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ORIGINAL ARTICLE

Overexpression of apolipoprotein CIII increases and CETP reverses diet-induced obesity in transgenic mice

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Objective: We recently described that hypertriglyceridemic apolipoprotein (apo) CIII transgenic mice show increased whole body metabolic rate. In this study, we used these apo CIII-expressing mice, combined or not with the expression of the natural promoter-driven *CETP* gene, to test the hypothesis that both proteins modulate diet-induced obesity.
Measurements and results: Mice expressing apo CIII, CIII/CETP, CETP and nontransgenic (NonTg) mice were maintained on a high-fat diet (14% fat by weight) during 20 weeks after weaning. At the end of this period, all groups exhibited the expected lipemic phenotype. Fasting glucose levels were neither affected by the high-fat diet nor by the distinct genotypes. However, apo CIII mice showed significantly higher glycemia (~35%) and lower insulin levels (~45%) in the fed state, compared with the NonTg mice. The apo CIII mice presented significantly increased body weight, lipid content of the carcass (~25%), visceral adipose tissue mass (about twofold) and adipocyte size (~25%) compared with the CETP and NonTg mice. The CETP expression in the apo CIII background normalized the subcutaneous adipose depot and visceral adipocyte size to the levels of NonTg mice. Plasma leptin levels were lower in CETP groups (25–50%) and higher in the apo CIII mice. Similar core body temperature in all groups and similar liver mitochondrial resting respiration rates in CIII and NonTg mice indicate no differences in basal energy expenditure rates among these mice fed a high-fat diet.
Conclusion: The elevation of plasma apo CIII levels aggravates diet-induced obesity and the expression of physiological levels of circulating CETP reverses this adipogenic effect, indicating a novel role for CETP in modulating adiposity.
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Keywords: CETP; apolipoprotein CIII; diet-induced obesity; hypertriglyceridemia; leptin; mitochondrial respiration

Introduction

Hypertriglyceridemia is a common feature in the general population. Although it can be caused by many factors, including dietary habits, alcohol intake, medication and different diseases, it is clear that a large number of individuals have a genetic tendency to hypertriglyceridemia.[1,2]

Apolipoprotein (apo) CIII is an 8.8-kDa plasma glycoprotein constituent of the triglyceride (TG)-rich lipoproteins, synthesized mainly by the liver and to a lesser extent by the intestine.[3,4] Apo CIII plays an important role in regulating plasma TG metabolism.[4–6] Transgenic mice expressing human apo CIII have elevated TG levels due to the presence of enlarged TG-rich lipoproteins in plasma with increased apo CIII and decreased apo E compared with controls.[7–9] Elevation in plasma TG is proportional to the level of *apo CIII* gene expression and to the amount of human apo CIII in plasma.[10,11] On the other hand, murine apo CIII gene deletion results in hypotriglyceridemia.[12] Apo CIII delays the clearance of TG-rich lipoproteins by two ways (i) decreasing the affinity of TG-rich lipoproteins for glycosaminoglycan-bound lipases[13] and (ii) reducing the receptor-mediated uptake of lipolyzed very low-density lipoprotein (VLDL) and chylomicron remnants.[8,14–16]

Cholesteryl ester transfer protein (CETP) is a plasma protein that promotes the heteroexchange of neutral lipids between circulating lipoproteins leading to the net mass transfer of cholesteryl ester from high-density lipoprotein (HDL) to the apo B-containing lipoproteins (chylomicron, VLDL and low-density lipoprotein).[17] Although mice normally lack CETP, humans and rabbits express moderate to high levels of this protein.[18] In humans, plasma CETP is

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synthesized by the liver, spleen and adipose tissue, with lower levels of expression in small intestine, adrenal, kidney and heart.[17] The tissue showing the most conserved expression in different species is probably the adipose tissue.[19] Transgenic mice expressing human[20,21] or simian[22] CETP exhibit a marked reduction in HDL cholesterol and increased diet-induced atherosclerosis.[22,23] In addition, CETP expression stimulates the selective uptake of HDL-derived cholesteryl ester by the liver and other tissues[24] and by human adipocytes[25,26] and protects against atherosclerosis in specific conditions such as hypertriglyceridemia[27] and sex-hormone deficiency.[28,29] By reducing HDL cholesterol levels, CETP has been considered a major target for developing inhibitors aimed at reducing atherosclerosis risk (for review see Clark[30]). However, unexpected negative outcomes from large-scale clinical trial testing the CETP-inhibitor torcetrapib have seriously questioned this strategy.[31,32]

The expression of apo C and CETP may also have an impact on adiposity. Recently, Zhou *et al*.[33] showed that adipose tissue-specific CETP transgenic mice had adipocytes significantly smaller than those of wild-type mice. Regarding the apo CIII, we showed recently that transgenic mice overexpressing apo CIII have higher mitochondrial resting respiration rates and whole-body oxidative metabolism, which allow these mice to keep body mass similar to the controls in spite of their severe hypertriglyceridemia and elevated food ingestion.[34] On the other hand, apo CIII deficiency in knockout mice aggravates diet-induced obesity,[35] probably through the increased intravascular lipolysis of the TG-rich lipoproteins.

