Trans-10, *cis*-12- and *cis*-9, *trans*-11-Conjugated Linoleic Acid Isomers Selectively Modify HDL-Apolipoprotein Composition in Apolipoprotein E Knockout Mice[1]

José M. Arbonés-Mainar,* María A. Navarro,* Sergio Acín,* Mario A. Guzmán,* Carmen Arnal,† Joaquín C. Surra,* Ricardo Carnicer,* Helen M. Roche,** and Jesús Osada*[2]

*Departamento de Bioquímica y Biología Molecular y Celular and †Patología Animal, Facultad de Veterinaria, Universidad de Zaragoza, E-50013 Zaragoza, Spain and **Nutrigenomics Research Group, Institute of Molecular Medicine, Trinity College, Dublin, Ireland

ABSTRACT *Trans*-10, *cis*-12-conjugated linoleic acid (CLA)-enriched diets promote atherosclerosis in mice despite increasing blood concentrations of HDL cholesterol. This suggests that under these conditions, the HDL apolipoproteins (apo) produced are abnormal. To test this hypothesis, apoE-deficient mice were fed a Western-type diet enriched with linoleic acid (control), *cis*-9, *trans*-11-CLA or *trans*-10, *cis*-12-CLA (1.0% wt/wt) for 12 wk, and the effects on HDL metabolism and apoC-III levels recorded. Compared with the control and *cis*-9, *trans*-11-CLA mice, those fed the *trans*-10, *cis*-12-CLA diet had significantly higher HDL cholesterol concentrations, and had a higher incidence of hypertriglyceridemia and hepatic steatosis. Plasma apoA-I and paraoxonase concentrations were significantly lower in the *trans*-10, *cis*-12-CLA group than in the *cis*-9, *trans*-11-CLA group. These reductions were associated with decreased hepatic expression of these proteins and a shift toward lipid-poor apolipoprotein particles. The plasma apoA-II concentration increased with its corresponding mRNA concentration in the liver, and was preferentially bound to HDL in the *trans*-10, *cis*-12-CLA mice, thus explaining the increased HDL cholesterol concentrations in this group. Significant, positive associations were found between apoA-II and C-III ($r = 0.883$, $P < 0.001$) and between apoA-II and atherosclerosis ($r = 0.68$, $P < 0.001$). These results indicate that *trans*-10, *cis*-12-CLA intake modifies HDL to form a proatherogenic apoA-II containing particle and promotes phenotypic changes compatible with metabolic syndrome. *Cis*-9, *trans*-11-CLA does not promote this detrimental effect. J. Nutr. 136: 353–359, 2006.

KEY WORDS: • *conjugated linoleic acid* • *apolipoprotein* • *high density lipoproteins* • *atherosclerosis* • *paraoxonase*

Epidemiological studies show that an inverse relation exists between HDL cholesterol (HDL-C)[3] concentrations and the risk of coronary heart disease (CHD) (1). The results of clinical trials suggest that high HDL-C levels help prevent cardiovascular events (2). However, current knowledge is insufficient to warrant the setting of target levels (3).

The antiatherogenic properties of regular HDL particles have been ascribed to their anti-inflammatory, antioxidant and profibrinolytic activities, and to their ability to promote cholesterol efflux from cells in artery walls (4). HDL particles are a heterogeneous class of lipoproteins with subtypes identified on the basis of their density, electrophoretic mobility, particle size, and apolipoprotein composition (5). There is growing evidence to suggest that not all HDL particles share the same biological properties. This may be due to these molecules bonding with different apolipoproteins, or to enzymes and transfer proteins that modulate HDL particle composition, fate, and function. For example, HDL particles enriched in apolipoprotein (apo) A-I or A-IV are considered antiatherogenic (6,7), whereas HDL-apoA-II is associated with proatherogenic effects (8). Thus, the HDL-C concentration on its own may not adequately reflect the consequences of the presence of different lipoparticles.

The term "conjugated linoleic acid" (CLA) refers to a group of positional and geometric isomers of linoleic acid. The *cis*-9, *trans*-11-CLA (c9,t11-CLA) isomer is the most abundant in meat and dairy products (9), although the *trans*-10, *cis*-12-CLA (t10,c12-CLA) isomer appears in almost equal concentrations in most processed CLA-containing foods and supplements (10).

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[2] To whom correspondence should be addressed. E-mail: josada@unizar.es.

[3] Abbreviations used: apo, apolipoprotein; C, cholesterol; CHD, coronary heart disease, CLA, conjugated linoleic acid; *c9,t11*-CLA, *cis*-9, *trans*-11 CLA isomer; *t10,c12*-CLA, *trans*-10, *cis*-12 CLA isomer; FCHL, familial combined hyperlipidemia; FPLC, fast protein liquid chromatography; MS, metabolic syndrome; PON-1, paraoxonase 1; PPAR, peroxisome proliferator-activated receptors; TG, triglycerides.

