Cholesteryl ester transfer protein (CETP) mRNA abundance in human adipose tissue: relationship to cell size and membrane cholesterol content

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Abstract Cholesteryl ester transfer protein (CETP) has a well-defined role in plasma neutral lipid transport. CETP synthesized by human adipose tissue may contribute to the plasma CETP pool. CETP mRNA abundance increases in subcutaneous adipose tissue in response to cholesterol feeding and we have hypothesized that CETP gene expression is regulated by a specific pool of cellular sterol. In the present study, we have quantified CETP mRNA levels in subcutaneous adipose tissue of 10 female subjects using a solution hybridization RNase protection assay. Particulate (membrane cholesterol) and lipid droplet cholesterol (core cholesterol) were determined by gas chromatography. CETP mRNA abundance in these adipose tissue specimens correlated significantly with membrane cholesterol expressed as a fraction of membrane protein (r = 0.67, P = 0.031). There was also a linear relationship between CETP mRNA abundance and membrane cholesterol to core triglyceride ratio (r = 0.77, P = 0.009) and a strong correlation between the percentage of cellular cholesterol in the membrane fraction (ratio of membrane to core cholesterol) and CETP mRNA abundance (r = 0.91, P = 0.0002). In contrast, there was a negative relationship between each of lipid droplet cholesterol and triglyceride and CETP mRNA levels. Human adipose tissue maintained in organ culture for several days was shown to secrete CETP into the culture medium. Incubation with cholesterol-rich chylomicron remnants elicited a dose-dependent increase in both membrane and core cholesterol and a concomitant increase in the level of CETP mRNA. These studies demonstrate that adipose tissue CETP mRNA abundance is a function of membrane cholesterol concentration rather than lipid droplet cholesterol and that CETP mRNA increases with adipocyte cholesterol enrichment via chylomicron remnants. CETP gene expression is highest in small lipid-poor adipocytes, suggesting that CETP synthesized and secreted by adipocytes may have a role in promoting cellular cholesterol accumulation.—**Radeau, T., P. Lau, M. Robb, M. McDonnell, G. Ailhaud, and R. McPherson.** Cholesteryl ester transfer protein (CETP) mRNA abundance in human adipose tissue: relationship to cell size and membrane cholesterol content. *J. Lipid Res.* 1995. **36:** 2552-2561.

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Cholesteryl ester transfer protein (CETP) mediates the hetero- and homo-exchange of neutral lipid between apoA-I- and apoB-containing lipoproteins, resulting in the net transfer of cholesteryl esters (CE) from HDL to apoB-lipoproteins. This protein may facilitate reverse cholesterol transport provided that the clearance mechanisms for CETP-modified triglyceride-rich lipoprotein remnants and CE-rich lipoproteins are intact. However, in metabolic situations associated with accumulation of very low density lipoprotein (VLDL) remnants due to defective lipolysis or impaired receptor-mediated clearance, excess CETP activity results in CE enrichment of apoB-lipoproteins and may have proatherogenic effects (1). We previously demonstrated that plasma CETP mass and VLDL-cholesterol concentrations are significantly correlated in normolipidemic as well as in hyperlipidemic subjects (2). In certain pathological conditions, LDL-cholesterol and HDL-cholesterol are, respectively, increased and decreased when plasma CETP mass is significantly elevated (3, 4).

CETP synthesis is controlled by both genetic and environmental factors. Numerous studies from this laboratory and others suggest that dietary cholesterol regulates CETP gene expression. In various animal species, including the rabbit (5), hamster (6), cynomolgus monkey (7, 8), and humans (9), dietary cholesterol increases CETP mRNA abundance in liver and/or adipose tissue and plasma CETP mass. Cholesterol feeding

Abbreviations: CETP, cholesteryl ester transfer protein; TG, triglyceride; CE, cholesteryl ester; HDL, high density lipoprotein; VLDL, very low density lipoprotein; LRP, LDL receptor-related protein; LDL, low density lipoprotein.

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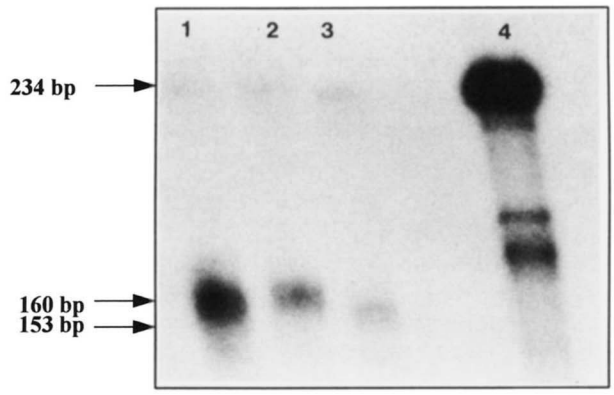


Fig. 1. Determination of CETP mRNA by Rnase protection assay. A representative autoradiogram of three adipose tissue mRNA analyses (60 μg each) is shown. One protected fragment of 160 bp corresponding to the wild-type CETP was resolved in adipose tissue RNA samples (lanes 1, 2, and 3). The anti-sense cRNA probe (234 bp) is also shown (lane 4). Expected size (153 bp fragment) for CETP Δ9 mRNA is also indicated.

also results in elevated levels of plasma CETP and increased hepatic CETP mRNA abundance in transgenic mice expressing the simian (10) or human (11) CETP gene, an effect that is dependent on the presence of proximal natural flanking region sequences of the human CETP gene (11). Liver appears to be the major site of CETP production in many animal species. However, Jiang et al. (6) first reported that adipose tissue was a major source of CETP mRNA in the hamster. Studies in nonhuman primates also demonstrated that adipose tissue expresses high levels of CETP (7, 8). In view of the size of this organ, adipose tissue may contribute significantly to plasma CETP mass in humans. However, the secretion of CETP by human adipocytes or adipose tissue has not been previously studied. CETP may play a restricted intracellular role for cholesterol transport in adipocytes and other cells, as suggested by the fact that certain animal species such as rodents and pigs express mRNA for CETP but do not secrete this protein into plasma (6).