In this work, we tested the hypothesis that apo CIII-overexpressing mice would be less prone to develop diet-induced obesity because (i) these mice have impaired plasma TG clearance, (ii) increased whole-body metabolic rate, (iii) apo CIII deficiency leads to augmented adiposity and (iv) overexpression of another apo C family member (apo CI) protected against a genetic type of obesity.[36] In addition, we hypothesized that CETP overexpression, perhaps through redistributing TG to HDL, in the long term, could decrease adipose tissue accumulation. Thus, coexpression of both proteins, apo CIII and CETP, could result in additive effects of reducing diet-induced adipose tissue formation.

Materials and methods

Animals and diets

All animal protocols were approved by the university's Committee for Ethics in Animal Experimentation (CEEA/UNICAMP). Human apo CIII transgenic mice (line 3707)[37] were crossbred with human natural promoter-driven CETP transgenic mice (line 5203)[21] to obtain CIII, CIII/CETP, CETP and nontransgenic (NonTg) littermates used in these studies. The mice founders were kindly provided by Dr Alan R Tall (Columbia University, New York, NY, USA). Heterozygous crossbreeding of apo CIII and CETP transgenic colonies has been carried out for 10 years using C57Bl6 mice, an obesity-prone strain of mice. The mice were housed in a temperature-controlled room at $22 \pm 1^{\circ}C$ on a 12-h light/dark cycle and were fed a high-fat diet during 20 weeks from weaning. This diet contained 21% protein, 59% carbohydrates and 14% saturated (lard) fat by weight, in a total of 446 Kcal/100 g, containing AIN93 mineral and vitamin mixtures. This diet also contained 0.01% cholesterol that was present in lard. Both male and female mice were used for the experiments. CETP-expressing mice were genotyped by assaying plasma CETP activity[38] and apo CIII transgenic had plasma TG levels above 300 mg/dl while NonTg TG levels were below 100 mg/dl.

Plasma lipids, glucose, insulin and leptin levels

Blood samples were obtained from the tail tip or from the retro-orbital plexus of anesthetized mice. Plasma cholesterol (Chod-Pap; Roche Diagnostic GmbH, Mannheim, Germany), TGs (Chod-Pap) and free fatty acids (FFA) (Wako Chemical, Neuss, Germany) were determined after an overnight fasting using enzymatic colorimetric assays according to the manufacturer's instructions. Ten microliter of whole blood precipitated with 5% trichloroacetic acid was used for glucose analysis using the glucose oxidase method (Glucose GOD PAP-Laborlab, SP, Brazil). Nonfasting plasma leptin concentrations were determined by ELISA (Crystal Chem Inc., Chicago, IL, USA) and insulin levels measured by radioimmunoassay, as described previously[39] using rat insulin standards.

Carcass chemical composition

Fed mouse carcasses (wet weight) were dehydrated to a constant weight at $65^{\circ}C$ during 72 h (dry weight). Total water content was calculated as wet weight minus dry weight. Total carcass fat (subcutaneous adipose tissue) was extracted with petroleum ether (LabSynth, SP, Brazil) using a soxhlet apparatus during 96 h and calculated by subtracting carcass weight before and after lipid extraction. The lean body mass was calculated as wet weight minus total lipid weight.

Determination of adipocyte area

Perigonadal white adipose tissue from male and female mice were dissected out and adipocytes were isolated according to the method of Rodbell,[40] with minor modifications, as follows. Krebs–Ringer bicarbonate buffer containing BSA (3%), glucose (6 mM) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (25 mM), pH 7.4, was utilized. After collagenase treatment (1 mg/ml), isolated fat cells were filtered through a nylon mesh, washed two times and the packed cells adjusted to approximately 10^5 cells/ml and placed on a Mallassez chamber for light microscopy and imaging capturing. Adipocyte sizes were analyzed with the software Image Pro Plus Analyser (Media Cybernetics, Silver Spring, MD, USA; version 3.0).

Mitochondrial respiratory rates

Mitochondria were isolated by conventional differential centrifugation[41] at 4°C. Liver homogenate was prepared in 250 mM sucrose, 1 mM EGTA, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.2) and 0.1% nonesterified fatty acid-free BSA and centrifuged at 600 *g* for 10 min. The supernatant was centrifuged at 7000 *g* for 10 min. The pellet was washed in the same medium devoid of BSA and containing 0.1 mM EGTA. The final mitochondrial pellet was diluted in 250 mM sucrose to a protein concentration of 60–80 mg/ml, measured by the Biuret method and BSA as the protein standard. Oxygen consumption was measured using a temperature-controlled computer-interfaced Clark-type oxygen electrode from Hansatech Instruments Ltd (King's Lynn, Norfolk, England) equipped with magnetic stirring, at 28°C. The experiments were done in standard medium containing 125 mM sucrose, 65 mM KCl, 4 mM potassium succinate, 2 mM inorganic phosphate, 1 mM magnesium chloride, 0.4 mM EGTA and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (pH 7.2). The state III respiration was initiated with the addition of 200 nmol adenosine diphosphate (ADP)/mg protein.