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Multiple biological effects have been ascribed to CLA including the control of body composition and energy metabolism, the modulation of the immune response, lipid metabolism, and insulin resistance (11–16). Several authors reported that the different CLA isomers have different effects on lipid metabolism in animals and humans (17–19). Notably, *t*10,*c*12-CLA increases HDL-C concentrations but also promotes atherosclerosis (20,21), suggesting that the HDL molecules produced are not fully effective in preventing this disease.

The aim of the present work was to determine how the different CLA isomers modify the relations among HDL composition, hepatic lipid metabolism, and atherosclerotic lesions in apoE-deficient mice. These well-characterized mice spontaneously develop atherosclerosis with features similar to those observed in humans (22).

MATERIAL AND METHODS

Mice and diets. Homozygous apoE knockout male mice ($n = 29$; 3 mo old), bred at the Unidad Mixta de Investigación, Zaragoza, were randomly assigned to a control ($n = 10$), *c*9,*t*11-CLA–enriched ($n = 10$) or *t*10,*c*12-CLA–enriched ($n = 9$) diet. All mice had similar baseline plasma cholesterol concentrations. These mice were housed in sterile, filter-top cages (3–4/cage), consumed food and water ad libitum, and were kept under a 12-h light:dark cycle.

The mice were fed the different experimental semipurified diets for 12 wk. All three diets were isocaloric and isonitrogenous;[4] fat provided 30% of the energy intake. The control diet contained 1.0% (wt/wt) linoleic acid, whereas the others contained an equivalent amount of either *c*9,*t*11-CLA or *t*10,*c*12-CLA, as previously described (19,23). All diets were prepared by Unilever and stored in an N_2 atmosphere at −20°C. Fresh food was provided daily. The study protocol was approved by the Ethics Committee for Animal Research of the University of Zaragoza.

Biochemical determinations. At the end of the 12-wk period the mice were killed by suffocation with CO_2. Blood was drawn from the heart, and the livers of all mice were quickly frozen in liquid N_2. Plasma total triglyceride (TG) and cholesterol concentrations were determined by microtiter assay (Triglyceride Kit 2016647, Roche Diagnostics and Infinity Cholesterol Reagent Kit 401-25P, Sigma Chemical).

Paraoxonase was determined as aryl esterase activity, as previously described (24); the results were expressed as μmol/(min·L) of hydrolyzed phenyl acetate. ApoA-I, A-II, and C-III were quantified by ELISA using specific polyclonal antibodies (Biodesign) as previously described (25). All samples were analyzed on the same day; all assays were performed in triplicate. The intra-assay CV was <4% in all cases.

Histological analysis. The bases of the mouse hearts were collected and processed for aortic cross-sectional analysis as previously described (26). A sample of liver from each mouse was stored in neutral formaldehyde and embedded in paraffin wax. Sections (4 μm) were stained with hematoxylin and eosin and observed using a Nikon microscope.

Gel filtration chromatography. Fast protein liquid chromatography (FPLC) employing a Superose 6B column (Amersham Biosciences) was used to separate the lipoproteins as previously described (26). Fractions containing apoA-I were considered as representative of regular HDL, and the cholesterol content was estimated using the Amplex® Red A12216 cholesterol assay (Molecular Probes) following the manufacturer's instructions. Nonesterified cholesterol was determined using the same method but omitting the cholesterol esterase. The intensity of fluorescence was measured with a microplate reader (SPECTRAfluor Plus, TECAN) at 595 nm using an excitation wavelength of 550 nm. The results were linear from 38 nmol/L to 10 μmol/L cholesterol.

Western blotting. The plasma and FPLC fractions were loaded onto 12% SDS-PAGE gels, electrophoresed, and transferred as previously described (27). Protein bands were detected using rabbit polyclonal anti-mouse apoA-IV antibodies (Santa Cruz Biotechnology). Paraoxonase 1 (PON1) protein bands were detected using a rabbit polyclonal antibody raised against a mouse oligopeptide (CYKNHRSSY-QTRLNAFREVTP) following standard immunization protocols (28).

RNA preparation and analysis. Liver RNA was isolated using Trigent reagent MRC following the manufacturer's instructions. Northern blot analysis was performed as previously described (24). The mouse clones for *pon-1* (4158951 IMAGE Clone) and *apoa5* (4196296 IMAGE Clone) were obtained from MGC Geneservice. The probes used were as follows: *Pon-1* (24), *apoa1* (24), *apoa5*, a 1805-bp *EcorI/XbaI* fragment, *apoc3*, a 410-bp *XbaI/XhoI* corresponding to the fourth exon of *apoc3*, an *apoa4* probe, and a 321-bp *BamHI/XhoI* fragment corresponding to the third exon of *apoa4*. A mouse *β-actin* fragment (24) was used to normalize the amount of RNA. Labeling and quantification were performed as previously described (24).

