Regulation by long chain fatty acids of the expression of cholesteryl ester transfer protein (CETP) in HepG2 cells

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FOOT NOTES

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Abbreviations: AA, arachidonic acid; aLA, α-linolenic acid; BSA, bovine serum albumin; C/EBP, CCAAT/enhancer binding protein; CETP, cholesteryl ester transfer protein; DgLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DMEM, Dulbecco's modified Eagle's medium, ELISA, enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; FA, fatty acid; gLA, γ-linolenic acid; LA, linoleic acid; LCAT, lecithin-cholesterol acyltransferase; LPL, lipoprotein lipase; OA, oleic acid; PBS, phosphate buffered saline; PUFA, polyunsaturated fatty acid; SA, stearic acid; SDS, sodium dodecyl sulphate; SREBP, sterol regulatory element-binding protein, SSPE, saline-sodium dihydrogenphosphate-ethylenediamintetraacetate buffer

ABSTRACT: Cholesteryl ester transfer protein (CETP) is an important determinant of lipoprotein function, especially high density lipoprotein (HDL) metabolism, and contributes to the regulation of plasma HDL levels. Since saturated and polyunsaturated fatty acids (FA) appear to influence the CETP activity differently, we decided to investigate the effects of FA on the expression of CETP mRNA in HepG2 cells using a RNA blot hybridization analysis. Long-chain FA (>18 carbons) at a 0.5 mM concentration were added to the medium and incubated with cells for 48 h at 37°C under 5% CO_2. After treatment with 0.5 mM arachidonic (AA), eicosapentaenoic (EPA), and docosahexaenoic acid (DHA), the levels of CETP mRNA were less than 50% of the control levels (AA, $P = 0.0005$; EPA, $P < 0.01$; DHA, $P < 0.0001$), with a corresponding significant decrease in the CETP mass. These results suggest that FA regulate the gene expression of CETP in HepG2 and this effect is dependent upon the degree of unsaturation of the acyl carbon chain in FA.

Cholesteryl ester transfer protein (CETP) is known to be a key protein in reverse cholesterol transport. By promoting the transfer and exchange of neutral lipids among plasma lipoproteins (1), it determines the plasma lipoprotein profile. A CETP deficiency markedly elevates plasma high density lipoprotein levels and decreases low density lipoprotein levels (2). The normal plasma CETP concentration of normolipidemic subjects ranges between 1.1 and 1.7 mg/L (3,4). CETP cDNA clones have been isolated from humans, cynomolgus monkeys, rabbits, and hamsters, with about an 80–95% sequence homology among the species (5–8). In humans, the organs with the most abundant expression of CETP mRNA are the liver, spleen, and adipose tissue, with lower levels of expression in the small intestine, adrenal glands, kidney, and heart (5,6).

The level of CETP mRNA is responsive to various environmental factors (9,10). High-fat and high cholesterol diets (i.e. atherogenic diets) have been shown to raise both the mass and mRNA levels of CETP (6,8,11), whereas marine lipids, mainly n-3 polyunsaturated fatty acids (PUFA) have been shown to reduce plasma cholesterol and triglyceride levels (12) and to decrease the CETP mass level in hypercholesterolemic subjects (13). A recent study also reported that plasma CETP activity increased in a palmitic acid-rich diet in comparison to a stearic acid (SA)-rich diet (14) and that dietary *trans* fatty acids (FA) (e.g. elaidic acid) significantly increased the CETP activity in comparison to diets rich in either SA, linoleic acid (LA) or palmitic acid (15,16).

In vitro studies with CaCo-2 cells (17) and HepG2 cells (18) have demonstrated that oleate or butyrate can up-regulate CETP secretion. Although the effects of various dietary FA on plasma CETP activity have been reported (19,20), whether various types of FA regulate the synthesis or expression of CETP mass and/or the mRNA has yet to be explained.

To elucidate the action of dietary FA on CETP expression, we investigated the effects of long-chain FA on the mRNA and protein levels of CETP using HepG2 cells as an *in vitro* model.

MATERIALS AND METHODS

Cell culture. The human hepatoma cell line HepG2 was purchased from the Riken gene bank (Wako, Japan), and the cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 265 μg/mL thymidine (all from Life Technologies Ltd., Grand Island, NY) and 10% (vol/vol) fetal calf serum (Intergen Co., Purchase, NY) at 37°C under 5% CO_2. For the experiments described herein, the cells were seeded in 60-mm diameter collagen-coated culture dishes (Sumitomo Bakelite Co., Ltd., Tokyo, Japan) at a density of 2 × 10^5/dish and then were cultured to approximately 90% confluency.

FA treatments. HepG2 cells were grown to 90% confluency in the same medium and then were incubated with 10% (vol/vol) fetal calf lipoprotein-deficient serum-DMEM supplemented (or not) with 0.5 mM of

FA: SA (18:0), oleic (18:1, OA), LA (18:2), alpha-linolenic (18:3, aLA), gamma-linolenic (18:3, gLA), arachidonic (20:4, AA), eicosapentaenoic (20:5, EPA) and docosahexaenoic (22:6, DHA) acids, which were dissolved in 10% essential FA-free bovine serum albumin (BSA) (all from the Sigma Chemical Co., St Louis, MO). Following a 48-h incubation at 37°C, cell-conditioned media were collected to determine the CETP mass, and the cells were dissolved in a denaturing solution for RNA extraction.

