

## Synthesis and Secretion of ApoC-I and ApoE during Maturation of Human SW872 Liposarcoma Cells<sup>1</sup>

Hanny Wassef, Lise Bernier, Jean Davignon, and Jeffrey S. Cohn<sup>2</sup>

*Hyperlipidemia and Atherosclerosis Research Group, Clinical Research Institute of Montréal, Montréal, QC, Canada*

**ABSTRACT** Little is known about the regulation of apolipoprotein (apo) C-I production by human adipocytes. The aim of the present study, therefore, was to investigate the effect of different tissue culture conditions on the synthesis and secretion of apoC-I and apoE in human SW872 liposarcoma cells. After 3–4 d in culture ( $0.5 \times 10^6$  cells/well, DMEM/F-12 medium with 10% fetal calf serum), cells reached confluence and became growth arrested. The molar ratio of apoE:apoC-I in the cell was  $8.9 \pm 0.6$  and in the medium was  $6.6 \pm 0.5$ . After 17 d in culture, SW872 cells contained significantly more cholesterol (100%) and triglyceride (3-fold) and secreted more apoC-I [4 vs. 17 d:  $0.11 \pm 0.01$  vs.  $0.23 \pm 0.01$  pmol/( $10^6$  cells  $\cdot$  24 h),  $P < 0.001$ ] and apoE [ $0.7 \pm 0.1$  vs.  $3.1 \pm 0.3$  pmol/( $10^6$  cells  $\cdot$  24 h),  $P < 0.001$ ]. Cellular apoC-I increased 7-fold and apoE increased 16-fold. Cell maturation was associated with significantly higher levels of apoE mRNA but not apoC-I mRNA. Increases in cell lipids, apoC-I, and apoE were not dependent on the presence of extracellular lipids because similar changes occurred in cells incubated with lipoprotein-deficient serum or in cells incubated without serum. Treatment (7 d) of cells during maturation with insulin (10 or 1000 nmol/L) significantly reduced the secretion of apoC-I and apoE. These results demonstrate that in maturing SW872 cells, cholesterol and triglyceride accumulation in the presence or absence of extracellular lipids, is associated with increased apoC-I and apoE production. Furthermore, apoC-I and apoE production are differentially regulated at the transcriptional level, and long-term treatment with insulin has an inhibitory rather than stimulatory effect on apoC-I and apoE production. *J. Nutr.* 134: 2935–2941, 2004.

**KEY WORDS:** • *adipose tissue* • *atherosclerosis* • *cholesterol* • *insulin* • *obesity*

Adipose tissue is not simply a depot of fat, but is an endocrine organ capable of secreting proteins that can affect whole-body metabolism and homeostasis (1). Several of these proteins [e.g., adiponectin, leptin, plasminogen activator inhibitor-1, apolipoprotein (apo)<sup>3</sup> E and apoC-I] have been implicated in the etiology of insulin resistance and atherosclerosis, and may be responsible for the direct link between increased adiposity and cardiovascular disease (2,3).

ApoE and apoC-I are proteins that play a central role in regulating plasma lipid metabolism (4,5). ApoE mediates the uptake of triglyceride-rich lipoproteins (TRL) by hepatic receptors (6), whereas apoC-I has an inhibitory effect (7). The genes for these proteins are adjacent to each other on chromosome 19 within a 45-kb cluster containing the pseudo apoC-I, apoC-IV, and apoC-II genes (8). The majority of plasma apoE and apoC-I is made by the liver. However, a

number of tissues are able to produce these proteins, e.g., the brain, spleen, lung, adrenals, ovary, kidney, and muscle (9–11). Adipose tissue also has the capacity to synthesize apoE and apoC-I. Zechner et al. (12) showed that differentiated mouse 3T3-L1 adipocytes and human adipose tissue biopsies contain apoE mRNA, and they both synthesize and secrete apoE. In 3T3-L1 cells, apoE is expressed in a differentiation-dependent manner; its regulation is related to intracellular free cholesterol levels. Lafitte et al. (13) demonstrated that the nuclear receptors, liver X receptor (LXR) $\alpha$  and LXR $\beta$ , and their oxysterol ligands are key regulators of adipocyte apoE expression through their interaction with conserved LXR response elements present in multi-enhancer regions ME.1 and ME.2 of the apoE/CI gene cluster.