Equal amounts of RNA from each mouse were used in quantitative real-time RT-PCR analyses. First-strand cDNA synthesis and the PCR reactions were performed using the SuperScript III Platinum Two-Step qRT-PCR Kit with SYBR Green (Invitrogen), according to the manufacturer's instructions. The following primers were used in real-time PCR: for *abca1*: sense, 5′-GGT TTG GAG ATG GTT ATA CAA TAG TTG T-3′, antisense 5′-TTC CCG GAA ACG CAA GTC-3′; for *apoa2*: sense, 5′-TGC TCG CAA TGG TCG CAC TG-3′, antisense 5′-TCT GAG GTC TTG GCC TTC TCC ACC-3′; for *endothelial lipase*: sense, 5′-TCC TGC ATA CCT ACA CGC TGT C-3′, antisense, 5′-GTC AAT GTG ACC CAC AGG CA-3′; for *hepatic lipase*: sense, 5′-TGA CCT GCA GAG CAT CGG CTT C-3′, antisense, 5′-TCT TGC CTG ACC GGT CCT TG-3′; for *sr-b1*: sense, 5′-GGC TGC TGT TTG CTG CG-3′, antisense, 5′-GCT GCT TGA TGA GGG AGG G-3′; and for *β-actin*: sense, 5′-CTG ACT GAC TAC CTC ATG AAG ATC CT-3′, antisense, 5′-CTT AAT GTC ACG CAC GAT TTC C-3′. Real-time PCR reactions were performed in an ABI PRISM 7700 Sequence Detector (Applied Biosystems). The relative amount of all mRNAs was calculated using the comparative $2^{-\Delta\Delta Ct}$ method. *β-Actin* mRNA was used as the invariant control.

Statistical analysis. The results are expressed as means ± SD. Comparisons were made using 1-way ANOVA and the Tukey-Kramer multiple comparison test (post hoc) when the distribution of the variables was normal. When the variables did not show such a distribution (according to the Shapiro-Wilks test) or failed to show homology of variance, comparisons were made using the Mann-Whitney U test. Correlations between variables were sought using the Pearson correlation test. All calculations were performed using SPSS version 11.0 software. Significance was set at $P \leq 0.05$.

RESULTS

Isomer-specific effects of CLA on the HDL-C concentration. FPLC fractions 16–21 corresponded to apoA-I–containing HDL (**Fig. 1**) and had cholesterol concentrations measurable with the colorimetric assay described (detection limit 100 μmol/L). These fractions were therefore understood to represent the regular HDL particle. Fractions 22–26 also contained apoA-I particles but had cholesterol concentrations of <100 μmol/L (recorded as lipid-free apoA-I). These could be determined only by the fluorescent assay (detection limit 0.013 μmol/L), but this again allowed these fractions to be considered to be representative of regular HDL. This regular HDL was then classified into 2 subclasses: cholesterol-rich HDL particles (containing cholesterol measurable by the standard colorimetric method) and cholesterol-poor HDL particles (with cholesterol concentrations below the detection limit of the standard colorimetric method [100 μmol/L]). Total, nonesterified, and esterified cholesterol concentrations (**Table 1**)

[4] The composition of the diet was (g/kg): cornstarch, 608; calcium caseinate, 158; fat, 123 (40% saturated, 33% monounsaturated and 27% polyunsaturated fatty acids); cellulose, 58.3; minerals, 40.7; vitamins, 11.7; and cholesterol, 1.5. The mineral and vitamin mixtures used were AIN-76 and AIN-76A respectively.

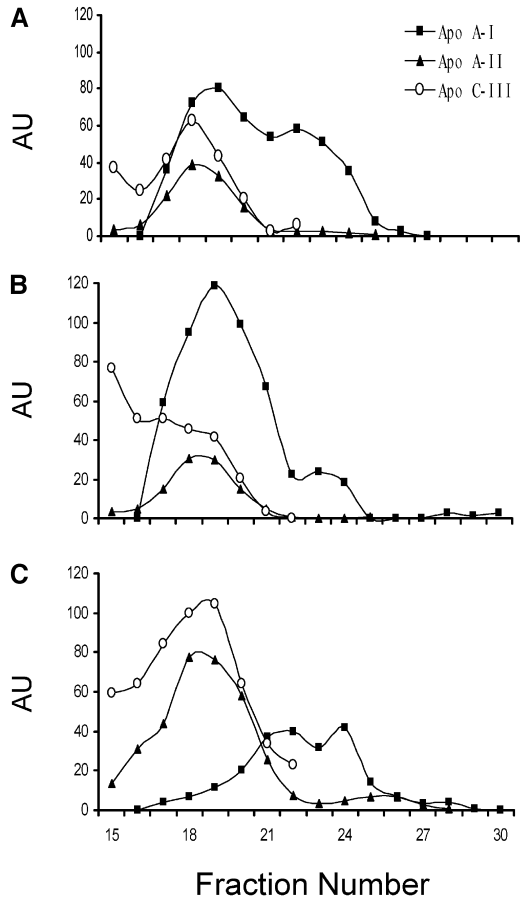

FIGURE 1 The effects of dietary *c*9,*t*11-CLA and *t*10,*c*12-CLA isomers on HDL apolipoprotein distribution in apoE-deficient mice. Lipoproteins were fractionated by FPLC (4 independent pools for each dietary condition). The apolipoprotein concentration of each fraction was determined in triplicate. The results are expressed in arbitrary units (AU) of absorbance per fraction. Representative patterns for the control (*A*), *c*9,*t*11-CLA (*B*) and *t*10,*c*12-CLA (*C*) diets are shown.