Human adipose tissue functions to store triglycerides and release free fatty acids as necessary for energy requirements. Relatively large amounts of free cholesterol are associated with triglyceride in the core lipid droplet of adipocytes (12). Cholesterol synthesis is limited in human adipocytes and the majority of cholesterol is provided by lipoproteins via specific receptors or by passive diffusion (12). We have hypothesized that CETP gene expression in human adipose tissue is teleologic and functions to promote cholesteryl ester accumulation by immature adipocytes. Based on in vivo experiments, demonstrating a rapid increase in adipose tissue CETP mRNA in response to dietary cholesterol, we also hypothesized that adipose tissue CETP mRNA abundance is a function of the size of the mobile cholesterol pool in the plasma membrane and have determined the effects of cholesterol enrichment of adipose tissue plasma membranes by incubation with chylomicron remnants on CETP gene expression.

MATERIALS AND METHODS

Subjects and specimen collection

Subcutaneous adipose tissue was obtained from 10 female subjects (39 ± 4.7 years) undergoing reduction mammoplasty (7 subjects) or abdominoplasty (3 subjects) procedures for cosmetic purposes. Subjects were in good health, non-diabetic, and not on lipid-active medication. The degree of adiposity varied (BMI (body mass index) 23–34). The study was approved by the

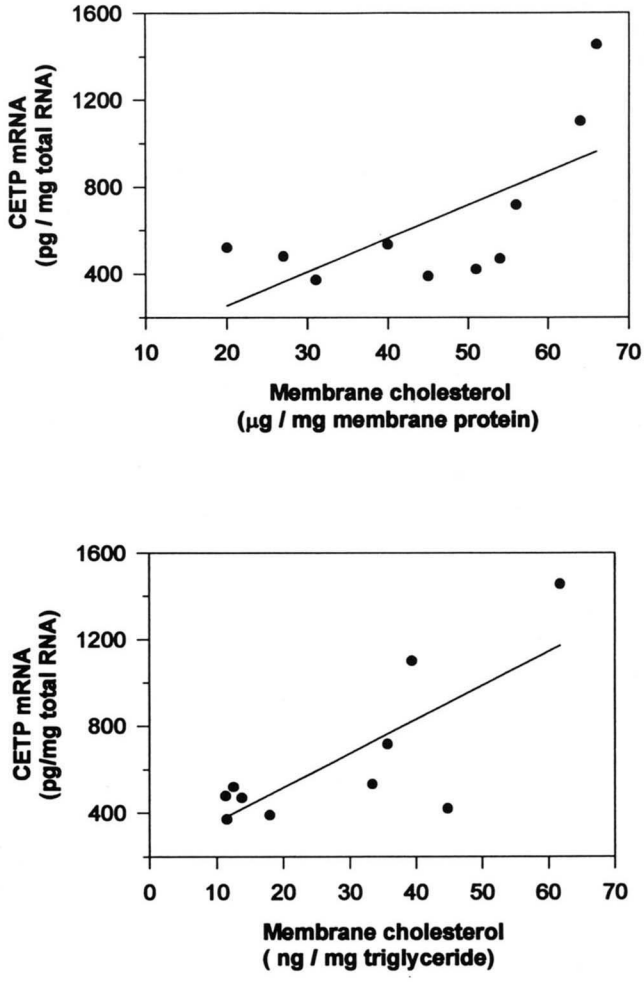


Fig. 2. Relationship between membrane cholesterol and CETP mRNA abundance. Panel A: A linear relationship is apparent between human adipose tissue CETP mRNA concentration and membrane cholesterol expressed per μg membrane protein. ($r = 0.67$, $P = 0.031$). Panel B: A significant linear correlation is apparent between CETP mRNA abundance and membrane cholesterol expressed per mg triglyceride ($r = 0.77$, $P = 0.009$)

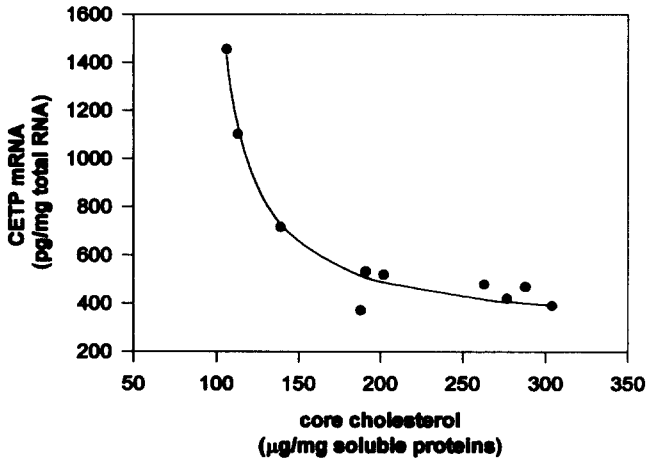


Fig. 3. Relationship between core cholesterol and CETP mRNA abundance. A nonlinear inverse correlation is evident between adipose tissue CETP mRNA and core cholesterol. (r = 0.90, P = 0.0004). The data are best fitted by a saturation curve ([CETP mRNA] = 300 • [core cholesterol]/[core cholesterol] - 80.5). Increases in core cholesterol above 180 µg/ml are not associated with further changes in CETP mRNA abundance.