Insulin secretion from isolated pancreatic islets

The pancreas was removed and islets were isolated by handpicking after collagenase digestion. Groups of 10 islets were first incubated for 30 min at 37°C in Krebs bicarbonate buffer containing 2.8 mmol/l glucose and equilibrated with 95% O_2/5% CO_2 at pH 7.4. The solution was then replaced with fresh Krebs bicarbonate buffer and islets were further incubated for 1 h with medium containing 22.2 mmol/l glucose. The incubation medium contained (in mmol/l): 115.0 NaCl, 5.0 KCl, 24.0 $NaHCO_3$, 2.56 $CaCl_2$, 1.0 $MgCl_2$ and 0.3% BSA.[42] The insulin released after 1 h was quantified as described previously[39] using rat insulin as the standard.

Statistical analysis

The results are presented as the mean ± s.e.m. for the number of determinations (*n*) indicated. The statistical analysis were done with the GraphPad InStat, Inc. software (version 3.00) using one way analysis of variance followed by the Tukey post test for multiple comparisons or Student's *t*-test for two group comparisons. Statistical significance was defined as $P<0.05$.

Results

Male and female littermate mice overexpressing apo CIII, combined or not with the natural promoter-driven CETP gene expression and control NonTg mice (CIII, CIII/CETP, CETP and NonTg), were placed on a high-fat diet (14% fat by weight) during 20 weeks after weaning.

Table 1 shows plasma lipid levels in male mice from the four genotypic groups fed the high-fat diet. They exhibited the expected lipemic phenotype, that is, increased TGs (about fivefold) and FFAs (30–50%) in apo CIII-expressing groups (CIII and CIII/CETP) compared with the apo CIII nonexpressing groups (CETP and NonTg). Interestingly, after 20 weeks on the high-fat diet, TG and FFA levels are similar to those found in these mice on a chow diet, while cholesterol levels are significantly elevated in all groups (data not shown). Also as expected, plasma total cholesterol levels were reduced by 20–40% in CETP transgenic mice compared with other groups. Similar results were found in female mice (data not shown).

Plasma insulin and glucose levels in fasting or fed states are shown in Table 2. Fasting glucose levels were neither affected by the high-fat diet nor by the distinct genotypes. However, male apo CIII transgenic mice showed significantly higher glycemia (35%) in the fed state compared with the NonTg mice. This is probably ascribable to the lower insulin levels (45%) in these mice, which are also observed in apo CIII females. The lower insulin levels and insulin/glucose ratios in apo CIII-expressing groups suggest impaired insulin secretion in the fed state. In fact, there was a significant reduction in the secretory capacity of the isolated pancreatic islet in response to high concentrations of glucose (22 mM): 2.1 ± 0.1 vs 2.8 ± 0.2 pg/islet/h, $n=10$–12, $P<0.02$, in CIII vs NonTg islet, respectively.

Body mass and composition in male and female mice overexpressing apo CIII and/or CETP and NonTg mice are shown in Table 3. After 20 weeks on a high-fat diet, the apo CIII transgenic mice had increased body weight in relation to both CETP-expressing groups (CETP and CIII/CETP), a feature more marked in males. Lean body mass and water content of the mice carcasses were similar in the four groups of mice. Lipid content of the carcass, which mainly represents the subcutaneous adipose tissue, was higher in the apo CIII mice compared with the other groups. Particularly impressive is the effect of the CETP expression in the apo CIII background in reducing (25%) the

Table 1 Plasma concentrations of lipids in male mice overexpressing apo CIII and/or CETP and NonTg mice after 20 weeks on a high-fat diet

	CIII	*CIII/CETP*	*CETP*	*NonTg*
CHOL (mg/dl)	265 ± 20[a] (10)	220 ± 20[b] (12)	144 ± 10[a,b] (11)	200 ± 15 (12)
TG (mg/dl)	530 ± 40[c,d] (10)	525 ± 26[e,f] (12)	95 ± 10[d,f] (11)	72 ± 5[c,e] (12)
FFA (mmol/l)	1.8 ± 0.3[g] (10)	1.8 ± 0.2[h] (12)	1.4 ± 0.1 (11)	1.1 ± 0.1[g,h] (12)

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; CHOL, cholesterol; FFA, free fatty acid; NonTg, nontransgenic; TG, triglycerides. Values represent mean ± s.e.m. (*n*). The same letters indicate the pair comparisons that are significantly different. [a,c,d,e,f]$P<0.001$; [b,g,h]$P<0.05$, analysis of variance.

