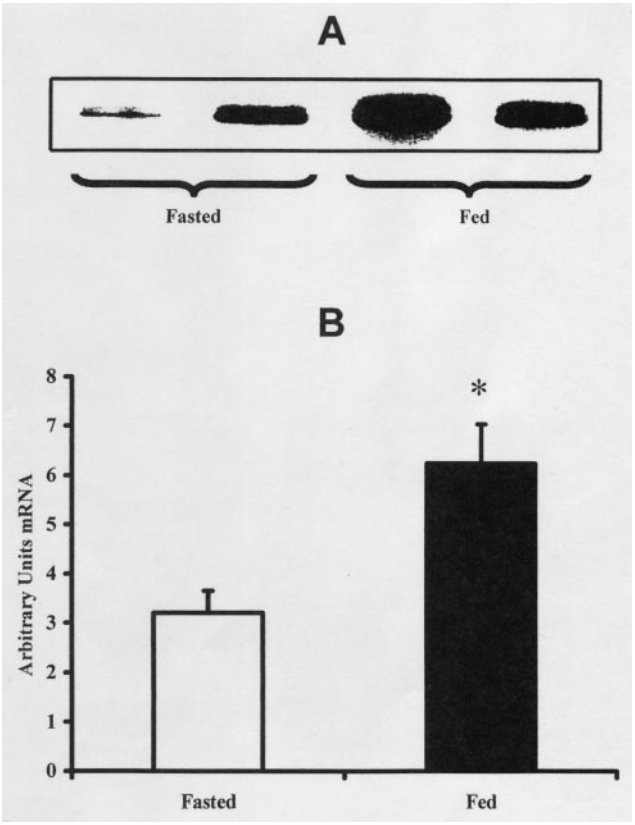

Figure 4 Cholesteryl ester transfer protein (CETP) mRNA in the liver of fasted and fed mice. CETP mRNA was determined by ribonuclease protection assays, using 30 μg of RNA, as described in Materials and methods. (A) Protected fragments (160 bp) were visualized by phosphorimage analysis and were (B) normalized to total RNA to obtain averages for the relative comparison between fasted and groups. $n = 40$ for fasted (20) and fed (20) groups. $^*P < 0.001$.

eters of CETP expression. The CETP in this fraction of liver homogenates may represent CETP that is destined for secretion (mature, fully glycosylated protein) or for degradation (just taken up by the liver). CETP mass in the particulate fraction correlated well with relative mRNA levels, and CETP appeared as a doublet on SDS-PAGE gels, with mobilities similar to those that have been reported for various glycosylation states of CETP.[23,30] Thus, CETP observed in the particulate fraction likely represents newly formed CETP in the process of maturation. Consistent with this hypothesis, the soluble fraction was found to have more of the lower mobility (fully glycosylated) protein, which also appeared to have a higher specific activity (compare soluble vs. particulate, activity/mass). Likewise, in the particulate fraction, the band with the greater mobility (less glycosylated) was generally more prevalent, and the protein appeared to have a lower specific activity. These bands are likely representative of proteins at different stages of glycosylation, as glycosylation is generally thought to be important for activity[31] and likely improves solubility. In this respect, our data suggest that the differential expression of CETP in the livers of fasted and fed animals is not at the site of release, but more likely is due to variations in mRNA levels that dictate the amount of protein production.

Table 2 Relationships between plasma CETA and the parameters of CETP expression

	Liver soluble mass	Liver particulate mass	Liver mRNA
Plasma CETA	0.39	0.59*	0.62*
Liver soluble mass	—	0.30	0.27
Liver particulate mass	—	—	0.72*

Bonferroni correlation coefficients (adjustment for multiple comparisons) between the variables were calculated for the relationships between plasma Cholesteryl ester transfer protein (CETP) activity, mass in the soluble and particulate fractions of liver homogenates, mass, and liver mRNA. The relationships between plasma Cholesteryl ester transfer activity (CETA), liver particulate mass, and liver mRNA remained significant when fasted and fed animals were analyzed separately. $n = 40$ in each comparison. $^*P < 0.001$.

It is unclear why the extraction of CETP from adipose tissue was unlike the liver. No CETP was detected in the soluble fraction of adipose tissue homogenates, even though the protein appeared to be fully glycosylated (*Figure 2*). This suggests that CETP in the soluble fraction of the liver may be bound to a carrier protein and/or lipoprotein that has increased the protein's solubility. The liver does manufacture, secrete, and take up lipoproteins that regularly associate with CETP in the plasma.[31] This tissue-specific function may alter the solubility of some CETP in the liver and explain the differences in tissue extraction. CETP in the particulate fraction of adipose tissue homogenates was relatively low when compared with that of the liver, even though reported comparisons of liver and adipose mRNA in this transgenic model under normal conditions were relatively similar (liver/adipose; approximately 2/1).[21] The reason for this difference in mRNA:protein ratio between the two tissues requires further study.

The results of this study suggest that the concerted, differential expression in the liver contributes to different CETP levels in the plasma of fed and fasted mice. The possible contribution of other factors, however, should not be discounted. Although the expression of CETP did not appear to be altered in adipose tissue, consistent with other dietary studies in this model,[21] CETP expression in adipose tissue could be a contributing factor involved in dictating plasma CETP levels. Pooling our samples did not allow us to relate adipose tissue expression to variations in plasma expression. CETP is also expressed in a number of other tissues in humans and this trangenic mouse model. The contribution of altered expression and secretion from these alternative sources is not addressed in this article and may contribute to different levels of CETP in fed and fasted states. Furthermore, the rate of CETP degradation may vary and influence plasma CETP levels in fed and fasted states.

Although this transgenic model expresses the human CETP gene with its natural flanking regions, the metabolic context of a mouse does not exactly reflect that found in the humans. There does appear to be species specificity when it comes to insulin's effects on CETP expression.[7,9,15,16] These factors must be considered when attempting to relate the findings of this study to humans. The fact that insulin was positively correlated with plasma CETP activity in the mice is interesting, but hyperinsulinemic euglycemic

clamps in humans lead to decreased CETP activity.[15,16] Furthermore, insulin was not related to plasma CETA when fed and fasted groups were analyzed separately. These observations suggest that either insulin does not play a major role in regulating the expression of the human CETP gene in this mouse model or that the metabolic environment in the mouse varies to the extent that insulin's regulatory effects on CETP expression are different in humans.

In summary, plasma CETP activity and mass were higher in fed animals than in fasted animals. These differences were associated with the concerted variations found in liver mRNA and liver particulate protein. Neither plasma insulin nor plasma glucose were related to the differences observed in CETP expression. Thus, although the liver expression appears to contribute to the altered plasma CETP levels in fed and fasted transgenic mice, this differential expression is likely due to factors other than plasma insulin concentrations. Although, insulin's putative effects were not readily apparent in our study, insulin may have a regulatory function that is not manifested in the present study design and/or may be an important regulator in other physiologic conditions.

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