Human Ethics Research Committee of the Ottawa Civic Hospital and written informed consent was obtained from all subjects. Tissue specimens were carefully dissected in the operating theatre immediately after collection to obtain a thin layer of subcutaneous fat tissue without any adhering breast tissue. Aliquots of adipose tissue were immediately frozen on dry ice and kept at -80°C until RNA or lipid analysis. A sample of fresh adipose tissue was collected in DMEM/Ham F12 medium (Gibco, Burlington, ON) and transported immediately to the laboratory for organ culture.

Adipose tissue subfractionation and lipid analysis

Adipose tissue (0.5–1 g) was homogenized in 5 ml of 20 mM Tris buffer, pH 7.4, containing 1 mM EDTA and 1 mM β-mercaptoethanol using a mechanical homogenizer (Cyclone IQ, VirTis Company, Gardiner, NY). Samples were centrifuged at 1600 rpm for 10 min at 4°C to separate the lipid layer and to pellet insoluble proteins. The infranatant was carefully removed and lipids were extracted as described by Folch, Lees, and Sloane Stanley (13). TG mass was determined by weighing the lipid extract. Lipids were also saponified in KOH/EtOH for 1 h at 60°C and then extracted with hexane. The infranatants were centrifugated for 1 h at 100,000 *g* at 4°C. Particulate fractions were resuspended and incubated overnight in 0.5 N NaOH. Proteins were assessed in particulate and cytosolic fractions as described by Lowry et al. (14). When analyzed, DNA concentrations were determined by fluorescence using Hoechst 33258 and a TKO100 minifluorometer (Hoefer Scientific Instruments, San Francisco, CA). Lipids were extracted from the particulate fractions as described by Bligh and Dyer (15). Cholesterol was determined by gas chromatography after trimethylsilyl (TMS) derivatization of particulate and saponified and nonsaponified core lipid fractions. Stigmasterol (Sigma, St. Louis, MO) was used as internal standard. Samples were analyzed using a HP 5890 gas chromatograph equipped with a HP Model 7673A autosampler and a hydrogen flame ionization detector (Hewlett-Packard, Palo Alto, CA). An Ultra-1 capillary column (25 m × 0.2 mm) coated with a thin film of methyl silicone (Hewlett-Packard) was used. The column temperature was programmed as follows: 160°C for 1 min, 20°C/min to 200°C, 200°C for 1 min, 10°C/min to 300°C, and finally 300°C for 10 min. The carrier gas was helium (constant flow = 0.6 ml/min). The hydrogen flame ionization detector was maintained at 360°C. Retention times for TMS-cholesterol and TMS-stigmasterol were 19.058 min and 21.195 min, respectively.

mRNA extraction and RNase protection assay

Total tissue RNA was extracted using the acidic guanidinium isocyanate method as described by Chomczynski and Sacchi (16). The mRNA purity was estimated by determining the ratio of absorbance at 260 and 280 nm and integrity was checked by agarose gel electrophoresis. The abundance of CETP mRNA was determined by a solution hybridization ribonuclease protection assay as described previously (5, 17). Fifty to sixty µg of total mRNA was hybridized to a human antisense RNA probe prepared from the human CETP cDNA (160 bp fragment, nucleotides 727–887; provided

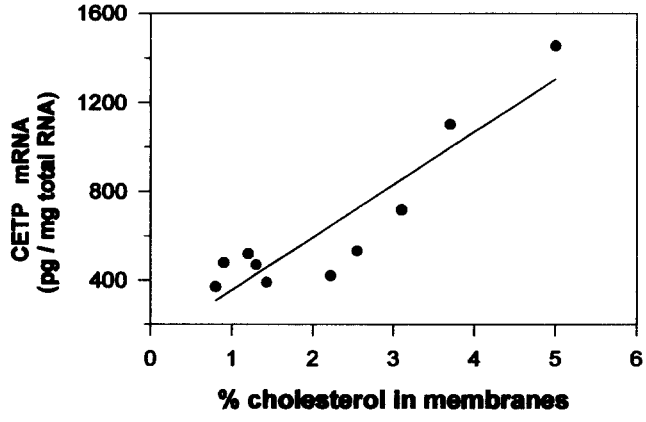


Fig. 4. Relationship between cellular cholesterol distribution and CETP mRNA expression in human adipose tissue. Cholesterol distribution is expressed as % of total cellular cholesterol in the membrane fraction. There is a strong positive relationship between this fraction of cellular cholesterol and CETP mRNA abundance (r = 0.90, P = 0.003).

by Dr. Alan Tall) subcloned into pBluescript KST. After 16–18 h of hybridization at 48°C, samples were digested by RNAse T2 for 2 h at 30°C and [^{32}P]RNA-RNA hybrids were analyzed on 5% polyacrylamide–urea sequencing gels. Protected fragments were visualized by autoradiography and quantitated by densitometry. RNA mass was determined by comparison with a standard curve of CETP mRNA hybridized simultaneously. For this purpose, sense strand RNA was synthesized by in vitro transcription and its mass was quantitated precisely by standard methods using [^{3}H]UTP (uridine triphosphate) incorporation.