were significantly greater in both HDL subclasses in mice fed the *t*10,*c*12-CLA diet compared with the control and *c*9,*t*11-CLA groups. The total HDL-C concentration did not differ significantly between the *c*9,*t*11-CLA and control groups. The nonesterified and esterified cholesterol concentrations were significantly lower in both HDL subclasses in the *c*9,*t*11-CLA group compared with the control group.

Effects of CLA isomers on plasma apolipoprotein concentrations and apolipoprotein distribution across the HDL subclasses. Mice fed the *t*10,*c*12-CLA diet had significantly lower plasma apoA-I concentrations and greater apoA-II concentrations than the control and *c*9,*t*11-CLA mice (**Table 2**). Indeed, there was a significant inverse association ($r = -0.73$, $P < 0.001$) between apoA-I and apoA-II concentrations. Mice fed the CLA diets experienced no significant change in plasma apoA-IV levels compared with the controls.

The distribution of apoA-I, A-II, A-IV, and C-III among the HDL subclasses isolated by FPLC was quantified by immunoassay (Fig. 1). Compared with the control diet, the *c*9,*t*11-CLA diet was associated with more apoA-I and less apoA-II in the cholesterol-rich HDL subfractions 16–21. The cholesterol-poor HDL particles (fractions 22–26), in contrast, contained less apoA-I and were devoid of apoA-II. The superimposed apoA-I and apoA-II peaks did not change in size or uniformity compared with those obtained for control mice. These results

TABLE 1

Isomer-specific effects of the CLA-enriched diets on plasma total, nonesterified, and esterified cholesterol concentrations in the cholesterol-rich and cholesterol-poor HDL particles in apoE knockout mice[1]

	Control (n = 10)	*c*9,*t*11-CLA (n = 10)	*t*10,*c*12-CLA (n = 9)
		μmol/L	
Cholesterol-rich HDL fraction			
Total cholesterol[2]	320 ± 190[b]	210 ± 150[b]	570 ± 260[a]
Nonesterified cholesterol	116 ± 3[b]	94 ± 3[c]	187 ± 2[a]
Esterified cholesterol	204 ± 3[b]	116 ± 3[c]	383 ± 2[a]
Cholesterol-poor HDL fraction			
Total cholesterol[2]	24 ± 14[b]	14 ± 10[b]	112 ± 53[a]
Nonesterified cholesterol	15 ± 0.3[b]	12 ± 0.3[c]	71 ± 1.5[a]
Esterified cholesterol	9 ± 0.3[b]	2 ± 0.3[c]	41 ± 1.5[a]

[1] Values are means ± SD. Means in a row without a common letter differ, $P \leq 0.05$ (one-way ANOVA and post hoc Tukey-Kramer multiple comparison test).
[2] Mann-Whitney U-test.

suggest that the *c*9,*t*11-CLA diet led to an increase in high cholesterol HDL-A-I particles and a reduction in high cholesterol HDL-A-I and A-II particles.

Interestingly, the *t*10,*c*12-CLA diet was associated with a markedly different distribution of apoA-I and ApoA-II in the HDL subfractions compared with the control and *c*9,*t*10-CLA diets. ApoA-I was reduced, whereas the apoA-II concentration was increased in the cholesterol-rich HDL fractions 16–21. Further, the peaks of these apolipoproteins were no longer superimposed, indicating that the appearance of HDL-A-II was due to the *t*10,*c*12-CLA–enriched diet. In contrast, almost all the apoA-I was present in the cholesterol-poor HDL particles (fractions 22–26) in mice after consumption of the *t*10,*c*12-CLA diet. These results indicate that important remodeling of HDL particles occurs after the administration of the different CLA isomers.