ApoC-I is a 57-amino acid protein (6.613 kDa) which, like apoE, regulates both systemic and cellular lipid metabolism (5,7). In vitro experiments demonstrated that apoC-I has the capacity to activate lecithin:cholesterol acyltransferase (LCAT), inhibit lipoprotein lipase, hepatic lipase, and phospholipase A<sub>2</sub>, and inhibit cholesterol ester transfer protein (CETP) activity. ApoC-I also inhibits the binding and/or uptake of triglyceride emulsions or VLDL by the LDL receptor, the LDL-like receptor protein (LRP) and the VLDL receptor (5,7). In mice, overexpression of human apoC-I leads to elevated plasma cholesterol and triglyceride concentrations, impaired hepatic uptake of VLDL particles, reduced whole-body

<sup>1</sup> Supported by an operating grant from the Canadian Institutes of Health Research (MOP-14684) and a CIHR/Pfizer Rx&D Investigator Salary Award to J.S.C. (DSC-64146). The financial support of Pfizer Canada to J.D. is also gratefully acknowledged.

<sup>2</sup> To whom correspondence should be addressed. E-mail: cohnj@ircm.qc.ca.

<sup>3</sup> Abbreviations used: apo, apolipoprotein; CETP, cholesterol ester transfer protein; FCS, fetal calf serum; LCAT, lecithin cholesterol acyltransferase; LPDS, lipoprotein-deficient serum; LPL, lipoprotein lipase; LRP, LDL-like receptor protein; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptor; TRL, triglyceride-rich lipoprotein(s).

nonesterified fatty acid uptake, and impaired uptake of fatty acids by adipose tissue (14–17).

In view of the aforementioned evidence that apoC-I plays an important role in regulating fatty acid uptake by adipose tissue, and given the lack of information pertaining to apoC-I production by adipocytes, we conducted the present study in which we investigated the effect of different tissue culture conditions on the synthesis and secretion of apoC-I and apoE by SW872 liposarcoma cells. The human liposarcoma SW872 cell line was used in previous studies as a human adipocyte cell model (18–20). Compared with mouse 3T3-L1 adipocytes, SW872 cells have the advantage of being of human origin and of not requiring an incubation cocktail (e.g., dexamethasone, insulin, and isobutylmethylxanthine) to differentiate into mature adipocytes. SW872 cells, when initially plated, have an immature adipocyte phenotype, and they constitutively express important adipocyte genes such as peroxisome proliferator-activated receptor (PPAR) $\alpha$ , PPAR $\gamma$ , LRP, lipoprotein lipase (LPL), CETP, CD36, and adipsin (20).

## MATERIALS AND METHODS

**Materials.** Human SW872 liposarcoma cells were obtained from the American Type Culture Collection. High-glucose DMEM was purchased from GIBCO. Fetal calf serum (FCS), dexamethasone, and bovine insulin were purchased from Sigma (St-Louis, MO). Bovine lipoprotein-deficient serum (LPDS) was obtained by ultracentrifugation at density 1.25 kg/L using solid KBr. LPDS was dialyzed against physiological saline (0.15 mol/L) and stored at 4°C.

**Cell culture.** SW872 cells were cultured in high-glucose DMEM supplemented with NaHCO<sub>3</sub> (3.7 g/L), 100  $\mu$ mol/L nonessential amino acids, 50 kU/L penicillin, 50 mg/L streptomycin, and 10% FBS, in a humidified incubator (37°C, 5% CO<sub>2</sub>). Media were replaced every 2–3 d. Cells (0.5  $\times$  10<sup>6</sup>) were seeded into 6-well plates containing 2 mL of medium. Maturation of SW872 cells was studied by allowing cells to grow for a maximum of 19 d. Media were changed every 48 h and analyses were conducted at 4, 10, and 17 d. Cells reached 100% confluence after 4 d. At each time point, media were removed and cells were washed twice with PBS before further manipulation. Cell maturation was studied in the absence of lipoproteins by incubating cells with 10% LPDS (100  $\mu$ mol/L nonessential amino acids, 50 kU/L penicillin, 50 mg/L streptomycin). Cells were also studied in the absence of serum, whereby the medium was supplemented with a growth-promoting cocktail containing 15 mmol/L NaHCO<sub>3</sub>, 15 mmol/L HEPES, 33  $\mu$ mol/L biotin, 17  $\mu$ mol/L pantothenate, 0.2 nmol/L triiodothyronine, 1.25  $\mu$ mol/L bovine insulin, 0.1 mmol/L NaOH, 0.1  $\mu$ mol/L dexamethasone, 100  $\mu$ mol/L nonessential amino acids, 50 kU/L penicillin and 50 mg/L streptomycin (individual components from Sigma Chemical). To study the effect of insulin, cells were grown for 7 d postconfluence in FCS-containing medium, washed with PBS, and incubated with either 0, 10, or 1000 nmol/L bovine insulin-supplemented medium (10% FCS) for a subsequent 7 d.