Adipose tissue culture

Adipose tissue samples (0.5–1 g) were carefully dissected to remove adhering tissue and capillaries. Tissue were then cut in small fragments and cultured in 5 ml of DMEM/Ham F12 medium. Samples were cultured in a petri dish at 37°C, 5% CO_2 for 4–6 days. Medium was changed every 2 days. Cell death and tissue viability were checked by measuring lactate dehydrogenase (LDH) specific activity in cell medium and homogenates. In addition, we measured specific activity of *sn*-glycerol-3-phosphate dehydrogenase (GPDH) which is highly expressed in adipocytes. Both activities were determined as described (18) and expressed as milliunits per minute and mg soluble proteins.

CETP synthesis and secretion

CETP secreted into the culture media was analyzed after 2, 4, and 6 days of organ culture by Western blot after immunoprecipitation with the human CETP specific monoclonal antibody (TP2). Aliquots (1.5 ml) of media were immunoprecipitated using TP-2 linked to CNBr-Sepharose (Pharmacia, Upsalla, Sweden). After a first incubation with 60 µl of CNBr-Sepharose gel suspension in PBS for 6–8 h, media were incubated overnight with 60 µl TP2-Sepharose gel. Gels were then washed several times with 1 ml PBS–0.1% Triton X-100 (Sigma), 1 ml PBS, and finally with 1 ml of 0.65 M Tris, pH 6.8. Gels were pelleted and resuspended in 30 µl SDS loading buffer to elute CETP. Eluates were analyzed on 4–20% SDS-PAGE electrophoresis and immunoblotted using ^{125}I-labeled TP2. Blots were then autoradiographed. Protein size was compared to recombinant human CETP (provided by Dr. A. Tall).

Cholesterol loading of human adipose tissue in organ culture

Chylomicron remnants were prepared from human plasma collected after an oral fat load (100 g fat, 1000 mg cholesterol). Plasma was centrifugated for 30 min at 4°C and 22,500 rpm (50.3 Ti rotor from Beckman Instruments, Palo Alto, CA). Chylomicron remnants were then washed once in phosphate buffered saline and diluted in this buffer. Absence of other lipoproteins was verified by agarose gel electrophoresis (Paragon®, Beckman, Palo Alto, CA). Cholesterol and TG concentrations were measured using automated enzymatic methodology (Technicon RA1000, Miles Inc., Tarrytown, NY). Fragments of adipose tissue were first conditioned for 2 days in DMEM/Ham F12 medium. Media were changed and tissue fragments were incubated with chylomicron remnants for 48 h. Aliquots were used for lipid analysis (in duplicate) or for mRNA extraction as described above. LDH and GPDH activity were used as indices of tissue viability.

TABLE 1. Lipid composition and CETP mRNA concentrations of adipose tissue specimens

Subject	Lipids	Proteins		Cholesterol		CETP
		Soluble	Membrane	Core	Membrane	
		µg/mg tissue				*pg/mg total RNA*
RE	784	6.6	0.50	0.91	0.028	717
JB	761	7.1	0.46	0.80	0.030	1102
GD	640	3.7	0.45	0.75	0.008	520
LS	731	2.6	0.18	0.75	0.010	469
IS[a]	712	8.2	0.66	0.87	0.044	1455
JF	619	3.1	0.28	0.82	0.007	479
AG[a]	520	3.7	0.18	0.70	0.006	371
MY	502	2.2	0.20	0.66	0.009	390
AV	357	2.8	0.28	0.75	0.016	420
EL[a]	479	3.1	0.42	0.60	0.016	533
Mean ± SD	610±141	4.3±2.14	0.36±0.16	0.76±0.009	0.017±0.012	

[a]Subcutaneous abdominal adipose tissue.

Northern blot analysis

Northern blot analysis for CETP was performed as described (19) except that the probe was labeled using a random labeling kit (Megaprime DNA from Amersham International plc, Amersham, UK). Membranes were blotted with a full-length CETP cDNA (1.2 kb) (kindly provided by Dr. A. Tall) and by a 497 bp β actin cDNA probe prepared using genomic DNA and polymerase chain reaction. Sense and anti-sense primers described by Levy and colleagues (20) were used. CETP mRNA levels were determined by densitometric scanning and corrected to β actin mRNA levels.

Data analyses and statistics

Data were expressed as mean ± SD. Cellular lipid data were expressed in terms of mg/mg soluble protein or membrane protein. Cell size and cell number were estimated as TG/mg protein and TG/mg soluble protein per mg of tissue, respectively. CETP mRNA was expressed as pg per mg of total RNA. Regression analyses were performed using the least squares method by Instat Graphpad® software version 1.11a. Curve fitting was performed using SigmaPlot® software version 2.0.

RESULTS

Effects of adiposity on adipose tissue CETP mRNA abundance

Body mass index (BMI) (kg/m^2) ranged between 23 and 34. Despite the presence of a range of normal weight and obese subjects, there was not a significant correlation between BMI and adipose tissue CETP mRNA abundance.

Relationship between intracellular cholesterol pools and adipose tissue CETP mRNA abundance

CETP mRNA abundance was determined by RNase protection assay using an antisense cRNA probe prepared from a 160 bp cDNA fragment. This fragment spans exon 7, exon 8, and the first 7 bp of exon 9. As shown (**Fig. 1**), an mRNA of approximately 160 bp was detectable by this assay. Thus, the 153 bp fragment corresponding to the exon 9-deleted CETP (CETP Δ9) mRNA, generated by alternative splicing (21), was not sufficiently different in size to permit resolution by this assay.