Northern blot analysis. Total RNA was isolated using an acid guanidinium thiocyanate-phenol-chloroform extraction method (21). The yield of purified RNA samples was determined by absorbance at 260 nm. RNA samples (15 μg per lane) were separated by electrophoresis in 1% agarose/5.5% formaldehyde gel containing 0.84 μM ethidium bromide, followed by capillary transfer to nylon membranes (Nytran-N, Schleicher & Schuell GmbH, Dassel, Germany) that were then cross-linked by exposure to ultraviolet light. The membranes were prehybridized (4 to 5 h) at 42°C, and then were probed overnight at 42°C with a CETP cDNA fragment (365–624 bp) which had been labeled with [α-^{32}P]dCTP (NEN Life Science Products, Inc., Boston, MA) using a commercial kit (Random Primer DNA Labeling Kit, Takara Co., Otsu, Japan) in 5 × saline-sodium dihydrogenphosphate-ethylenediaminteraacetate buffer (SSPE), 5 × Denhardt's, 10% dextran sulfate, 50% deionized formamide, 1% sodium dodecyl sulfate (SDS), 0.2 mg/ml of heat-denatured salmon sperm DNA, and 1 mg/ml of BSA. The blot was washed twice at 42°C and twice further at

50°C for 30 min with 2 × saline-sodium citrate buffer (SSC), 0.2% SDS and then was exposed to an imaging plate (Fuji Film, Tokyo, Japan). The mRNA levels were quantitated by estimating the photostimulated luminescence per area of the corresponding band with an imaging analyzer (BAS 2000, Fuji Film). The data obtained were normalized for a glyceraldehyde phosphate dehydrogenase level. Northern hybridization detected CETP mRNA in HepG2 dose dependently ($r = 0.977$, $P < 0.0001$) when the total RNA per lane varied in mass from 0 to 20 μg.

Measurement of the CETP mass. The CETP mass secreted by HepG 2 cells into 4 mL culture medium/60-mm i.d. dish was measured using an enzyme-linked immunosorbent assay (ELISA) (22). One milliliter of a cultured medium was concentrated to about 100 μL using Microcon-10 (Amicon, Inc., Beverly, MA), and then the CETP mass of the concentrated medium was determined using the CETP (Chugai) ELISA kit (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). The results obtained were then adjusted to the original volume.

Analysis of the FA content in HepG2 cells. The cells were washed three times with PBS without Ca^{2+}/Mg^{2+} before the cell lipids were extracted by the method of Folch et al. (23). The lipids were analyzed after methylation using HCl-methanol with margaric acid (17:0) as an internal standard as described in a previous paper (24). The FA content was then measured by gas–liquid chromatography (PerkinElmer Auto System GC, Palo Alto, CA) on an Rscot Sillier 5CP capillary column (0.25 mm i.d. × 50

m; Nihon Chromato Works Ltd., Tokyo, Japan). The protein concentration of the cells was determined with a Micro BCA Protein Assay Reagent kit (Pierce Laboratories Inc., Rockford, IL) using BSA as a standard.

Statistical analysis. The results are presented as the mean ± SE. Statistical comparisons of the experimental groups and a linear regression analysis were performed using one-way analysis of variance, and each group was compared with each other by Fisher's protected least significant difference test. The level of significance was set at $P < 0.05$. Analyses were performed using the StatView J4.51.1 software package (Abacus Concepts Inc., Berkeley, CA).

RESULTS

Expression of CETP by FA treatment. To investigate the influence of FA on the CETP mRNA levels, HepG2 cells were treated with 0.5 mM of various kinds of FA. After 48 h of incubation, both the cell growth and morphology were normal in these treatments. Figures 1A and 1B show the effect of various FA on the levels of mRNA and protein of CETP in the HepG2 cells, respectively. After treatment with 0.5mM of AA, EPA, and DHA, the expression of CETP mRNA was less than 50% that of the control (Fig. 1A, $P < 0.0001$). Decreases in the CETP mRNA levels correlated with the increases in the degree of unsaturation of FAs. As shown in Fig. 1B, AA, EPA, and DHA also induced a significant decrease, relative to the control, in

the CETP mass levels that correlated with the expression of CETP mRNA (AA, $P = 0.0005$; EPA, $P < 0.01$; DHA, $P < 0.0001$). On the other hand, in the presence of 0.5 mM of SA, OA and LA, each of which contains 18 acyl carbons, the induction of the CETP mass was facilitated as the degree of unsaturation increased (OA vs. SA, $P < 0.05$; LA vs. SA, $P < 0.005$), even though the mRNA level decreased in these cases.

Correlation between the CETP protein mass and the mRNA level. To analyze the correlation between the CETP mass and the mRNA level, we plotted the levels of the CETP mass and mRNA obtained after treatment with both the n-6 and n-3 series of FA, respectively (Figs. 2, 3). These plots indicated that the n-6 and n-3 series each reduced both the CETP mass and mRNA level as the degree of unsaturation increased. The degree of unsaturation was strongly related to the expression of CETP if FA were classified into two groups consisting of the n-6 and n-3 series. Furthermore, the CETP mass and the mRNA levels both showed a positive correlation in this experiment (n-6, $r = 0.986$; n-3, $r = 0.998$).