**Cells analysis.** Cells were washed with PBS, and total cellular lipids were extracted using three 1-h incubations with 1.0 mL hexane/isopropanol (3:2, v:v). Solvent extracts were centrifuged (10 min at 2000  $\times$  g), and supernatants were transferred and dried under nitrogen. Total cholesterol and triglyceride were quantified by enzymatic reaction using reagents from Roche Diagnostics (Indianapolis, IN). Cellular lipid accumulation was also monitored microscopically after lipid staining with Oil Red O (21). Media were collected and apolipoproteins were quantified by a sandwich ELISA (22,23) using goat anti-human polyclonal antibodies obtained from Biodesign International and horseradish peroxidase-conjugated goat anti-human antibodies obtained from Academy Bio-Medical Company. ELISAs were specific for human apolipoproteins, and zero values were obtained when fresh media containing bovine serum were assayed. ApoC-I and apoE detected in cell media therefore represented a measure of secreted apolipoprotein. Re-uptake of human apolipoproteins was negligible because the media contained an excess of bovine apoC-I

and apoE provided by the FCS. To quantify cellular apolipoproteins, SW872 cells were washed with PBS, harvested, and dissolved in a lysis buffer (50 mmol/L TRIS-Base, 150 mmol/L KCl and 1% Tween). Cellular apolipoproteins were then quantified by ELISA. To measure total cell proteins, cells were washed with PBS and solubilized using 0.1 mol/L NaOH. Total cell proteins were quantified according to Lowry et al. (24), using bovine serum albumin as a standard. RNA was extracted according to the manufacturer's instructions with Trizol obtained from Invitrogen and then quantified using a spectrophotometer. A reverse transcriptase reaction was then carried out and the resultant cDNA was amplified using real-time PCR with the SYBR green mastermix purchased from Qiagen and analyzed with Mx4000 software. S14 (used as a standard), apoC-I, apoE, PPAR $\alpha$ , PPAR $\beta$ , and PPAR $\gamma$  primers were designed using the Primer 3 software (25) and were made by Invitrogen.

**Statistics.** Values in the text are means  $\pm$  SEM. One-way ANOVA was performed using SigmaStat 2.0 (Jandel). Data that were not normally distributed were analyzed nonparametrically using Kruskal-Wallis 1-way ANOVA on ranks. Post-hoc multiple comparison tests included Tukey's test (parametric) or Dunn's method (nonparametric). Differences were considered significant at  $P < 0.05$ .

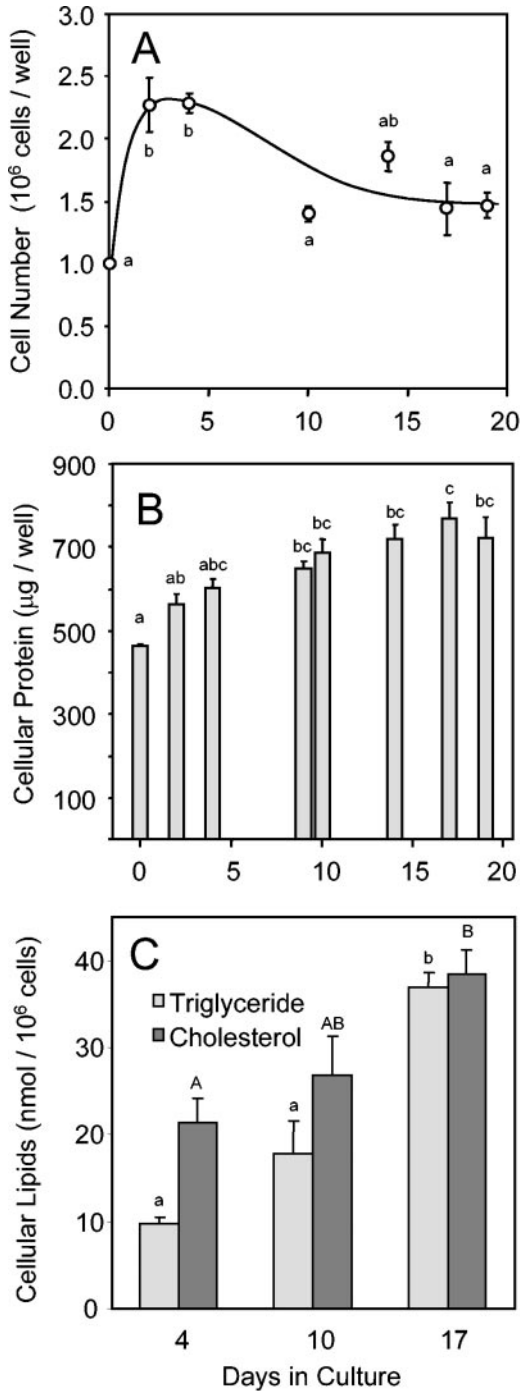
## RESULTS

Human SW872 cells were cultured in the presence of 10% FCS, and cell maturation was monitored for 17 d. When first plated, the cells were elongated and fibroblastic in appearance. After several days in culture, they became more rounded. At 100% confluence, they became growth arrested, as evidenced by a plateau (3–4 d after plating) in the number of cells per dish (Fig. 1A). Cellular protein increased gradually with time (Fig. 1B). Cells became vacuolized and Oil Red O staining provided evidence of lipid accumulation (data not shown). Seventeen days after plating, cells contained  $\sim$ 100% more cholesterol and 3 times more triglyceride than when they reached confluence at d 4 (Fig. 1C).