Cholesterol was determined in the particulate (membrane) fraction and in the lipid droplet (core cholesterol). Cholesterol in the cytosolic fraction accounted for less than 0.5% of total tissue cholesterol content. No difference was found between total and free cholesterol in the lipid droplet fraction, confirming that little CE is stored in the lipid core of adipocytes. There was a significant linear relationship between membrane cholesterol expressed as mg membrane protein and CETP mRNA (r = -0.67, P = 0.031) (**Fig. 2A**). A positive correlation was found between CETP mRNA concentration and membrane cholesterol expressed per mg core triglyceride (r = 0.77, P = 0.009) (Fig. 2B). In contrast, core (lipid droplet) cholesterol per mg soluble protein was inversely correlated with CETP mRNA concentrations (r = -0.90, P = 0.0004) (**Fig. 3**). This was a nonlinear correlation and the data were best fitted to a saturation curve. CETP mRNA abundance was clearly highest in tissue containing relatively low levels of core cholesterol. At progressively higher core cholesterol concentrations, the level of CETP mRNA remained relatively constant (circa 300 pmol/mg total RNA).

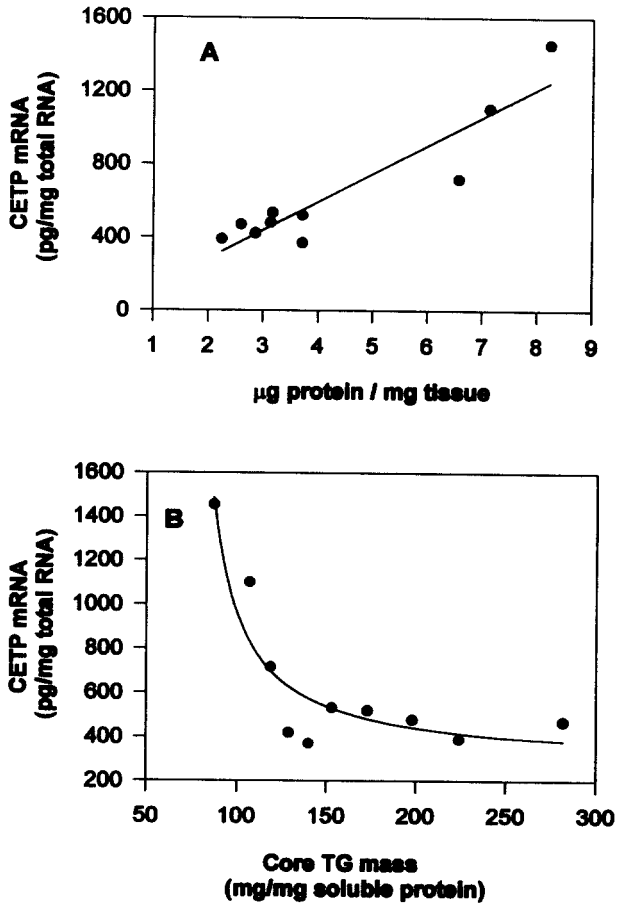


Fig. 5. Effects of adipose tissue cell size on CETP mRNA abundance. Panel A. An increase in cell number, expressed as an increase in µg protein/mg adipose tissue is associated with a linear increase in CETP mRNA abundance (r = 0.92, P = 0.0001). Panel B. Cell size estimated as the adipose tissue TG/protein ratio was inversely correlated with CETP mRNA abundance. The data are best fitted to a saturation curve ([CETP mRNA] = 300 • [Triglyceride]/[triglyceride] - 62.8). Within the size range of smaller cells, there was a steep negative relationship between cell size expressed as the adipose tissue TG/protein ratio with no further change in CETP mRNA abundance as cell size increased further.

No relationship with CETP mRNA abundance was found when core cholesterol was expressed per mg TG. There was, however, a very significant linear relationship between adipose tissue CETP mRNA concentration and the percentage of total cellular cholesterol present in the membrane fraction ($r = 0.91$, $P = 0.0002$) (**Fig. 4**).

The data for individual subjects are illustrated (**Table 1**). As indicated by the standard deviation of mean values, variability of membrane cholesterol among subjects is higher than that of soluble protein, membrane protein, core cholesterol, and triglyceride. The variation observed in membrane cholesterol per mg membrane protein and per mg TG as well as in the percentage of cholesterol in the membrane fraction was mainly due to individual differences in membrane cholesterol rather than other parameters.

Effect of cell number and cell size on CETP mRNA abundance

Regression analyses were also performed using adipose tissue mass, core TG and membrane cholesterol. As shown (Table 1), intracellular triglyceride accounts for the vast majority of adipocyte mass. However, adipose tissue cell size and cell number vary considerably for different individuals. The soluble protein content of adipose tissue is a marker of cell number and, in other studies in our laboratory, has been shown to correlate with the DNA content of human adipose tissue ($r = 0.87$, $P = 0.001$, n = 10). Cell size, usually expressed as µg TG/cell, was determined as adipose TG/mg soluble protein. Membrane cholesterol per mg tissue was positively correlated with protein/mg adipose tissue ($r = 0.91$, $P = 0.0002$) and inversely correlated with TG/mg soluble protein ($r = -0.70$, $P = 0.024$), consistent with the expected inverse relationship between adipose tissue cell size and cell number. The adipose tissue TG/mg protein ratio correlated negatively with % total cholesterol in the membrane fraction ($r = -0.70$, $P < 0.02$). Thus, as expected, small lipid-poor cells have relatively more cellular cholesterol in the membrane fraction.