Table 2 Plasma glucose (mg/dl) and insulin (ng/ml) levels in fasted and fed male and female mice overexpressing apo CIII and/or CETP and NonTg mice after 20 weeks on a high-fat diet

	CIII	*CETP/CIII*	*CETP*	*NonTg*
Glucose (mg/dl) (fasted)				
Male	98±10 (10)	73±6.0 (12)	75±7.0 (11)	87±8.0 (12)
Female	74±7.0 (6)	73±6.0 (11)	65±7.0 (8)	65±4.0 (12)
Glucose (mg/dl) (fed)				
Male	371±26 (10)[a]	299±25 (12)	297±19 (12)	277±14 (13)[a]
Female	245±22 (7)	243±20 (11)	225±8 (10)	230±9 (14)
Insulin (pg/ml) (fed)				
Male	693±78 (7)[b]	731±102 (8)[c]	942±213 (7)	1299±173 (7)[b,c]
Female	641±50 (6)[d,e,f]	1093±158 (7)[d]	1160±189 (6)[e]	1035±146 (6)[f]
Insulin/glucose (fed)				
Male	1.93±0.3 (7)[g]	2.70±0.4 (8)[h]	3.15±0.6 (7)	4.90±0.5 (7)[g,h]
Female	2.81±0.3 (6)[i,j]	4.96±1.1 (7)	5.28±1.0 (6)[i]	4.84±0.7 (6)[j]

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; NonTg, nontransgenic. Values represent mean±s.e.m. (*n*). The same letters indicate the pair comparisons that are significantly different. [a,b,g,h]$P<0.05$ (analysis of variance) and [c,d,e,f,i,j] (Student *t*-test).

Table 3 Body mass and composition (% body weight) in male and female mice overexpressing apo CIII and/or CETP and NonTg mice after 20 weeks on a high-fat diet

Genotype	*Body weight (g)*	*Lean body mass*	*Water*	*Lipid**
Male				
CIII	38.5±1.8[a,b]	50.0±1.0	35.0±1.0	21.5±1.1[c,d]
CIII/CETP	31.0±1.6[a]	55.0±2.4	38.0±1.6	15.2±1.7[c]
CETP	31.5±1.7[b]	56.1±3.0	39.0±2.2	15.0±2.3[d]
NonTg	33.0±2.5	54.0±2.8	38.5±2.0	16.0±2.5
Female				
CIII	27.0±1.5	56.0±2.0	39.0±1.3	16.0±1.7[e]
CIII/CETP	24.0±0.8	60.0±0.7	41.5±0.5	11.2±0.8[e]
CETP	24.3±0.5	60.0±1.0	41.0±0.9	11.2±1.1
NonTg	25.0±2.2	57.0±2.7	39.5±1.7	13.5±2.4

Abbreviations: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; NonTg, nontransgenic. *lipid extracted from mice carcasses indicate subcutaneous lipid depots. Values represent mean±s.e.m. for six animals in each group. The same letters indicate the pair comparisons that are significantly different. $P<0.05$ (Student *t*-test).

subcutaneous adipose tissue mass to the levels of CETP and NonTg mice (Figure 1).

Total visible visceral adipose tissue mass, including perigonadal, mesenteric and retroperitoneal depots were excised and weighed after the 20-week period of high-fat diet and are shown in Figure 2. Apo CIII mice had 1.5-to twofold greater visceral adipose tissue weight compared with the CETP and NonTg mice. The CIII/CETP group had an intermediate visceral adipose tissue weight, between CIII and CETP groups, showing that the CETP expression was not sufficient to correct the higher visceral adipose tissue weight of apo CIII transgenic mice. Thus, it is clear that the overexpression of *apo CIII* gene-induced fat accumulation in the visceral depots, in both sexes, as shown in pooled analysis (apo CIII expressing vs nonexpressing groups).

The adipocyte sizes from the four groups were measured in cells isolated from collagenase-treated perigonadal adipose tissue and is shown in Figure 3. Adipocytes from apo CIII mice had significantly greater area (20–25%) than those from the other three groups. CETP expression in the apo CIII background completely normalized the larger adipocyte size of apo CIII mice.

Total lipid extracted from the livers showed no differences in hepatic fat content among the four groups (mg/g of liver): 68±6, 57±3, 66±10, 62±5 for CIII, CIII/CETP, CETP and NonTg male mice, respectively. Similar values were found in females.

Nonfasting plasma leptin levels (Figure 4) were lower in CETP transgenic groups (males and females) and higher in the apo CIII group (males), confirming the adipose tissue mass results. Leptin plasma levels paralleled better than the pattern of subcutaneous better than visceral adipose tissue mass observed in male and female mice, probably because the leptin production rates are higher for subcutaneous than for omental adipose tissue.[43]

We previously reported that, under low-fat chow diet, adult apo CIII transgenic mice exhibited an increased energy-dissipating process measured as higher mitochondrial