Cell maturation was associated with a significant increase in cellular levels of PPAR $\beta/\delta$  mRNA (Fig. 2A). Neither PPAR $\alpha$  nor PPAR $\gamma$  mRNA levels increased significantly with time, although PPAR $\gamma$  mRNA levels were already elevated in confluent cells at 4 d. This was consistent with the fact that SW872 cells resemble differentiated adipocytes and express other functionally important adipocyte genes including LRP, LPL, CETP, and CD36 (20). At d 17, SW872 cells had significantly higher LRP mRNA levels (0.46  $\pm$  0.05 vs. 0.19  $\pm$  0.03,  $P < 0.001$ ) and higher LPL mRNA levels (0.05  $\pm$  0.01 vs. 0.04  $\pm$  0.01,  $P < 0.01$ ) compared with d 4. Cellular levels of apoE mRNA increased significantly during cell maturation; however, levels of apoC-I mRNA decreased significantly (Fig. 2B).

Levels of both apoC-I and apoE were higher in the medium than in cells. Both cellular and medium levels of apoC-I (Fig. 3A) and apoE (Fig. 3B) increased significantly during cell maturation. From d 4 to 17, cellular apoC-I and apoE increased 7- and 16-fold, whereas medium apoC-I and apoE increased 2- and 4-fold, respectively. At d 4, the molar ratio of apoE:apoC-I in the cell was 8.9  $\pm$  0.6 and in the medium, 6.6  $\pm$  0.5. At d 17, the molar ratio of apoE:apoC-I in the cell was 21.6  $\pm$  2.6 ( $P < 0.001$ ), and in the medium, 13.3  $\pm$  1.6 ( $P < 0.001$ ). SW872 cells therefore contained and secreted more apoE than apoC-I. SW872 cells secreted 6–7 molecules of apoE for each molecule of apoC-I at 100% confluence (i.e., d 4) and secreted 13–14 molecules of apoE for each molecule of apoC-I after 17 d in culture.

To determine the extent to which lipoproteins in the medium were able to affect the production of apoC-I and apoE during maturation of SW872 cells, experiments were carried out in which cells were grown in the presence of 10% FCS or

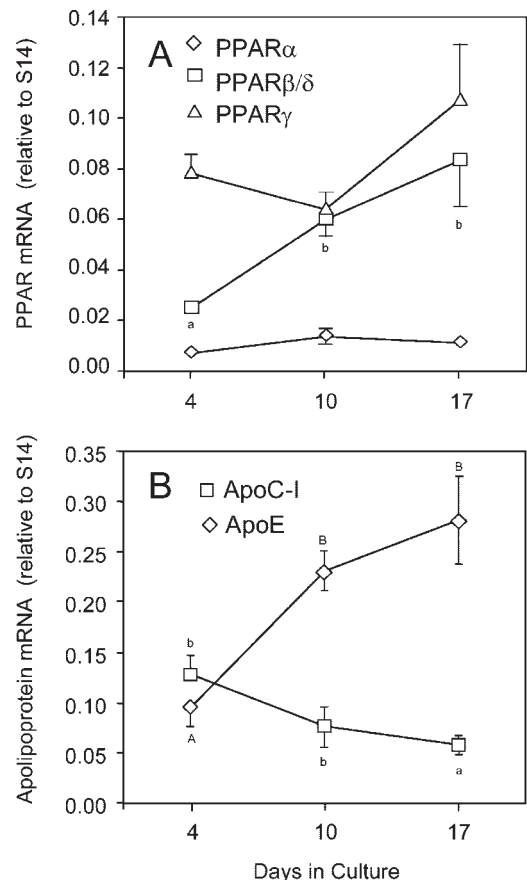


**FIGURE 1** Number of cells per dish at different time-points (*panel A*), cellular protein/dish (*panel B*), and cellular lipid (*panel C*) during the time course of maturation of SW872 liposarcoma cells. At d 0, cells were plated into 6-well tissue culture plates ( $0.5 \times 10^6$  cells / well) and allowed to grow in the presence of DMEM/F-12 (3:1, v:v) and 10% FCS. The medium was changed every 48 h. Cells reached confluence after 3–4 d in culture and became growth arrested. Values are means  $\pm$  SEM,  $n = 3$  (*A* and *B*) or 5 (*C*). Means for a variable without a common letter differ ( $P < 0.05$ ), by one-way ANOVA and the post-hoc Tukey's test.