There was a significant linear relationship between CETP mRNA abundance and soluble protein per mg tissue ($r = 0.92$, $P = 0.0001$) (**Fig. 5A**). Adipose tissue TG per mg protein was inversely correlated with CETP mRNA abundance ($r = -0.74$, $P = 0.0128$). As shown in Fig. 5B, these data were best fitted to a hyperbolic regression curve. Small lipid-poor cells expressed higher amounts of CETP mRNA and, as cell size increased, CETP mRNA expression by adipose tissue decreased to a fairly constant minimum value (ca. 300 pg/mg total RNA). The nonlinear relationship between CETP mRNA abundance and cell size suggests that this relationship is not simply a function of the inverse relationship between cell size and cell number in adipose tissue.

Release of CETP by human adipose tissue cultured in vitro

In order to determine whether CETP synthesis and secretion are effectively maintained by human adipose tissue in organ culture, small fragments of tissue were cultured for 2–6 days. High LDH activity (350 mU/mg protein) in the medium was evident over the first 2 days of culture, indicating the initial disruption and demise of some cells. However, between 2 and 4 days of organ culture, LDH activity in the medium returned to normal (95 and 47 mU/mg protein). Moreover, in adipose tissue homogenates, the LDH activity increased with time from 1322 mU/mg protein before culture to 2138 mU/mg protein at 6 days of tissue culture. The *sn*-glycerol-3-phosphate dehydrogenase activity (GPDH), which is a more specific enzymic marker of the metabolic integrity of mature adipocytes, was also well maintained throughout tissue culture (1958 mU/mg proteins before culture versus 1557 mU/mg protein after 6 days in tissue culture) demonstrating good cell viability. Immunoreactive CETP continued to be secreted into the culture medium after 2 and 4 days although secretion decreased after 6 days in culture (**Fig. 6**). Thus, human adipose tissue in primary culture remains viable and continues to secrete CETP for a significant length of time.

Effect of cholesterol loading on CETP mRNA expression by adipose tissue

Incubation of adipose tissue fragments with human cholesterol-rich (10–20 µg/ml) chylomicron remnants resulted in a significant increase in membrane and core cholesterol. CETP mRNA abundance increased in response to cholesterol loading (**Fig. 7A and B**). Incubation of chylomicron remnants with adipose tissue over a 2-day period resulted in a dose-related increase in membrane cholesterol per µg DNA (+14% and +22%) as well as in core cholesterol (+15% and +20%). CETP

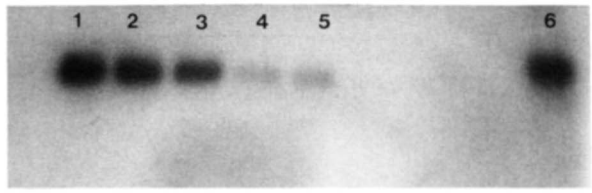
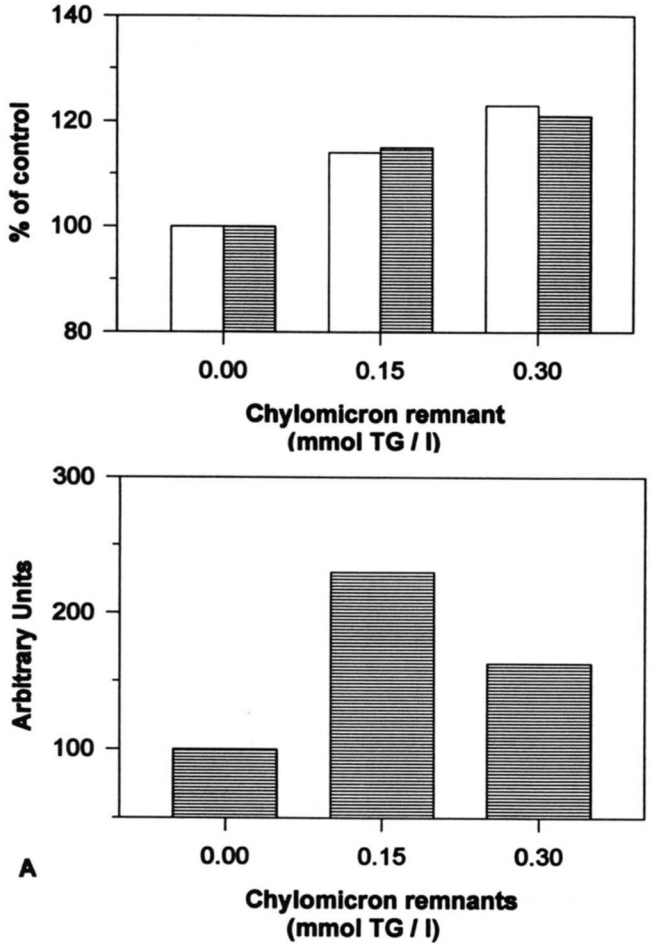


Fig. 6. Secretion of CETP by human adipose tissue in organ culture. Adipose tissue culture was carried out as described in Methods. Medium CETP or recombinant CETP were immunoprecipitated using the Mab, TP-2 and analyzed by Western blot. Lane 1: culture medium at 2 days; lanes 2 and 3: culture medium at 4 days; lanes 4 and 5: culture medium at 6 days; lane 6: recombinant human CETP. Secretion of CETP by human adipose tissue was well maintained for 6 days of culture.