Although apoC-III is not exclusively bound to HDL, its plasma concentration was determined to explain the variable TG concentrations after the administration of the different CLA isomers. The *t*10,*c*12-CLA diet induced hypertriglyceridemia (46.5 ± 6.0 compared with 17.8 ± 0.9 mmol/L for control group; $P < 0.05$). In contrast, the *c*9,*t*11-CLA diet reduced the TG concentration (13.3 ± 1.5 compared with 17.8 ± 0.9 mmol/L for the control group). Compared with the

TABLE 2

Isomer-specific effects of CLA-enriched diets on plasma apolipoprotein levels in apoE knockout mice[1]

	Control (n = 10)	*c*9,*t*11-CLA (n = 10)	*t*10,*c*12-CLA (n = 9)
		%	
Apo A-I	100 ± 14[a]	109 ± 11[a]	76 ± 13[b]
Apo A-II	100 ± 14[b]	69 ± 8[c]	139 ± 14[a]
Apo A-IV	100 ± 12[a]	98 ± 6[a]	109 ± 13[a]
Apo C-III	100 ± 9[b]	90 ± 8[c]	118 ± 9[a]

[1] Values are means ± SD. Means in a row without a common letter differ, $P \leq 0.05$ (one-way ANOVA and post hoc Tukey-Kramer multiple comparisons test).

controls, plasma apoC-III concentrations were significantly lower in the *c*9,*t*11-CLA group and significantly higher in the *t*10,*c*12-CLA group (Table 2). Indeed, plasma apoC-III concentrations correlated with plasma TG ($r = 0.722$, $P < 0.001$).

Surprisingly, there was a significant, positive association ($r = 0.883$, $P < 0.001$) between plasma apoA-II and apoC-III. To further explore this association, apoC-III was determined in the cholesterol-rich and cholesterol-poor HDL fractions. ApoC-III levels were increased in the high cholesterol HDL fraction in mice fed the *t*10,*c*12-CLA diet, reflecting the redistribution of HDL-apoA-II (Fig. 1C). HDL-apoA-IV composition was not altered by the dietary intervention (data not shown).

Paraoxonase concentrations. A progressive reduction in serum PON-1 levels occurred in mice after consumption of the *c*9,*t*11-CLA and *t*10,*c*12-CLA diets (**Fig. 2***A*). Similarly, the hepatic expression of *pon*-1 mRNA was significantly reduced by the *c*9,*t*11-CLA diet and to an even greater extent by the *t*10,*c*12-CLA diet (Fig. 2*B*). Despite these alterations, the aryl esterase activity of PON-1 was not affected (data not shown).

Hepatic gene expression. The *c*9,*t*11-CLA-fed mice had higher hepatic *apoa1*, *apoc3*, *apoa4*, and *apoa5* mRNA expressions than the controls (**Table 3**). In contrast, those receiving the *t*10,*c*12-CLA had significantly reduced hepatic *apoa1*, *apoc3*, and *apoa5* mRNA expressions. The levels of *apoa4* and *apoa2* mRNA were significantly increased in the *t*10,*c*12-CLA mice. The mRNA expression of the cholesterol transporter gene *abca1* was significantly reduced in mice fed either CLA diet. Neither diet had any effect on hepatic *sr-b1* receptor expression. Hepatic and endothelial lipase expression were not affected (data not shown).

Histological analysis. The control mice displayed macrovesicular steatosis restricted to the hepatocytes of liver zone 2, and microvesicular steatosis in hepatocytes outside this region (**Figs. 3***A* and *B*). The *c*9,*t*11-CLA diet substantially alleviated the fatty infiltrates (Figs. 3C and *D*), whereas the *t*10,*c*12-CLA diet dramatically worsened the liver's steatotic appearance (Figs. 3*E* and *F*).

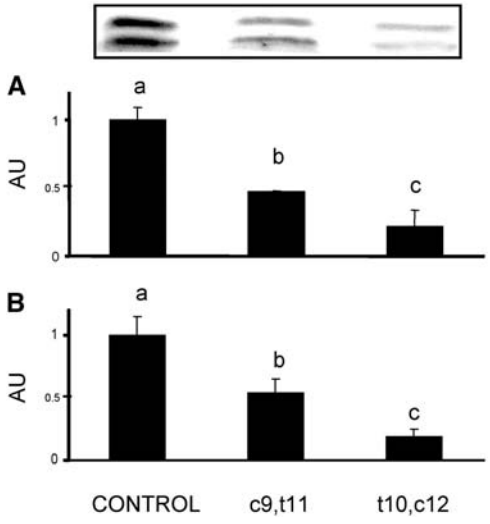

FIGURE 2 The effect of dietary *c*9,*t*11-CLA and *t*10,*c*12-CLA isomers on PON 1 regulation in apoE-deficient mice. (*A*) Quantification of plasma enzyme concentration by densitometry; *inset*: Western bands. (*B*) Hepatic mRNA expression of *pon1* gene. The results are expressed as arbitrary units (AU) and are means ± SD, $n = 9$–10. Means without a common letter differ, $P \leq 0.05$ (Mann-Whitney U-test).