in the presence of 10% FCS devoid of lipoproteins (i.e., in 10% lipoprotein-deficient serum, LPDS). As demonstrated before (Fig. 1–3), maturation in the presence of 10% FCS was associated with an increase in cellular lipids (Fig. 4A), an

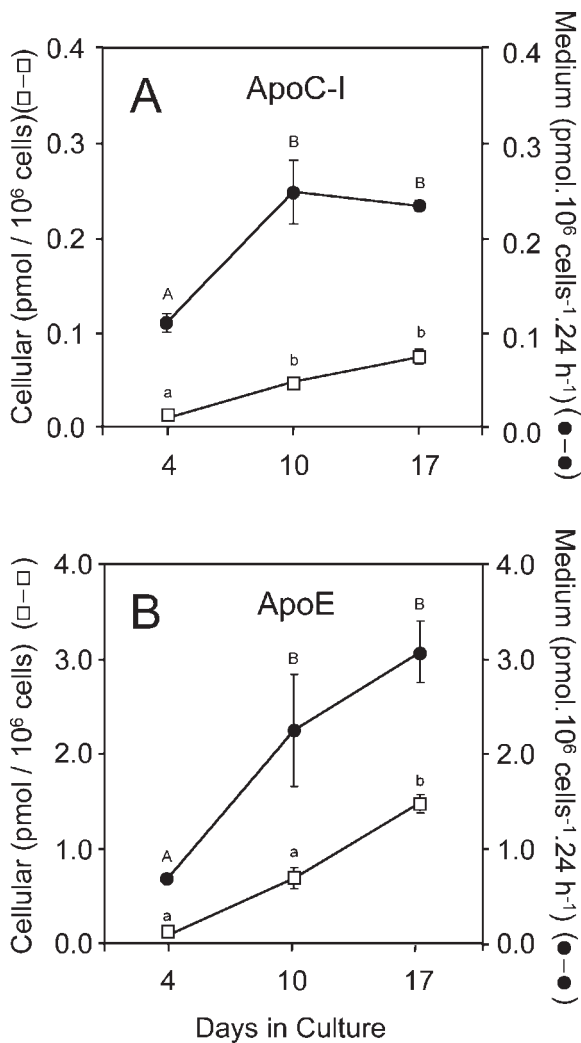
increase in apoE mRNA but not apoC-I mRNA (Fig. 4B), and significant increases in both cellular and medium apoC-I (Fig. 4C) and apoE levels (Fig. 4D). Cellular triglyceride and apoE mRNA levels were lower in LPDS-incubated cells; however, this was not associated with any reduction in levels of apoC-I or apoE compared with FCS-incubated cells.

In view of the small effect of the absence of lipoprotein lipids in the medium on apoC-I and apoE production during SW872 maturation, an experiment was carried out with serum-free medium containing a cocktail of compounds (i.e., insulin, dexamethasone, triiodothyronine, biotin, and pantothenate) designed to promote the maturation of SW872 cells and also the production of apoC-I and apoE. The cells accumulated both triglyceride and cholesterol (Fig. 5A). At 100% confluence (d 4), they contained levels of triglyceride and cholesterol similar to those of SW872 cells incubated in medium containing 10% FCS (Fig. 1C). At d 10 and 17, however, they contained 4 and 7 times more triglyceride, respectively, and also slightly more cholesterol than cells in standard medium. The lipid accumulation was associated with an increase in apoE mRNA, although not apoC-I mRNA (Fig. 5B). The increase in cellular apoE mRNA with growth-promoting medium was  $\sim 3$  times greater than that occurring with standard medium (Fig. 5B). Cellular and medium apoC-I and apoE increased significantly (Fig. 5C and D), and this increase was



**FIGURE 2** Change in cellular PPAR (*panel A*) and apolipoprotein (*panel B*) mRNA levels during maturation of SW872 cells over 4, 10, or 17 d. Levels of mRNA were quantitated by real-time RT-PCR and were expressed relative to S14 mRNA. Results are means  $\pm$  SEM,  $n = 6$ . Means for a variable without a common letter differ ( $P < 0.05$ ), by one-way ANOVA and the post-hoc Dunn's test.





**FIGURE 3** Effect of SW872 cell maturation on cellular and medium apoC-I (panel A) and apoE levels (panel B). Apolipoproteins were measured by ELISA in cells dissolved in lysis buffer or in medium recovered from cells after 48 h of incubation. Results are means  $\pm$  SEM,  $n = 6$ . Means for a variable without a common letter differ ( $P < 0.05$ ), by one-way ANOVA and the post-hoc Dunn's test.

also greater in magnitude than that observed in the presence of FCS-containing medium (Fig. 3A and B).