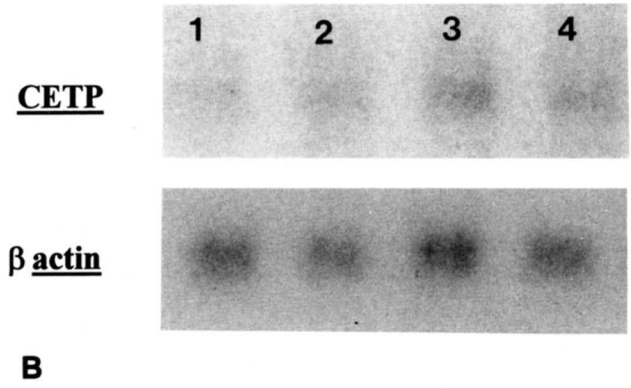


Fig. 7. Effect of cholesterol loading on CETP mRNA expression by adipose tissue in culture. Fragments of adipose tissue were maintained in culture for 2 days and incubated with cholesterol-rich chylomicron remnants for a further 2 days. Panel A: Incubation with chylomicron remnants resulted in an increase in membrane (open bars) as well as core cholesterol (hatched bars) in adipose tissue. Data are expressed as a percentage of control values (mean of two separate experiments). Panel B: Northern blot analysis of CETP and β actin mRNA in adipose tissue fragments cultured in the presence or absence of chylomicron remnants. Controls were performed in duplicates (lanes 1 and 2). Two concentrations of chylomicron remnants of 0.15 mmol/l and 0.30 mmol/l are shown in lanes 3 and 4, respectively. Densitometric scanning of CETP mRNA and correction for β actin mRNA levels demonstrated increased CETP mRNA expression in adipose tissue cultured in the presence of chylomicron remnants (mean of two separate experiments).

mRNA was identified by using negative control cells (human skin fibroblasts) and positive control cells (freshly isolated human adipose tissue). Increases of 130% and 63% in CETP mRNA were found (mean of two experiments), (Fig. 7B). Secretion of CETP into the culture medium was not determined in these experiments.

DISCUSSION

Human adipose tissue is a significant site of CETP synthesis in humans, and CETP gene expression in adipose tissue appears to be regulated in part by cholesterol. We have previously demonstrated that cholesterol feeding increases plasma CETP mass and adipose tissue CETP mRNA abundance in human subjects (9). Plasma CETP concentrations are also increased in certain hyperlipoproteinemic states such as chylomicronemia and apoE2/2-linked dysbetalipoproteinemia (2). Increased plasma CETP associated with these metabolic conditions may reflect enhanced delivery of lipoprotein cholesterol to adipose tissue and increased CETP gene expression by adipocytes (2, 9). Jiang et al. (11) have demonstrated that dietary cholesterol increases CETP gene expression in mice bearing the human CETP transgene and that response to dietary cholesterol is dependent on the presence of upstream natural flanking sequences of the CETP gene. Interestingly, this dietary cholesterol-responsive region of the human CETP gene bears no nucleic acid homology to the classic sterol response element.

Dietary cholesterol is delivered to adipose tissue via chylomicron remnants and other lipoproteins (22–26). Despres and colleagues (25) have hypothesized that CETP may play a role in the selective uptake of HDL and LDL cholesteryl ester by adipocytes. A significant amount of adipose tissue cholesterol may also be provided by chylomicron remnants dependent on dietary conditions. Free cholesterol released during lipolysis may be taken up by adipocytes. Adipocytes also bind and internalize chylomicron remnants via the LDL-receptor related protein (LRP) (26). Cholesterol accumulation by adipose tissue involves initial cholesterol enrichment of the plasma membrane. We have hypothesized that CETP gene expression in human adipose tissue is regulated by plasma membrane cholesterol. Adipocyte core

cholesterol is a relatively inert pool of free cholesterol solubilized in the lipid droplet and thus less likely to be regulatory for gene expression. In this study, we have determined CETP mRNA abundance in relation to intracellular cholesterol pools in fresh adipose tissue collected from normal human subjects and in adipose tissue maintained in culture.

A significant amount of total body cholesterol (> 25% in normal subjects) is stored in adipose tissue (12). De novo cholesterol synthesis is extremely limited in adipocytes and the majority of cholesterol is provided by lipoprotein-derived cholesterol. Newly synthesized cholesterol and cholesterol derived from lipoproteins are rapidly integrated into the plasma membrane (22, 27). The cholesteryl ester pool is insignificant in human adipose tissue due to high cholesterol esterase activity (28). In this study, adipose tissue cholesterol was determined in the two major pools. The larger pool (core cholesterol) consists of intracellular free cholesterol and is associated with the TG-rich lipid droplet. Lower levels of free cholesterol (0.8–5% of total cellular cholesterol) were present in the crude particulate fraction, which represents mainly plasma membrane cholesterol. Membrane cholesterol content in adipose tissue was shown to vary significantly amongst individuals. Possible ex vivo exchange of cholesterol between cellular pools was prevented by immediate freezing of adipose tissue and by performing all lipid analyses at 4°C.

In vitro experiments have demonstrated that plasma membrane cholesterol levels can be increased as much as 50% by incubation with exogenous lipoproteins (29). A similar result was evident in these studies when small fragments of adipose tissue maintained in culture were incubated with cholesterol-rich chylomicron remnants. Thus, adipocyte membrane cholesterol is a modifiable pool of cholesterol and may also vary dependent on certain metabolic states. These studies demonstrate that CETP mRNA abundance is significantly correlated with membrane cholesterol expressed as mg membrane protein, suggesting that cholesterol enrichment of membrane fraction may directly or indirectly regulate CETP gene expression. The data presented here demonstrate a strong linear association between the membrane cholesterol:core cholesterol ratio and CETP mRNA level amongst the individuals studied ($r = 0.91$, $P = 0.0002$). Similarly, cholesterol enrichment of adipose tissue in culture, albeit of both membrane and core cholesterol pools, resulted in a significant increase in CETP mRNA abundance.