TABLE 3

Modification of hepatic gene expression after the consumption of the c9,t11-CLA and t10,c12-CLA–enriched diets in apoE-deficient mice[1,2]

	Control ($n = 10$)	*c*9,*t*11-CLA ($n = 10$)	*t*10,*c*12-CLA ($n = 9$)
		AU	
apoa1	0.47 ± 0.04[b]	0.82 ± 0.06[a]	0.16 ± 0.06[c]
apoc3	0.33 ± 0.02[b]	0.65 ± 0.17[a]	0.23 ± 0.07[c]
apoa4	0.36 ± 0.002[c]	0.68 ± 0.11[b]	0.78 ± 0.21[a]
apoa5	0.57 ± 0.08[b]	0.72 ± 0.05[a]	0.29 ± 0.03[c]
apoa2	0.68 ± 0.16[b]	0.57 ± 0.09[b]	0.86 ± 0.10[a]
abca1	0.83 ± 0.14[a]	0.59 ± 0.05[b]	0.56 ± 0.05[b]

[1] Values are means ± SD. Means in a row without a common letter differ, $P \leq 0.05$ (Mann-Whitney U-test).

[2] The expressions of *apoa1, apoc3*, *apoa4* and *apoa5* were determined by Northern blotting; *apoa2* and *abca1* mRNAs levels were determined by qRT-PCR.

DISCUSSION

This work shows the opposite effects of the 2 dietary CLA isomers on high HDL-C and apolipoprotein composition in apoE-deficient mice. The *t*10,*c*12-CLA diet increased plasma HDL-C levels, but this was accompanied by a greater presence of atherosclerotic lesions (19). The increased HDL-C levels in these apoE knockout mice agrees with that seen in hamsters (20) and humans (21). These results indicate that compared with the amount of cholesterol transported by apoA-I and apoA-II (95% of the HDL protein mass) (5), apoE plays a minor role; its absence does not seem to influence the effect of the CLA isomers on HDL. The apparent conflicting effect of *t*10,*c*12-CLA on HDL-C levels and atherosclerosis may be explained by a divergent effect of the individual CLA isomers on HDL-apolipoprotein composition, which ultimately determines HDL function. Mice fed *t*10,*c*12-CLA had lower plasma apoA-I and higher apoA-II concentrations. The opposite was observed in mice fed the *c*9,*t*11-CLA diet. These effects of CLA on apoA-I are important because this apolipoprotein is largely responsible for the antiatherogenic effects of HDL (6). In addition, apoA-II may antagonize the action of apoA-I and thus impede cholesterol efflux from cells (29). Interestingly, transgenic mice overexpressing apoA-II have increased susceptibility to atherosclerosis, despite marked increases in their HDL-C levels (8,30,31). Therefore, increased apoA-II is associated with atherosclerosis (**Fig. 4**). In the present work, the *t*10,*c*12-CLA diet had a similar effect. If ApoA-II were absent, as in rabbits (32), no differences would be seen in the effects of the CLA isomers. This may explain the lack of any such difference in their influence on atherosclerosis in rabbits (33).

The distribution of the different apolipoproteins varied in the cholesterol-rich and cholesterol-poor HDL particles depending on the diet fed. Most apoA-I and apoA-II was bound preferentially to high-cholesterol HDL particles in the control and *c*9,*t*11-CLA groups. The latter diet favored the presence of HDL-A-I particles, which are considered antiatherogenic (34). However, the *t*10,*c*12-CLA group favored the appearance of HDL-A-II, and the HDL-A-I concentration was very low. The cholesterol-poor HDL contained mainly apoA-I, without apo A-II. The presence of cholesterol-rich HDL-AII particles in the plasma might explain the higher cholesterol concentrations in the *t*10,*c*12 mice (35,36). Pooling the data for all diets showed that plasma apoC-III levels were positively correlated with