Because the maturation-promoting medium (described above) contained a large amount of insulin, experiments were carried out to investigate the effect of insulin alone on SW872 apoC-I and apoE production (Fig. 6). Insulin at concentrations of 10 and 1000 nmol/L in medium containing 10% FCS did not affect apoE mRNA, but the higher concentration reduced apoC-I mRNA levels (Fig. 6A). This was associated with a significant decrease in medium apoC-I and apoE (Fig. 6B and C), with no difference in cellular apoC-I and a small 20% decrease in cellular apoE in incubations with 1000 nmol/L insulin (Fig. 6B).

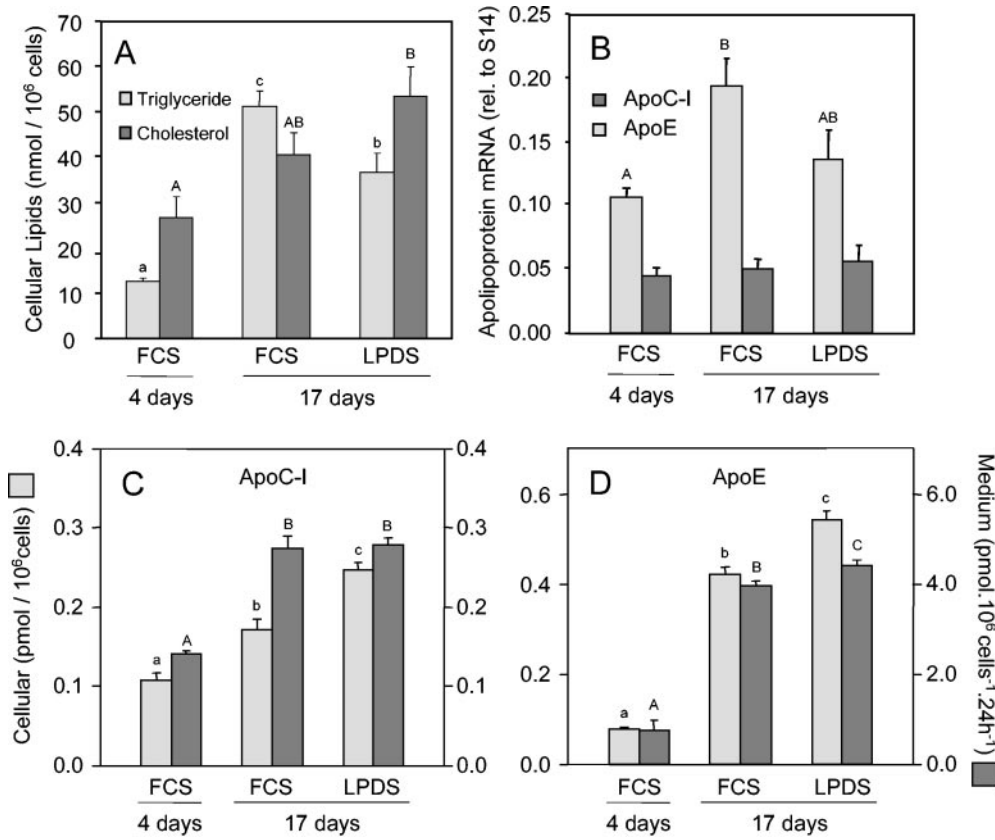
## DISCUSSION

The results of the present study showed that both apoC-I and apoE are synthesized and secreted by SW872 cells. At confluence, after 3 to 4 d in culture, cells contained  $\sim 1$  molecule of apoC-I for every 9 molecules of apoE, and the

medium contained  $\sim 1$  molecule of apoC-I for every 7 molecules of apoE. When SW872 cells were allowed to mature for 17 d in culture in the presence of 10% FCS, cellular lipid accumulation (100% increase in cholesterol, 3-fold increase in triglyceride) was associated with a significant increase in both cellular and medium levels of apoC-I and apoE. Increase in the synthesis and secretion of apoE was greater than that of apoC-I (Fig. 3) and after 17 d in culture, the molar ratio of apoE:apoC-I in the cell was  $21.6 \pm 2.6$  and in the medium was  $13.3 \pm 1.6$ . Similar changes were observed when cells were incubated with lipoprotein-deficient serum (Fig. 4) or when cells were incubated without serum (Fig. 5), demonstrating that increased production of both apoC-I and apoE was closely linked to the accumulation of cellular cholesterol and triglyceride, independent of the presence or absence of lipid in the medium.