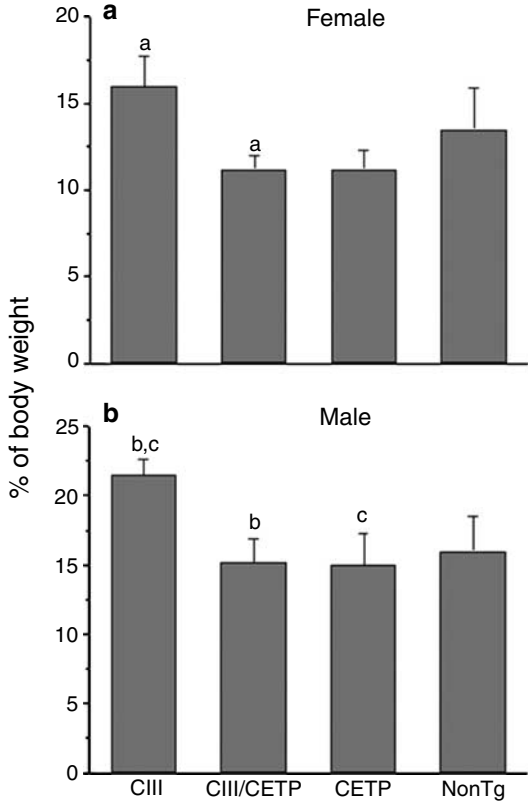

Figure 1 Relative weight of subcutaneous adipose tissue depot (carcass lipid content, % of body weight) in female (**a**) and male (**b**) mice overexpressing apo-CIII and/or CETP and nontransgenic (NonTg) mice on a high-fat diet for 20 weeks. Mean ± s.e.m. ($n = 6$). The same letters indicate the pair comparisons that are significantly different: $^{a,b,c}P < 0.05$ (Student *t*-test).

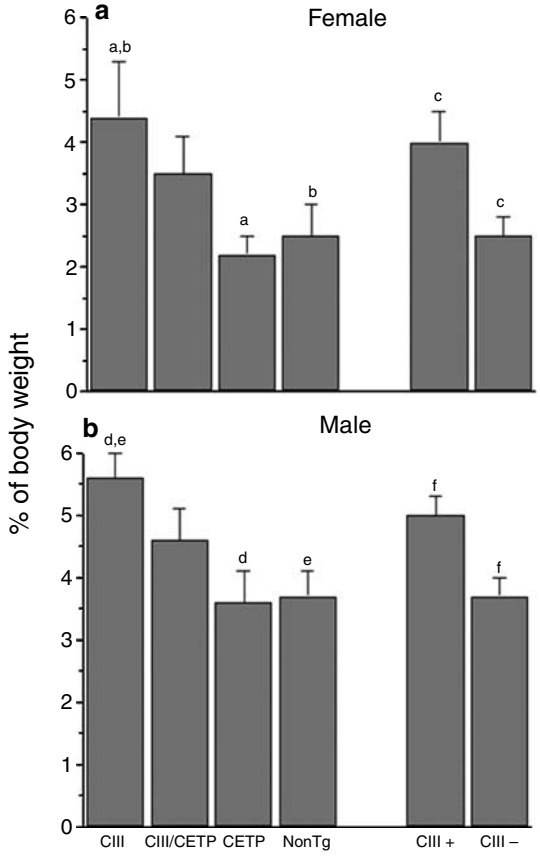

Figure 2 Relative weight of visceral adipose tissue depot (% of body weight) in female (**a**) and male (**b**) mice overexpressing apo-CIII and/or CETP and nontransgenic (NonTg) mice on a high-fat diet for 20 weeks. Mean ± s.e.m. ($n = 7$–14). Black bars indicate pooled values from CIII-expressing mice. (+) CIII + CIII/CETP- and CIII-nonexpressing mice. (−) CETP + NonTg. The same letters indicate the pair comparisons that are significantly different: $^{a,b}P = 0.052$ (analysis of variance (ANOVA), females); $^{d,e}P < 0.05$ (ANOVA, males); $^{c,f}P < 0.01$ (Student *t*-test).

resting respiration, higher whole body metabolism and higher rectal temperature.[34] This adaptation may explain why these mice, although hypertriglyceridemic and hyperphagic, maintained their body weight. Here, we report that apo CIII and NonTg male mice on a low-fat diet have similar adipose tissue stores, 1.1 ± 0.3 and 0.9 ± 0.3 for perigonadal and 0.8 ± 0.1 and 0.8 ± 0.2% for visible subcutaneous depots (% body weight), respectively. Since in the present experimental conditions of high-fat diet, the apo CIII mice presented higher body and adipose tissue mass, we re-evaluated their mitochondrial respiration rates (Table 4). It can be observed that, under high-fat diet, the apo CIII transgenic and NonTg mitochondria present similar resting respiration rates that are higher than the NonTg mitochondria fed a low-fat diet. Thus, high-fat diet induced an increase in resting respiration in NonTg mitochondria but did not increase further the already higher mitochondrial resting respiration in apo CIII transgenic mice. Therefore, we can conclude that there are no differences in cell-energy dissipation between apo CIII and NonTg mice fed a high-fat diet.

We also measured core body (rectal) temperature in the four genotypic groups as an index of body metabolic rate (Figure 5). No statistical differences were observed among the groups, suggesting that the main differences observed in adipose tissue stores among these groups fed a high-fat diet are not related to differential body metabolic rates.