Incorporation and metabolism of FA. As shown in Table 1, each FA added to the media increased the cellular FA concentration in comparison to the control, which was incubated without FA, thus indicating that FA were incorporated into HepG2 after 48 h of incubation. The metabolism of PUFA in humans and other animals proceeds by both chain elongation, such as biosynthesis of dihomo-γ-linolenic acid (DgLA, 20:3n–6) from gLA (18:3n–6), and desaturation, such as biosynthesis of gLA (18:3n–6) from LA (18:2n–6).

For example, in the n–9 series, the concentration of OA (18:1n–9) and 20:3n9, the metabolites of SA (18:0n–9), increased from 639.7 to 1424.9 nmol/mg protein and from undetectable levels to 78.2 nmol/mg protein after 48 h of incubation, respectively. In the n–6 series, DgLA and AA (20:4n–6), the metabolites of LA, were undetectable but rose to 172.1 nmol/mg protein and from 38.0 to 65.2 nmol/mg protein, respectively. Similarly in the n–3 series, 20:4n–3, the undetectable metabolite of aLA, increased to 203.8 nmol/mg protein. By the addition of LA, DgLA increased to 172.1 nmol/mg protein, while AA rose to a level that was 27.2 nmol/mg protein more than the control. Similarly, 20:4n–3 increased to 203.8 nmol/mg protein, while EPA (20:5n–3) was not detectable after treatment with aLA. These results supported the findings of previous reports in which the low activity of Δ5-desaturase in HepG2 resulted in the accumulation of DgLA or 20:4n–3 (24).

DISCUSSION

These studies demonstrate that FA influence both the expression of CETP mRNA and protein in HepG2 cells. The human hepatoma-derived cell line HepG2 reveals many characteristic differences for normal differentiated hepatocytes, and very low levels of CETP gene expression and CETP activity in the medium have been reported (18,25,26). In the present study, using a northern blot analysis we succeeded in detecting low amounts of

radioactivity in bands corresponding to CETP mRNA.

FA of various chain lengths and degrees of unsaturation have been reported to affect the expression of enzymes and proteins involved in lipid metabolism (27–31). For example, previous studies reported that long-chain FA increase lipoprotein lipase (LPL) mRNA but reduce the degree of LPL activity, presumably therefore regulating the posttranslational processing of LPL (29). Skretting *et al.* (30) also found that lecithin-cholesterol acyltransferase (LCAT) activities and mRNA levels in HepG2 cells decreased due to a posttranscriptional mechanism following sodium butyrate treatment. Faust *et al.* (17) reported that CETP secretion measured by the CETP activity was regulated in response to the OA concentration in CaCo-2 cells.

We therefore investigated whether various types of FA, particularly those with different degrees of unsaturation, influenced the expression of CETP. The results demonstrated that increasing degrees of unsaturation of FA reduced the CETP mRNA levels; for example, the expression of the CETP mRNA decreased to less than 50% of that of the control with 0.5 mM of AA, EPA, or DHA (Fig. 1A). The CETP mass levels also decreased significantly based on the levels of CETP mRNA expression. These results were also supported by the findings of previous studies, which suggested the mRNA level is a major determinant of secreted CETP protein (11,26). The increasing FA concentration in HepG2 cells after FA treatments also indicated that the FA influenced the expression of CETP mRNA and protein

(Table 1). Although the mechanism of regulation of CETP protein secretion by FA remains unknown, some interesting findings have appeared. Agellen *et al.* (26) indicated that CCAAT/enhancer binding protein (C/EBP) -α levels influence CETP mRNA expression, since C/EBP-α binds to the site of CETP gene promoter and activates the CETP promoter activity. *Raclot et al.* (27) found that C/EBP-α mRNA level is influenced by PUFA in the following order: DHA-rich diets > EPA-rich diets > OA-rich diets. Our observations are in accord with this finding, which may, in part, suggest that the mechanisms for the modulation of the mRNA levels by PUFA occur indirectly through the expression levels of C/EBP because the transcriptional levels of CETP are partly dependent on C/EBP. Ritsch A *et al.* (32), however, reported that C/EBP had no specific influence on the expression of CETP in HepG2. Another possible mechanism for the regulation of the CETP gene expression by FA may be the influence of apolipoprotein regulatory protein-1 (ARP-1) and/or v-erbA-related protein-3 (Ear-3)/chicken ovalbumin upstream promoter transcription factor (COUP-TF) (33), which are orphan receptors, and sterol regulatory element-binding proteins (SREBP) (34,35). These transcriptional factors, especially SREBP-1 (34,35), play an important role in controlling CETP gene expression. Kim *et al.* (36) described how SREBP-1 expression in the liver nuclei of fish oil-fed mice decreased by 57% compared with safflower oil-fed mice. Yahagi *et al.* (37) reported that dietary PUFA drastically decreased the mature, cleaved form of SREBP-1 protein in the nucleus in

the liver of wild-type mice, presumably due to the reduced cleavage of the SREBP-1 precursor protein. Therefore, the regulation of the CETP gene expression by FA may be mediated by the cooperative interaction with SREBP-1 and other transcriptional factors.