Zechner et al. (12) showed previously that differentiation of mouse 3T3-L1 preadipocytes into mature adipocytes results in increased production of apoE. More recently, gene chip experiments showed that apoC-I expression is also significantly increased during adipocyte differentiation (26,27). The present results extend these observations by showing that human SW872 cells, which already display a differentiated adipocytic phenotype, i.e., they express measurable amounts of PPAR $\gamma$  mRNA (Fig. 2) and other adipocytic genes in the basal state (20), increase their apoE production as they accumulate lipid. Cell maturation is also associated with an increase in synthesis and secretion of apoC-I, independent of any additional increase in PPAR $\gamma$  mRNA (Fig. 2). These data suggest that increased apoC-I and apoE production is not only a feature of adipocyte differentiation, but is a characteristic of the transformation of SW872 cells from immature to mature adipocytes. ApoC-I and/or apoE may play an important role in the regulation of adipocyte lipid accumulation, consistent with their documented effects on lipid uptake and/or efflux in other cell types. For example, apoC-I is an inhibitor of lipoprotein receptors of the LDL-receptor family (5,7), and has an inhibitory effect on the tissue uptake of FFA (16,17). ApoE might be important in regulating receptor-mediated uptake of adipocyte lipid (4), as supported by the observation that adipose tissue mass is significantly reduced in obese *ob/ob* mice that are also made deficient in apoE (28). ApoE can also stimulate LRP-mediated selective uptake of HDL cholesteryl esters by adipocytes (20). On the other hand, newly synthesized endogenous apoE mediates cholesterol efflux from macrophages (29,30), and it is possible that apoE plays a similar role in maturing adipocytes. Clearly, these different possibilities can only be substantiated by future experiments.

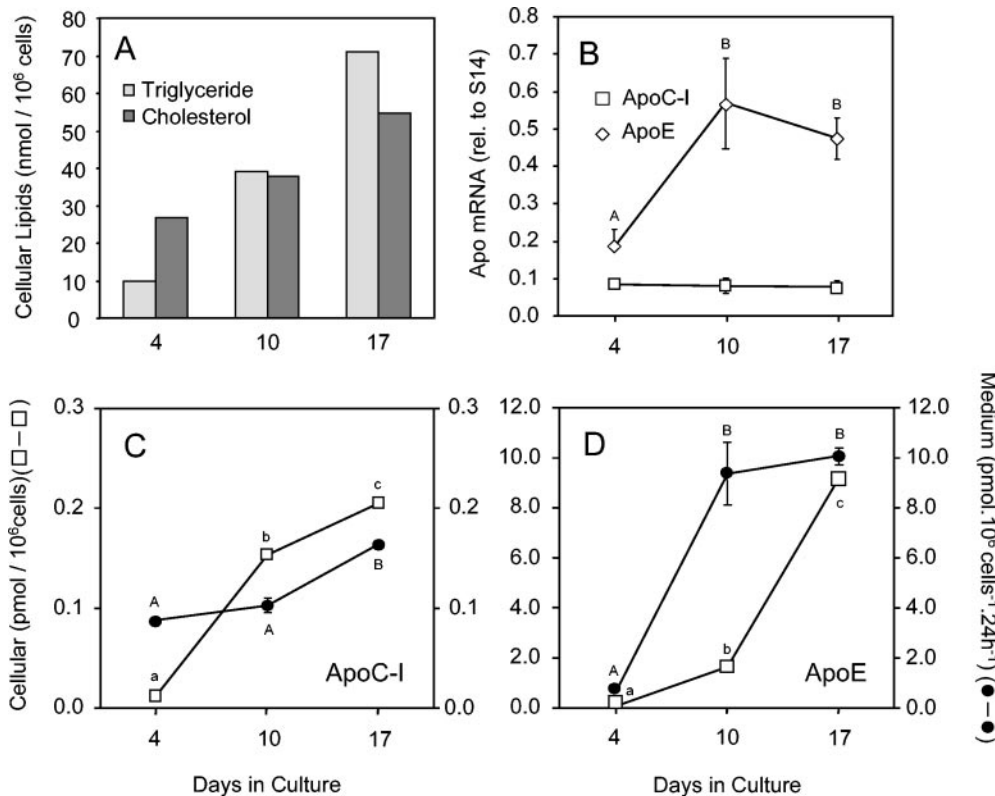
The apoC-I and apoE genes are adjacent to each other on the same chromosome (8) and are regulated by common control regions (13,31,32). Thus, apoC-I and apoE gene expression in the liver is driven by 2 enhancer regions, hepatic control regions (HCR) 1 and 2, which are  $\sim 9$  and  $\sim 20$  kb, respectively, downstream of the apoC-I gene (32,33). Macrophage and adipose tissue apoC-I and apoE gene expression is in turn directed by 2 multienhancer regions (ME.1 and ME.2) that are situated on either side of the apoC-I gene (34). Furthermore, 2 LXR response elements located within ME.1 and ME.2 appear to be responsible for the induction of macrophage apoC-I and apoE gene expression by ligands for LXR and retinoid X receptor (35). On the basis of these results, we initially hypothesized that any change in the level of apoC-I mRNA would be mimicked by a similar change in apoE mRNA. Furthermore, we expected that changes in apoC-I or apoE mass in maturing SW872 cells would be mimicked by similar changes in mRNA levels. In fact, parallel changes in