Discussion

In this study, we used transgenic mice overexpressing the human *apo CIII* gene, combined or not with the expression of the natural promoter-driven *CETP* gene, to test the hypothesis that both proteins would prevent diet-induced adipose tissue accumulation. Contrary to our hypothesis, the overexpression of apo CIII increased diet-induced visceral and subcutaneous adipose tissue stores. However, according to our hypothesis, the expression of CETP decreased the subcutaneous adipose tissue mass, visceral adipocyte size and plasma leptin levels of apo CIII mice fed a high-fat diet.

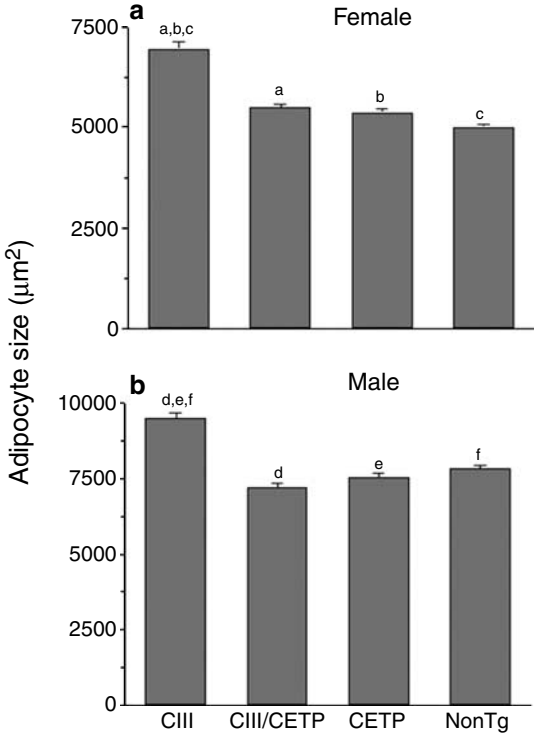

Figure 3 Adipocyte size (μm^2) of perigonadal adipose depot in female (**a**) and male (**b**) mice overexpressing apo-CIII and/or CETP and NonTg mice after 20 weeks on a high-fat diet. Mean ± s.e.m. 200–500 cells from six mice per group for females and 400–700 cells from six mice per group for males. The same letters indicate the pair comparisons that are significantly different: $P<0.001$ (analysis of variance).

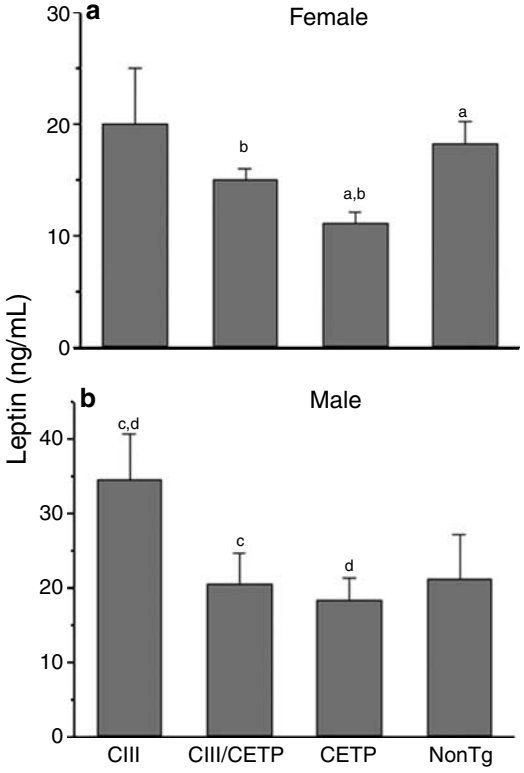

Figure 4 Nonfasting leptin plasma levels (ng/ml) in female (**a**) and male (**b**) mice overexpressing CIII and/or CETP and NonTg mice on a high-fat diet for 20 weeks. Mean ± s.e.m. ($n=6$). The same letters indicate the pair comparisons that are significantly different: $P<0.05$ (Student *t*-test).

We considered the possibility that high plasma concentrations of insulin, the most potent endogenous lipogenic hormone, could have been responsible for the differential size of the lipid depots in these mice. Along with others, we have demonstrated previously that apo CIII mice, under low-fat diet, have normal glucose and insulin tolerance[44,45] and normal glucose-stimulated insulin secretion by isolated pancreatic islet.[44] Here, we also report that under low-fat diet, they have adipose tissue stores similar to the controls. Since chronic consumption of high-fat diet could lead to insulin resistance and compensatory hyperinsulinemia could increase adipogenesis (for review see Biddinger and Kahn[46]), we measured glucose and insulin plasma levels in these mice fed a high-fat diet. Postprandial glucose levels were higher while insulin levels were lower in apo CIII compared with NonTg mice. Reduced glucose stimulated insulin secretion in these apo CIII mice was confirmed *in vitro* in isolated pancreatic islet. It is possible that an ectopic fat accumulation in β-cells could have affected insulin secretion. Yin *et al*.[47] reported that reduction of ectopic lipid deposition alleviated β-cell damage in miniature swine fed a high-fat/high-sucrose diet. Also important and well documented is the deleterious effect of long-term elevated plasma levels of FFAs on insulin secretion, which is present in the apo CIII-expressing groups as compared with CETP and NonTg mice (Table 1). Thus, both conditions, high-circulating FFA and a putative ectopic lipid deposition, may have contributed to lower insulin secretion in apo CIII-expressing mice.