On the other hand, the concentration of FA in lipoprotein particles influences the CETP-mediated cholesteryl ester transfer activity *in vitro* (20,38,39); that is, a low concentration of FA stimulates and promotes CETP activity while increasing the FA concentrations above an optimal level, thus inhibiting the CETP activity in a dose-dependent manner. Both the length and degree of unsaturation of the FA acyl carbon chain also affect the CETP activity. The CETP activity is thus likely to be influenced by double bonds in the *trans* instead of the natural *cis* configuration (15,16); however, we did not investigate the effects of *trans* fatty acids on the gene expression of CETP. Further studies are needed to reveal whether a different configuration (*cis*- or *trans*-) of FA possibly controls the expression of CETP mRNA.

Our present data indicate that FA regulate gene expression of CETP in HepG2, and this effect is dependent upon the degree of unsaturation of the acyl carbon chain in FA. FA may therefore be convenient modulators of both the CETP expression level and the cholesteryl ester transfer activity.

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REFERENCES

1 Barter, P.J., Gorjatschko, L., and Calvert, G.D. (1980) Net mass transfer of esterified cholesterol from human low density lipoproteins to very low density lipoproteins incubated in vitro, *Biochim. Biophys. Acta 619*. 436-439.

2 Inazu, A., Brown, M.L., Hesler, C.B., Agellon, L.B., Koizumi, J., Takata, K., Maruhama, Y., Mabuchi, H., and Tall, A.R. (1990) Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation, *N. Engl. J. Med. 323*. 1234-1238.

3 Marcel, Y.L., McPherson, R., Hogue, M., Czarnecka, H., Zawadzki, Z., Weech, P.K., Whitlock, M.E., Tall, A.R., and Milne, R.W. (1990) Distribution and concentration of cholesteryl ester transfer protein in plasma of normolipemic subjects, *J. Clin. Invest. 85*. 10-17.

4 Ritsch, A., Auer, B., Foger, B., Schwarz, S., and Patsch, J.R. (1993) Polyclonal antibody-based immunoradiometric assay for quantification of cholesteryl ester transfer protein, *J. Lipid Res. 34*. 673-679.

5 Drayna, D., Jarnagin, A.S., McLean, J., Henzel, W., Kohr, W., Fielding, C., and Lawn, R. (1987) Cloning and sequencing of human cholesteryl ester transfer protein cDNA, *Nature 327*. 631-634.

6 Jiang, X.C., Moulin, P., Quinet, E., Goldberg, I.J., Yacoub, L.K., Agellon, L.B., Compton, D., Schnitzer-Polokoff, R., and Tall, A.R. (1991) Mammalian adipose tissue and muscle are major sources of lipid transfer protein mRNA. *J. Biol. Chem. 266*. 4631-4639.

7 Nagashima, M., McLean, J.W., and Lawn, R.M. (1988) Cloning and mRNA tissue distribution of rabbit cholesteryl ester transfer protein. *J. Lipid Res. 29*. 1643-1649.

8 Pape, M.E., Rehberg, E.F., Marotti, K.R., and Melchior, G.W. (1991) Molecular cloning, sequence, and expression of cynomolgus monkey cholesteryl ester transfer protein. Inverse correlation between hepatic cholesteryl ester transfer protein mRNA levels and plasma high density lipoprotein levels. *Arterioscler. Thromb. 11*. 1759-1771.

9 Tall, A.R. (1993) Plasma cholesteryl ester transfer protein. *J. Lipid Res. 34*. 1255-1274.

10 Lagrost, L. (1994) Regulation of cholesteryl ester transfer protein (CETP) activity: review of in vitro and in vivo studies. *Biochim. Biophys. Acta 1215*. 209-236.

11 Quinet, E.M., Agellon, L.B., Kroon, P.A., Marcel, Y.L., Lee, Y.C., Whitlock, M.E., and Tall, A.R. (1990) Atherogenic diet increases cholesteryl ester transfer protein messenger RNA levels in rabbit liver. *J. Clin. Invest. 85*. 357-363.

12 Leaf, A., and Weber, P.C. (1988) Cardiovascular effects of n-3 fatty acids. *N. Engl. J. Med. 318*. 549-557.

13 Bagdade, J.D., Ritter, M.C., Davidson, M., and Subbaiah, P.V. (1992) Effect of marine lipids on cholesteryl ester transfer and lipoprotein composition in patients with hypercholesterolemia. *Arterioscler. Thromb. 12*. 1146-1152.

14 Schwab, U.S., Maliranta, H.M., Sarkkinen, E.S., Savolainen, M.J., Kesaniemi, Y.A., and Uusitupa, M.I. (1996) Different effects of palmitic and stearic acid-enriched diets on serum lipids and lipoproteins and plasma cholesteryl ester transfer protein activity in healthy young women. *Metabolism 45*. 143-149.

15 van Tol, A., Zock, P.L., van Gent, T., Scheek, L.M., and Katan, M.B. (1995) Dietary trans fatty acids increase serum cholesterylester transfer protein activity in man. *Atherosclerosis 115*. 129-134.

16 Khosla, P., Hajri, T., Pronczuk, A., and Hayes, K.C. (1997) Replacing dietary palmitic acid with elaidic acid (t-C18:1 delta9) depresses HDL and increases CETP activity in cebus monkeys. *J. Nutr. 127*. 531S-536S.

17 Faust, R.A., and Albers, J.J. (1988) Regulated vectorial secretion of cholesteryl ester transfer protein (LTP-I) by the CaCo-2 model of human enterocyte epithelium. *J. Biol. Chem. 263*. 8786-8789.