The requirement for exogenous lipoprotein-derived cholesterol would be expected to be greatest for immature lipid-poor adipocytes and other studies in this laboratory demonstrate that adipose tissue CETP functions in the selective uptake of lipoprotein cholesteryl ester (M. McDonnell, P. Lau, T. Radeau, R. W. Milne, and R. McPherson, unpublished observations). The present data demonstrate that CETP mRNA expression is a function of adipose tissue cell size. The positive correlation between CETP mRNA abundance and membrane cholesterol/core triglyceride indicates that CETP gene expression is greatest in small lipid-poor adipocytes. Similarly, cell size, estimated as mg TG/mg soluble protein is inversely correlated with CETP mRNA abundance in fresh adipose tissue. Regression analysis demonstrates that these data fit a saturation curve. Tissues containing small lipid-poor cells express higher amounts of CETP mRNA as compared to tissue consisting of large lipid-rich cells. An inverse relationship was also found between CETP mRNA levels and core cholesterol per mg protein as expected since core cholesterol accumulation is a function of triglyceride concentration. The inverse relationship between core cholesterol and CETP mRNA abundance in these patient samples is likely a function of differences in cell size. On the other hand, membrane cholesterol was independent of core cholesterol but was inversely correlated with TG/mg protein, reflecting the greater surface to core ratio of smaller cells. Higher CETP gene expression in small lipid-poor adipocytes was also demonstrated by the relationship obtained between CETP mRNA and protein/mg tissue. In other studies, we have demonstrated a good correlation between soluble protein and DNA concentrations in human adipose tissue. Thus, protein/mg tissue provides a reliable estimation of adipose tissue cell number. The anticipated inverse relationship between adipose tissue cell size and cell number (12) was demonstrated in this study by the correlation between TG/mg soluble protein and soluble protein/mg of tissue. However, in contrast to the relationship of adipose tissue CETP mRNA to TG/mg protein, the association with soluble protein/mg tissue was linear. As expected, CETP mRNA abundance increased linearly with cell number.

Exposure of cultured adipose tissue to cholesterol-rich chylomicron remnants was associated with an increase in core cholesterol as well as membrane cholesterol and likely reflects uptake of chylomicron remnant cholesterol via the LRP or other pathways. Thus, the resultant increase in CETP mRNA abundance cannot be definitely attributed to the change in the plasma membrane pool of cholesterol. However, the core cholesterol pool consists of free cholesterol solubilized in the lipid droplet and might not be expected to be regulatory for gene expression. Little is known regarding cholesterol exchange between core and membrane cholesterol; however, specific proteins may be involved (30). CETP may promote cholesterol accumulation in human adipose tissue via selective uptake of cholesteryl ester from lipoproteins (25) and thus it is not unex-

pected that expression is greatest in lipid-poor cells. CETP may also play a role in intracellular lipid trafficking as suggested by the presence of CETP mRNA in the tissues of certain species that do not secrete CETP into plasma (6).

CETP mRNA abundance is greatest in the liver in many mammalian species, including primates, but the size of this organ is considerably less than that of adipose tissue for most individuals. The extent to which adipose tissue contributes to the plasma CETP pool in humans is not known. As described by Inazu et al. (21), the CETP gene is subject to alternative splicing and this appears to play an important regulatory role in CETP function and secretion. The protein coded for by the wild-type CETP mRNA is secreted and active in neutral lipid transfer. Alternative splicing results in deletion of exon 9 (CETP Δ9). In contrast to the wild-type CETP, the protein encoded by CETP Δ9 is poorly secreted and is inactive in neutral lipid transfer (21). Importantly, CETP Δ9 forms a intracellular heterodimer with wild-type CETP preventing its secretion (31). In this study, we have determined CETP mRNA expression by RNAse protection assay or by Northern blot. Under the conditions of these assays, we were not able to obtain data on the differential expression of Δ9-CETP and wild-type CETP mRNA. In studies in progress, using competitive reverse transcriptase PCR, we are determining the effects of cholesterol enrichment of human adipocytes on differential splicing of the primary CETP RNA transcript.

In the cholesterol-fed hamster, we reported a direct relationship between adipose tissue CETP mRNA abundance and plasma concentrations of CETP (32). In the present study, we have demonstrated for the first time that human adipose tissue in organ culture secretes CETP. Thus, it is possible that adipose tissue contributes to the high levels of circulating CETP associated with certain types of dyslipoproteinemia or with cholesterol feeding (2). It has recently been reported that plasma CETP is increased in obesity and is correlated with body mass index (4, 33). As compared to lean subjects, obese subjects have cholesterol-enriched adipocytes (12) although not necessarily significantly greater cell size (34). No data comparing membrane cholesterol in obese and lean subjects have been reported and higher levels of circulating CETP in obese subjects may simply reflect the greater size of this CETP-secreting organ rather than differences in cell size or membrane cholesterol content.

In summary, we have demonstrated that CETP gene expression in human adipose tissue is a function of cell size and membrane cholesterol concentration. Adipocyte CETP may play a role in cholesterol acquisition from lipoproteins or may function in regulating the distribution of cholesterol between the plasma membrane and the core lipid droplet. Human adipose tissue secretes CETP and may contribute to elevated plasma levels of CETP in certain dyslipoproteinemic states.

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