One possibility to explain the concomitant larger adipose depots and low insulin levels in apo CIII mice is an increased adipocyte sensitivity to insulin. However, other insulin-independent mechanisms may be involved. Recently, Araujo *et al*.[48] have shown that decreasing insulin signal by using an insulin receptor substrate-1 antisense oligonucletide leads to insulin resistance and concomitant increase in epididymal fat weight through an activation of a growth-related pathway exclusively in white adipose tissue.

Similar core body temperature in all groups and similar mitochondrial respiration rates in CIII and NonTg mice fed a high-fat diet suggest that there are no differences in body energy expenditure rates among these mice. Although we cannot exclude a differential spontaneous physical activity pattern, these data suggest that the lipid availability to adipose storage is most probably implicated in the distinct pattern of adiposity found in CIII- and/or CETP-expressing mice.

Table 4 Effect of high-fat diet on the mitochondrial respiration rates in liver mitochondria isolated from apo CIII transgenic and NonTg mice

	Phosphorylating respiration (state III)	*Resting respiration (state IV)*	*Respiratory control (state III/IV)*
CIII (high-fat diet)	140.4±4.7	34.8±0.7[a]	4.0±0.08[d]
NonTg (high-fat diet)	138.5±2.6	33.9±1.2[b]	4.1±0.12[c]
NonTg (low-fat diet)	128.9±4.3	27.5±0.3[a,b]	4.6±0.10[c,d]

Abbreviations: apo, apolipoprotein; NonTg, nontransgenic. Mean±s.e.m. ($n=9$). Respiration rates given in nanoatoms oxygen/mg protein/min. The state III respiration was initiated with the addition of 200 nmol adenosine diphosphate (ADP)/mg protein. The same letters indicate the pair comparisons that are significantly different. [a,b,c,d]$P<0.05$ (analysis of variance).

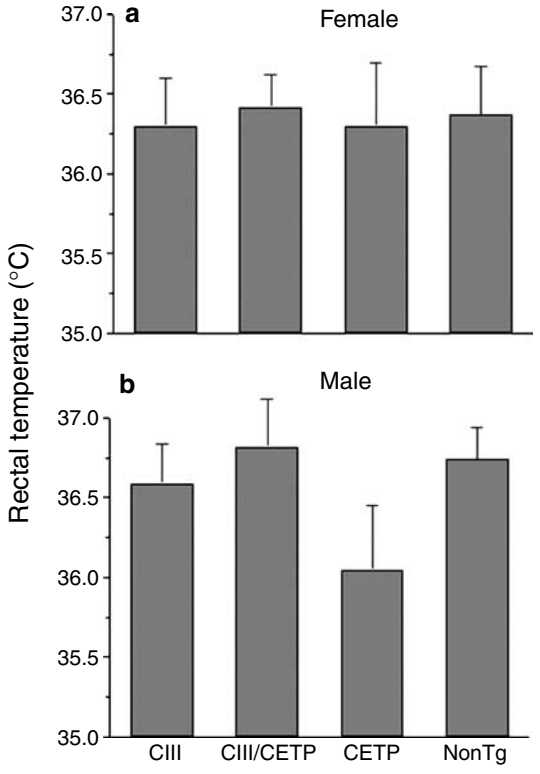

Figure 5 Rectal temperature (°C) in female (**a**) and male (**b**) mice overexpressing apo C-III and/or CETP and NonTg mice on a high-fat diet for 20 weeks. Mean±s.e. $n=7$–14 for female and $n=10$–14 for male mice.