18 Sperker, B., Mark, M., and Budzinski, R.M. (1993) The expression of human plasma cholesteryl-ester-transfer protein in HepG2 cells is induced by sodium butyrate. Quantification of low mRNA levels by polymerase chain reaction. *Eur. J. Biochem. 218*. 945-950.

19 Lagrost, L., and Barter, P.J. (1991) Effects of various non-esterified fatty acids on the particle size redistribution of high density lipoproteins induced by the human cholesteryl ester transfer protein. *Biochim. Biophys. Acta 1082*. 204-210.

20 Lagrost, L., and Barter, P.J. (1991) Effects of various non-esterified fatty

acids on the transfer of cholesteryl esters from HDL to LDL induced by the cholesteryl ester transfer protein. *Biochim. Biophys. Acta 1085*. 209-216.

21 Chomczynski, P., and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem. 162*. 156-159.

22 Kiyohara, T., Kiriyama, R., Zamma, S., Inazu, A., Koizumi, J., Mabuchi, H., and Chichibu, K. (1998) Enzyme immunoassay for cholesteryl ester transfer protein in human serum. *Clin. Chim. Acta 271*. 109-118.

23 Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957) A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem. 266*. 497-509.

24 Fujiyama-Fujiwara, Y., Ohmori, C., and Igarashi, O. (1989) Metabolism of gamma-linolenic acid in primary cultures of rat hepatocytes and in Hep G2 cells. *J. Nutr. Sci. Vitaminol. 35*. 597-611.

25 Faust, R.A., and Albers, J.J. (1987) Synthesis and secretion of plasma cholesteryl ester transfer protein by human hepatocarcinoma cell line, HepG2. *Arteriosclerosis 7*. 267-275.

26 Agellon, L.B., Zhang, P., Jiang, X.C., Mendelsohn, L., and Tall, A.R. (1992) The CCAAT/enhancer-binding protein trans-activates the human cholesteryl ester transfer protein gene promoter. *J. Biol. Chem. 267*. 22336-22339.

27 Raclot, T., Groscolas, R., Langin, D., and Ferre, P. (1997) Site-specific

regulation of gene expression by n-3 polyunsaturated fatty acids in rat white adipose tissues. *J. Lipid Res. 38*. 1963-1972.

28 Auwerx, J., Schoonjans, K., Fruchart, J.C., and Staels, B. (1996) Transcriptional control of triglyceride metabolism: fibrates and fatty acids change the expression of the LPL and apo C-III genes by activating the nuclear receptor PPAR. *Atherosclerosis 124*. Suppl:S29-S37.

29 Amri, E.Z., Teboul, L., Vannier, C., Grimaldi, P.A., and Ailhaud, G. (1996) Fatty acids regulate the expression of lipoprotein lipase gene and activity in preadipose and adipose cells. *Biochem. J. 314*. 541-546.

30 Skretting, G., Gjernes, E., and Prydz, H. (1997) Sodium butyrate inhibits the expression of the human lecithin: cholesterol acyltransferase gene in HepG2 cells by a post-transcriptional mechanism. *FEBS Lett. 404*. 105-110.

31 Subbaiah, P.V., Subramanian, V.S., and Liu, M. (1998) Trans unsaturated fatty acids inhibit lecithin: cholesterol acyltransferase and alter its positional specificity. *J. Lipid Res. 39*. 1438-1447.

32 Ritsch, A., Doppler, W., Pfeifhofer, C., Sandhofer, A., Bodner, J., and Patsch, J.R. (1999) Cholesteryl ester transfer protein gene expression is not specifically regulated by CCAAT/enhancer-binding protein in HepG2-cells. *Atherosclerosis. 146*. 11-18.

33 Gaudet, F., and Ginsburg, G.S. (1995) Transcriptional regulation of the cholesteryl ester transfer protein gene by the orphan nuclear hormone receptor apolipoprotein AI regulatory protein-1. *J. Biol. Chem. 270*.

29916-29922.

34 Chouinard, R.A. Jr., Luo, Y., Osborne, T.F., Walsh, A., and Tall, A.R. (1998) Sterol regulatory element binding protein-1 activates the cholesteryl ester transfer protein gene in vivo but is not required for sterol up-regulation of gene expression. *J. Biol. Chem. 273*. 22409-22414.

35 Gauthier, B., Robb, M., Gaudet, F., Ginsburg, G.S., and McPherson, R. (1999) Characterization of a cholesterol response element (CRE) in the promoter of the cholesteryl ester transfer protein gene: functional role of the transcription factors SREBP-1a, -2, and YY1. *J. Lipid Res. 40*. 1284-1293.

36 Kim, H.J., Takahashi, M., and Ezaki, O. (1999) Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. *J. Biol. Chem. 274*. 25892-25898.

37 Yahagi, N., Shimano, H., Hasty, A.H., Amemiya-Kudo, M., Okazaki, H., Tamura, Y., Iizuka, Y., Shionoiri, F., Ohashi, K., Osuga, J., Harada, K., Gotoda, T., Nagai, R., Ishibashi, S., and Yamada, N. (1999) A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. *J. Biol. Chem. 274*. 35840-35844.