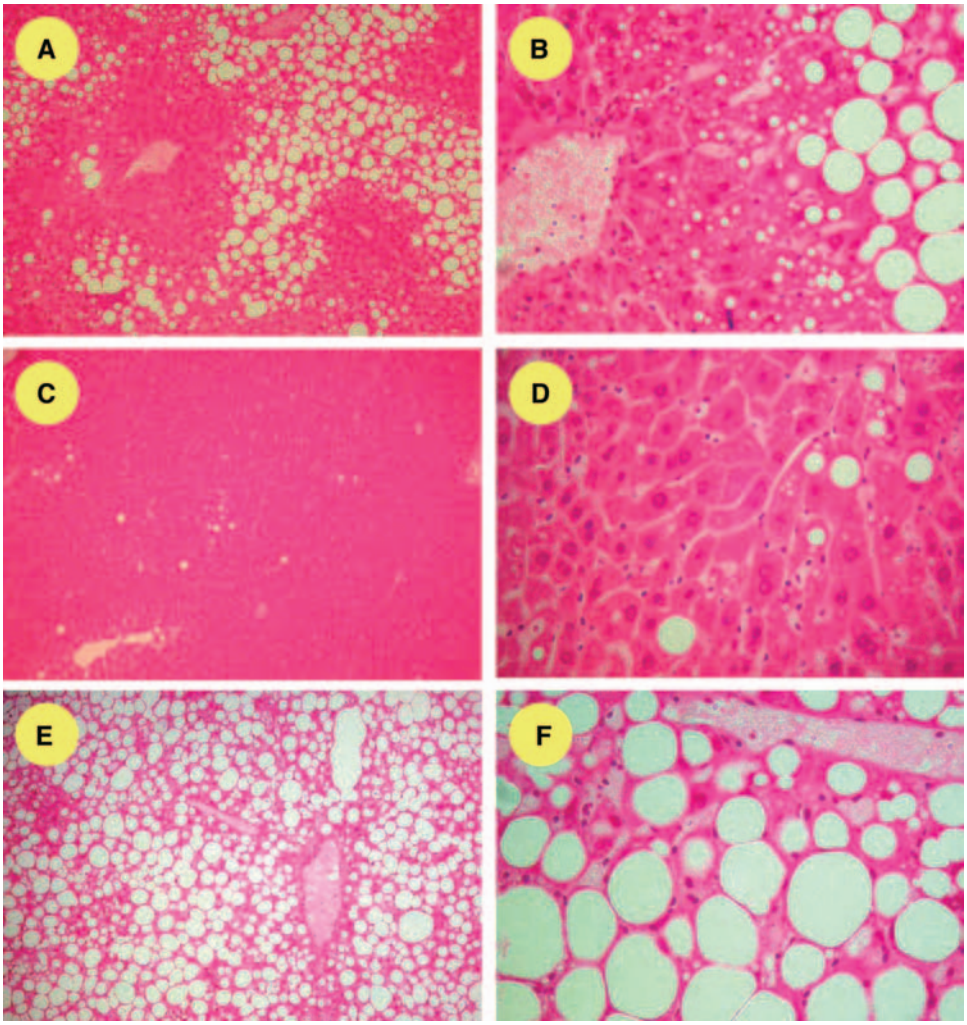

FIGURE 3 Representative liver micrographs from apoE-deficient mice consuming the control, *c*9,*t*11-CLA, and *t*10,*c*12-CLA-enriched diets at 2 different magnifications (*A*, *C*, *E*: X100; *B*, *D*, *F*: X400). Liver sections (4 μm) from all 29 mice were stained with hematoxylin and eosin and evaluated without knowledge of diet group. Control (*A*) and (*B*); *c*9, *t*11-CLA (*C*) and (*D*); and *t*10,*c*12-CLA (*E*) and (*F*).

apoA-II. The apoC-III, an inhibitor of lipoprotein lipase (37), appeared in the same FPLC fractions as apoA-II. These results indicate that in this model, the presence of apoA-I and A-II bound to HDL, plus the level of apoC-III, are important determinants in the relation between TG and HDL-C.

The *t*10,*c*12-CLA diet also reduced paraoxonase expression, which may be related to apoA-II overexpression (29). In the present study, the plasma PON-1 concentration and hepatic mRNA were reduced by both CLA diets, although more so by the *t*10,*c*12-CLA regimen. There were no differences in the aryl esterase activity of paraoxonase among any of the groups; however, the concentration of this enzyme was reduced in mice fed either of the CLA diets. The control mice had higher plasma PON-1 concentrations but with relatively reduced activity. This could be because linoleic acid, the main fatty acid of the control diet, is a potent destabilizing agent as well as a PON-1-arylesterase inhibitor ($K_i = 3.8$ mmol/L) (38). Because the final concentration of linoleic was ~12 μmol/L in the present assay, the possibility of enzyme inactivation seems the most plausible explanation for reduced activity in the control group. PON1 is differentially modulated by linoleic acid isomers at several levels. Thus, the CLA isomers caused a reduction in the protein mass brought about by variation in the liver mRNA levels. When linoleic acid was present, enzyme activity was substantially inactivated despite the fact that the enzyme concentration was twice that in the CLA-fed groups.

The *apoa1/c3/a4/a5* gene cluster is jointly organized in the genome (39) and regulates lipoprotein metabolism and atherosclerosis (40). The *c*9,*t*11-CLA–rich diet increased *apoa1*, *c3*, *a4*, and *a5* mRNA expression in the liver, but the *t*10,*c*12-CLA diet downregulated the expression of all of these genes with the exception of *apoa4*. Alterations in hepatic mRNA expression were partly reflected in plasma apolipoprotein levels, in particular those of apoA-I and apoA-IV. However, for C-III, there was an inverse correlation between hepatic expression and the plasma protein level, suggesting that it is regulated via another post-transcriptional CLA-mediated mechanism. Interestingly, a similar expression pattern was observed for *apoc3* and *apoa5*, despite their opposing roles in plasma TG homeostasis (41). This suggests that the expression of *apoa5* and *apoc3* is coordinated at the mRNA level. These findings, plus the absence of any change in lipase expression, suggest that the phenotypic variations in HDL produced by CLA are a consequence of alterations in the apolipoprotein gene cluster and *apoa2* gene expression, which ultimately affects lipid metabolism.