The previously reported protection from obesity conferred by the apo C1 overexpression was related to a 50% decrease in fatty acid uptake by white adipose tissue stores.[36] On the other hand, lack of apo CIII significantly increased plasma TG lipolysis and fatty acid release to tissue uptake, leading to obesity.[35] We show here that the overexpression of the apo CIII does not protect but actually exacerbates diet-induced obesity. The increased plasma FFA concentrations observed in apo CIII mice fed a low-fat diet[8,44] suggested a saturated plasma FFA-removal mechanism in these mice. However, lack of further increase in TG and FFA plasma levels after 20 weeks of high-fat diet actually shows that these mice can handle the extra dietary fat supply, suggesting no saturation of plasma FFA tissue removal. Direct inhibitory effect of apo CIII on lipoprotein lipase (LPL) activity is still elusive. Although Ebara *et al*.[13] have shown an inhibitory effect of apo CIII mice plasma on the *in vitro* LPL activity, Aalto-Settala *et al*.[8] have shown that VLDL from apo CIII and NonTg mice are equally lipolyzed *in vitro* by purified LPL. The intravascular lower TG removal observed in apo CIII mice can be entirely explained by a decrease in the binding of apo CIII transgenic VLDL to a proteoglycan matrix model[16] or to glycosaminoglycan,[13] where LPL is anchored. This delays, however, does not necessarily inhibit VLDL–TG hydrolysis. The main reason these mice are hypertriglyceridemic is the decreased cellular uptake of the TG-rich lipoprotein remnants.[8,16] Hence, their prolonged residence time in plasma provides continuously more FFA to the peripheral tissues than if the remnant particles had been normally removed by liver. Thus, FFA availability to the adipose and other extrahepatic tissues is actually increased in these apo CIII mice fed a high-fat diet. Additionally, Conde-Knape *et al*.,[49] comparing the expression of apo CIII in apo B48-only or apo B100-only mice, found that apoB48 TG-rich lipoproteins were more sensitive to apo CIII-mediated inhibition of plasma clearance than apoB100 particles. Accordingly, the apoB48 particles were more resistant to TG-enriching effect of apo CIII, since they remained longer in plasma and were more lipolyzed. This reinforces the proposition that the apo CIII overexpression increases the FFA availability to the extrahepatic tissues, especially from intestinal TG-rich particles. Interestingly, the apo B48/CIII mice were also hyperglycemic as we report here for the apo CIII mice fed a high-fat diet.

The CETP-mediated lowering of adipose tissue effect may be related to several processes. First, as a result of CETP-mediated redistribution of TG in HDL particles, part of the FFA release is shifted to the liver since HDL–TG is a better substrate for hepatic lipase than for extrahepatic LPL. In this way, less FFA would be available to the white adipose tissue uptake in CETP-expressing mice. This FFA flow shift from periphery to the liver may not be relevant when TG levels are low, as in CETP transgenic and NonTg mice; however, this may be very important when TG levels are markedly elevated, as in apo CIII-expressing mice. Since there are no differences in liver fat content among all mice groups, the extra FFA taken up by the liver in CETP mice would have been metabolized or reutilized for VLDL secretion and recirculation. This could explain, at least in part, the

adiposity-reducing effects of the CETP expression in the apo CIII background.

Although the CETP transgenic line used in this work express very low levels of CETP in the adipose tissue,[21] other possibilities that can be raised are local actions mediated by circulating CETP in decreasing TG accumulation and/or increasing lipolysis in adipose cells. Lay *et al*.[50] have shown that enlarged adipocytes from several models of rodent obesity (Zucker rats, fat mice and ob/ob mice) have reduced membrane cholesterol concentrations in different fat stores, demonstrating that lower cholesterol is characteristic of adipocyte hypertrophy *per se*. More importantly, reducing the cholesterol content of adipocytes via different ways (statins or cyclodextrins) modulates the expression of genes involved in energy metabolism such as upregulation of fatty acid synthase (*FAS*) and glucose transporter-1 (*GLUT-1*) and downregulation of *GLUT-4* and uncoupling protein-3 (*UCP-3*). This provides evidence that cholesterol might be a link between fat-cell size and metabolic activity. Along with others, we have recently shown that CETP expression increases adipocyte uptake of exogenous cholesterol.[24,26] Thus, by increasing cholesterol content of adipocytes, CETP could indirectly contribute to decrease FAS and increase UCP-3 expression favoring a reduction in fat deposition in CETP-expressing mice.

Other strong evidence for CETP-mediated reduction in adipocyte size was recently provided by Zhou *et al*.,[33] using an adipose tissue-specific promoter (aP2)-driven CETP transgenic mice. These mice presented physiological plasma concentrations of CETP, smaller adipocytes and reduced mRNA expression of the adipogenic genes *LPL*, *PPARγ* and *SREBP-1c* compared with controls. Thus, two independent CETP-expressing mouse models show the same adiposity-reducing effect of CETP.

A few human studies have positively correlated circulating CETP levels and obesity.[51,52] However, hyperinsulinemia and/or insulin resistance are common confounding covariants in obese subjects. Recently, Teran-Garcia *et al*.[53] reported that *CETP* gene polymorphisms in normal-weight population contribute to differential changes in adiposity on excessive calorie consumption. Individuals with the V405V genotype had the highest percentage increase in visceral fat after overfeeding (130%). The 405V CETP allele has been consistently related to decreased CETP mass and activity.[54] Therefore, our results are in accordance with these human data and suggest that plasma CETP levels or gene polymorphism screening may be useful to identify individuals predisposed to develop diet-induced obesity.

Altogether, the results presented here showed that while elevation of plasma apo CIII levels aggravates diet-induced obesity, the expression of physiological levels of circulating CETP reverses this adipogenic effect, indicating a novel role for circulating CETP in modulating adiposity. Probable mechanisms seem to be related to the FFA availability to white adipose tissue uptake and to local expression of lipogenic genes.

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