38 Sammett, D., and Tall, A.R. (1985) Mechanisms of enhancement of cholesteryl ester transfer protein activity by lipolysis. *J. Biol. Chem. 260*.

6687-6697.

39 Nishida, H.I., Arai, H., and Nishida, T. (1993) Cholesterol ester transfer mediated by lipid transfer protein as influenced by changes in the charge characteristics of plasma lipoproteins. *J. Biol. Chem. 268*. 16352-16360.

40 Lagrost, L., Barter, P.J. (1991) Effects of various non-esterified fatty acids on the transfer of cholesteryl esters from HDL to LDL induced by the cholesteryl ester transfer protein. *Biochim. Biophys. Acta 1085*. 209-216.

LEGENDS

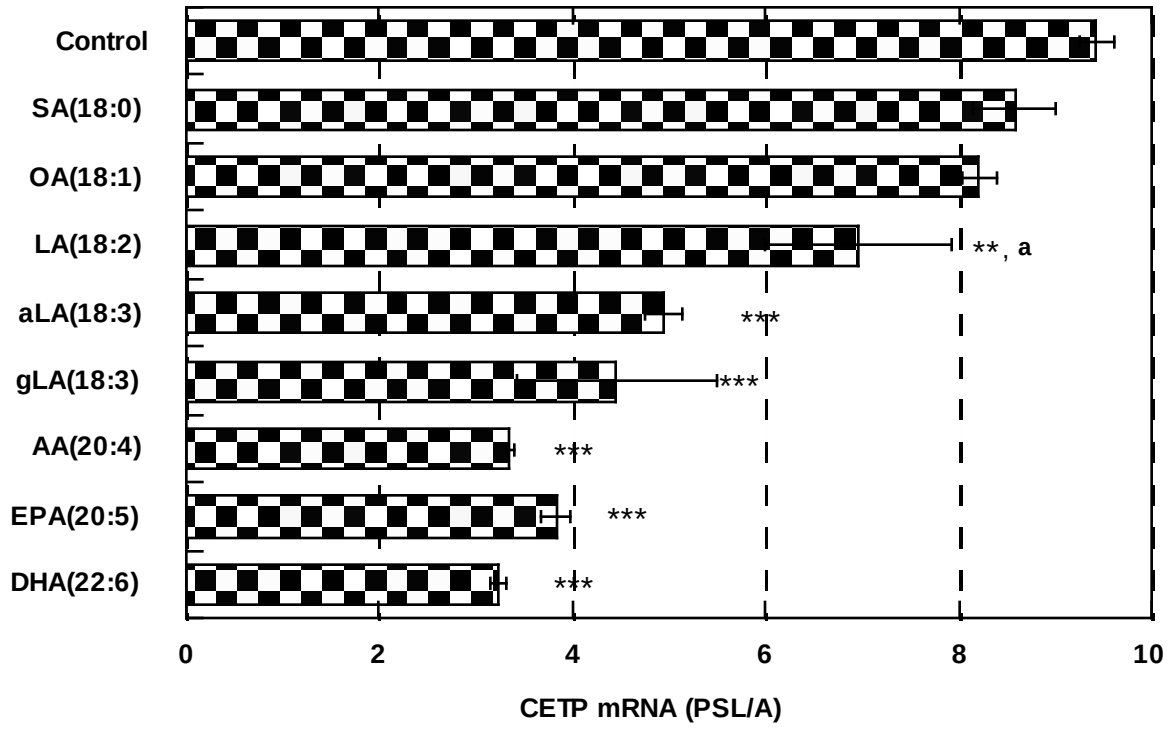
Fig. 1 Comparative effects of various saturated and unsaturated fatty acids on the expression of the cholesteryl ester transfer protein (CETP) mRNA (A) and mass (B). HepG2 cells were incubated with 0.5 mM of various fatty acids or 1.25% bovine serum albumin (as a control) for 48 h in Dulbecco's Modified Eagle's Medium with 10% lipoprotein deficient serum. The CETP mRNA and mass were measured as described in the text. Each data point represents the mean ± SE from three dishes. In A, statistical differences are shown as ** $P < 0.005$, *** $P < 0.0001$ relative to the control, and in B * $P < 0.05$, ** $p < 0.01$, *** $P = 0.0005$, **** $P < 0.0001$ and in both [a] $p < 0.05$, [b] $p < 0.005$ relative to stearic acid (SA). OA, oleic acid; LA, linoleic acid; aLA, α-linolenic acid; gLA, γ-linolenic acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Fig. 2 Relationship between the CETP mRNA and mass secreted into the medium after the treatment of n-6 fatty acids. A linear regression analysis demonstrates a positive correlation between CETP mRNA and mass (r = 0.986). Each data point represents the mean ± SE from three dishes. For abbreviations see Figure 1.

Fig. 3 Relationship between the CETP mRNA and mass secreted into the medium after the treatment of n-3 fatty acids. A linear regression analysis

demonstrates a positive correlation between CETP mRNA and mass ($r = 0.998$). Each data point represents the mean ± SE from three dishes. For abbreviations see Figure 1.