Other studies demonstrated that *t*10,*c*12-CLA is associated with hyperglycemia, hyperinsulinemia, and insulin resistance (23,42,43), reduced plasma leptin concentrations (42,43), and liver steatosis (43,44). In the present study, the mice fed *t*10,*c*12-CLA had hypertriglyceridemia, reduced apoA-I and increased apoA-II concentrations, significantly larger

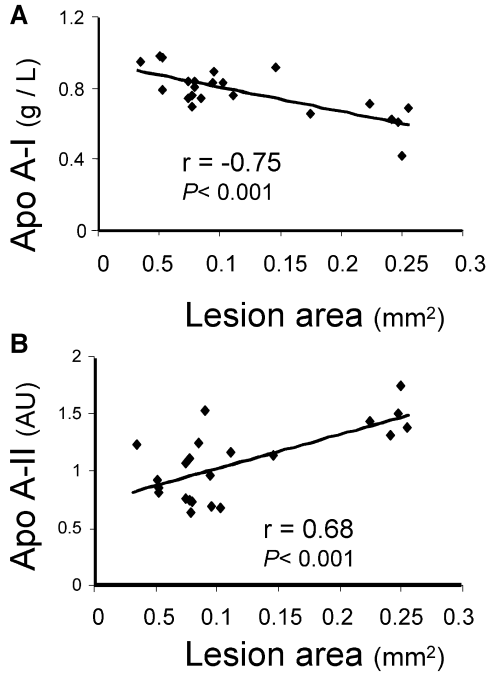

FIGURE 4 Correlations between aortic lesion areas and plasma apoA-I or A-II concentrations in apoE-deficient mice consuming control, *c*9,*t*11-CLA, and *t*10,*c*12-CLA-enriched diets. AU, arbitrary units.

atherosclerotic lesions (Fig. 4), and a redistribution of apolipoproteins among the different HDL particles. This phenotype is similar to that of familial combined hyperlipidemia (FCHL) or metabolic syndrome (MS) (45). All of these traits of FCHL were described and characterized in apoA-II transgenic mice (30,46). The present results provide an unexplored mechanism that explains the link between hypertriglyceridemia and increased apoA-II levels via the existence of an increased presence of apoC-III in the same HDL particles. Further, these apolipoprotein changes can be explained by expression changes at the *apoa1/c3/a4/a5* gene cluster and *apoa2* gene, polymorphic variations that have been associated with FCHL (30). Together, these finding suggest that the administration of the *t*10,*c*12-CLA isomer to apoE-deficient mice provides an excellent FCHL and MS model for studying the relation between atherosclerosis, hepatic steatosis, and HDL apolipoprotein metabolism.

The dietary interventions had markedly different effects on the lipid content of the liver. The effects of CLA may be explained through interactions with a number of transcription factors, particularly the peroxisome proliferator-activated receptors (PPAR). Indeed both *c*9,*t*11-CLA and *t*10,*c*12-CLA are PPARα and PPARγ ligands (42,47). PPARα activates lipolytic pathways and apolipoprotein production, ultimately reducing the fat content of the liver (48). Thus, *c*9,*t*11-CLA could promote PPARα activation, leading to reduced liver fat (Fig. 3) and increased *apoa4* and *a5* mRNA levels (Table 3). However, this does not explain the changes in *apoa1, apoa2, c3* or *abca1* mRNA levels, which are regulated by Foxo1 (49) or hepatic nuclear factor-4α (50). The *t*10,*c*12-CLA isomer promoted profound hepatic steatosis, an effect reported in other studies (42–44), reinforcing the notion that the absence of apoE does not modify the response to this isomer. This steatotic effect was attributed to increased hepatic TG, cholesterol, cholesterol esters, and free fatty acids (51), reflecting increased hepatic fatty acid synthesis (52). However, the mechanisms by which the liver becomes steatotic in response to *t*10,*c*12-CLA appear to be complex because *t*10,*c*12-CLA also induces β-oxidation (53); this would not be associated with lipid accumulation. Indeed, the reduced *apoc3* and *apoa1* and increased *apoa2 and apoa4* mRNA levels in this study (Table 3) suggest the involvement of PPARα, but this does not explain the changes in *apoa5* or *abca1* or fat accumulation (Fig. 3). The hepatic expression of *abca1* was reduced with both CLA isomers, concordant with the liver X receptor-α downregulation promoted by these isomers (23). Despite reduced hepatic *abca1* mRNA expression, plasma cholesterol levels were modified in a CLA isomer–specific manner, suggesting that CLA also regulates phenotypic expression of this transporter at different levels, and that HDL-C levels are the result the action of many genes. Overall, these data indicate that for each gene involved, multiple transcription factors are required.

In conclusion, the present work shows that apoE-deficient mice fed *t*10,*c*12-CLA displayed characteristics of FCHL or MS. It would appear that simply measuring HDL-C levels is insufficient for determining the risk of cardiovascular disease due to HDL apolipoprotein changes. The results also suggest that diets rich in *t*10,*c*12-CLA may be proatherogenic, whereas those rich in *c*9,*t*11-CLA may be antiatherogenic.

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