Fig. 1 A


Control
SA(18:0)
OA(18:1)
LA(18:2)
aLA(18:3)
gLA(18:3)
AA(20:4)
EPA(20:5)
DHA(22:6)
**, a
***
***
***
***
***
0
2
4
6
8
10
CETP mRNA (PSL/A)

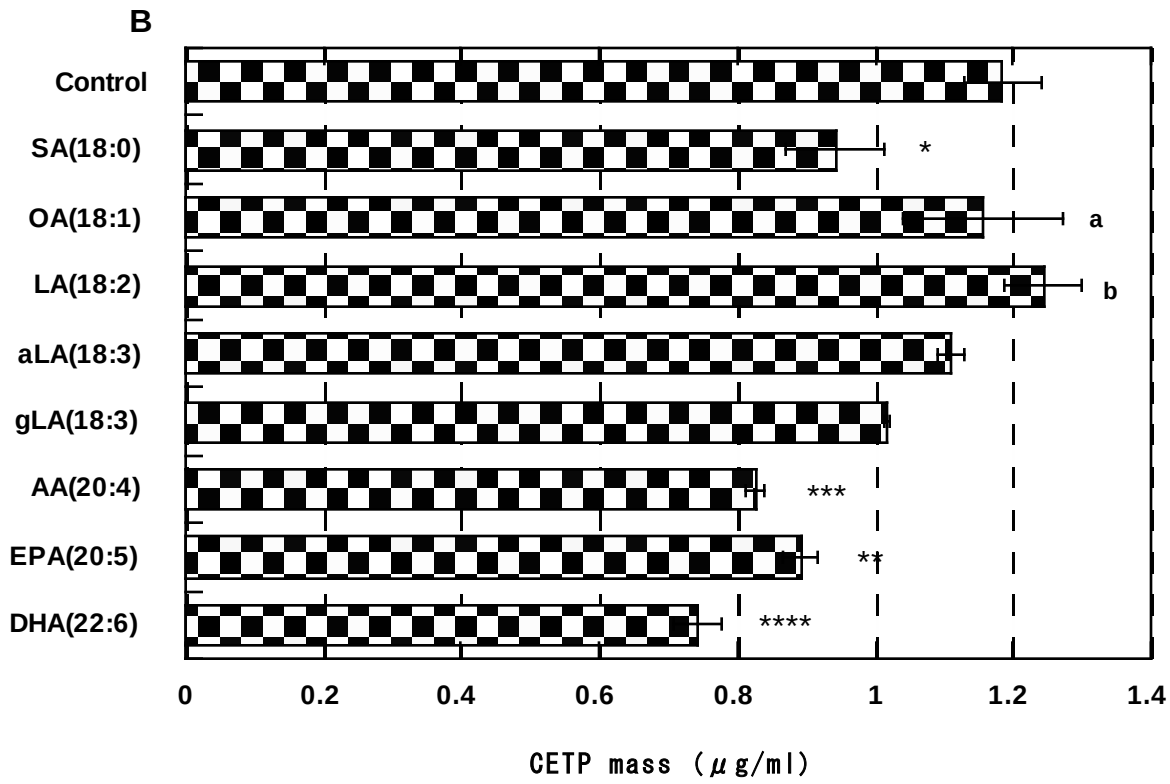

B
Control
SA(18:0)
OA(18:1)
LA(18:2)
aLA(18:3)
gLA(18:3)
AA(20:4)
EPA(20:5)
DHA(22:6)
*
a
b
***
**
****
0
0.2
0.4
0.6
0.8
1
1.2
1.4
CETP mass (μg/ml)

Fig. 2

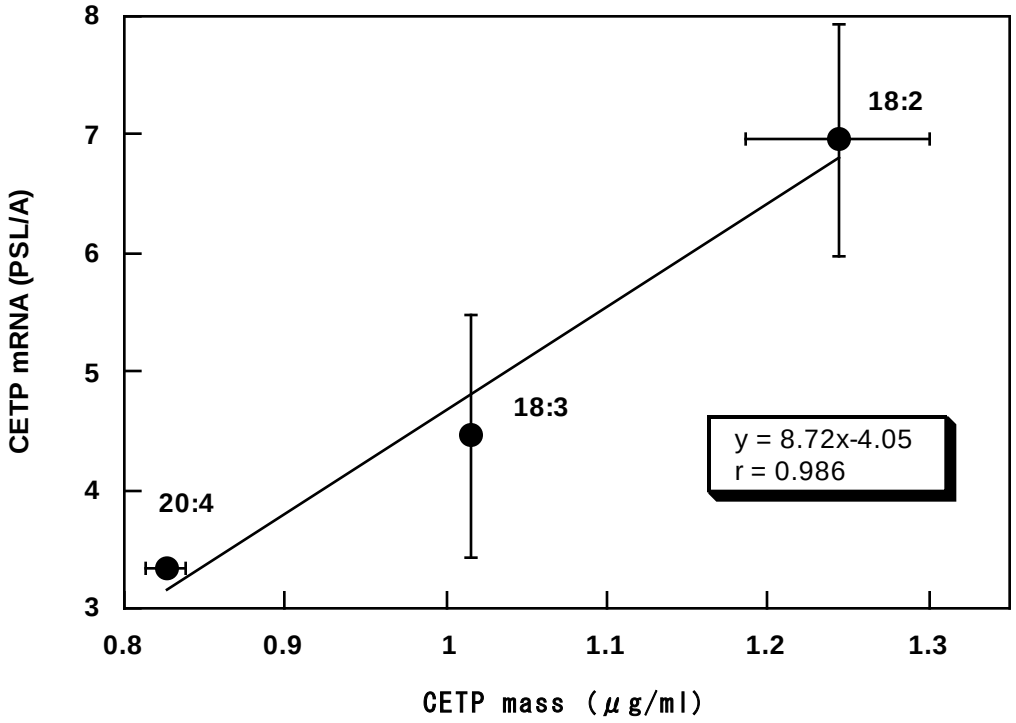

8
7
6
5
4
3
CETP mRNA (PSL/A)
0.8
0.9
1
1.1
1.2
1.3
CETP mass (μg/ml)
18:2
18:3
20:4
y = 8.72x-4.05
r = 0.986

Fig. 3

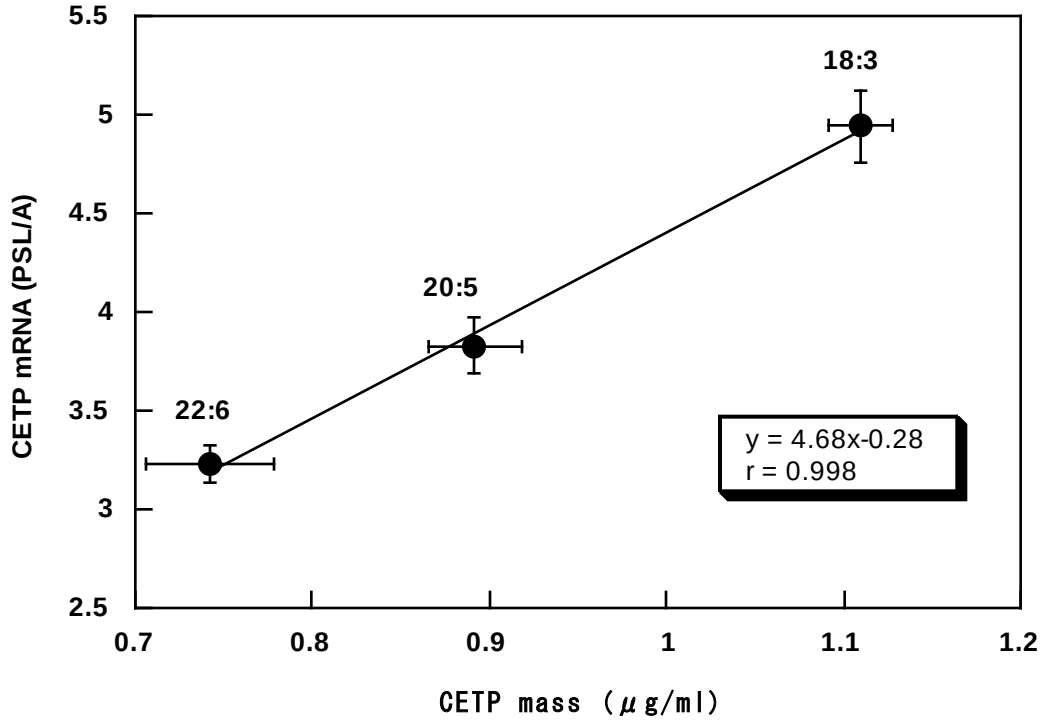

5.5
5
4.5
4
3.5
3
2.5
CETP mRNA (PSL/A)
0.7
0.8
0.9
1
1.1
1.2
CETP mass (μg/ml)
18:3
20:5
22:6
y = 4.68x-0.28
r = 0.998

TABLE 1

The composition of PUFA in HepG2 cells

Added PUFAs	18:0	18:1n9	18:2n6	18:3n3	20:3n9	20:3n6	20:4n6	20:4n3	20:5n3	22:4n6	22:6n3
Control	128.9±15.5	639.7±86.7	14.2±1.9	nd	nd	nd	38.0±4.9	nd	nd	nd	nd
18:0	540.4±34.1[e]	1424.9±73.6[d]	9.6±4.9	nd	78.2±4.3	nd	46.7±2.5	nd	nd	nd	nd
18:1n9	131.9±23.0	1738.4±281.6[e]	9.85±.5	nd	88.8±14.5	nd	34.9±5.5	nd	nd	nd	nd
18:2n6	216.4±29.4[a]	502.0±68.4	1275.4±154.2[e]	nd	nd	172.1±21.2	65.2±9.4[b]	nd	nd	nd	nd
18:3n3	179.0±7.6	446.7±18.8	14.3±0.8	1115.3±50.5	nd	nd	34.7±1.3	203.8±6.5	nd	nd	nd
20:4n6	153.1±2.3	228.9±5.9[a]	7.1±1.2	nd	nd	nd	633.1±12.2[e]	nd	nd	226.1±9.8	nd
20:5n3	234.9±9.6[c]	422.9±19.0	15.0±3.2	nd	nd	nd	42.8±1.7	nd	619.7±42.7	nd	513.7±37.5
22:6n3	148.4±26.9	352.9±63.6	11.5±1.7	nd	nd	nd	28.9±4.9	nd	9.4±5.9	nd	911.0±146.4

Note. Data are expressed as nmol/mg cell protein and are the means ± SE from 3 dishes. nd, not detectable. a ; $p<0.05$, b ; $p<0.01$, c ; $p<0.005$, d ; $p<0.0005$, e ; $p<0.0001$; significantly different from the control according to the ANOVA and Fisher's PLSD test.