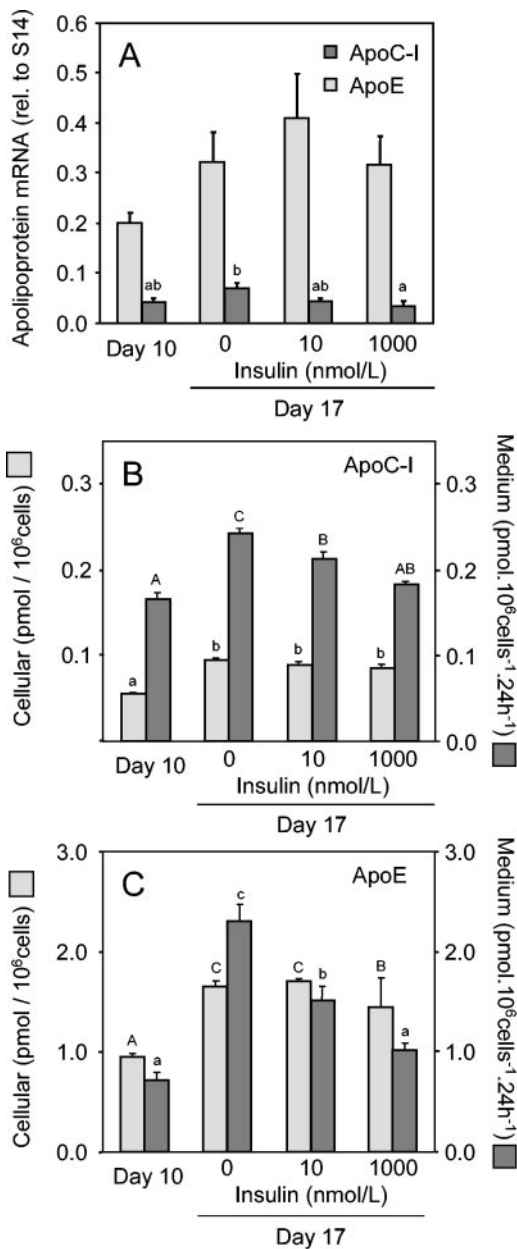
**FIGURE 4** Cellular triglyceride and cholesterol levels (*panel A*), apoC-I and apoE mRNA levels as determined by real-time RT-PCR and expressed relative to S14 mRNA (*panel B*), cellular and medium apoC-I levels as determined by ELISA (*panel C*), and cellular and medium apoE levels (*panel D*) during SW872 cell maturation and apoC-I and apoE production in the presence or absence of serum lipoproteins. Cells were plated into 6-well tissue culture plates ( $0.5 \times 10^6$  cells/well) in the presence of DMEM/F-12 (3:1, v:v) and 10% FCS. At 100% confluence (d 4), cells were allowed to continue to mature in the presence of 10% FCS or in the presence of 10% LPDS. Cells and media were harvested 13 d after reaching confluence (i.e., at d 17). Results are means  $\pm$  SEM,  $n = 6$ . Means for a variable without a common letter differ ( $P < 0.05$ ), by one-way ANOVA and the post-hoc Tukey's test.

apoC-I and apoE mRNA were not observed in either the maturation experiments or those with insulin. Increased levels of apoE mRNA, but not apoC-I mRNA, were consistently

observed in cells incubated in the presence or absence of extracellular lipids (Fig. 2B and 5B), whereas insulin treatment was associated with a decrease in apoC-I mRNA, but not apoE



**FIGURE 5** Effect of serum-free medium supplemented with a growth-promoting cocktail on cellular triglyceride and cholesterol levels (*panel A*), apoC-I and apoE mRNA levels expressed relative to S14 mRNA (*panel B*), and cellular and medium apoC-I (*panel C*) and apoE levels (*panel D*) during SW872 cell maturation. Cells were plated into 6-well tissue culture plates ( $0.5 \times 10^6$  cells / well) in the presence of DMEM/F-12 (3:1, v:v) containing 1.25  $\mu$ mol/L insulin, 0.1  $\mu$ mol/L dexamethasone, 0.2 nmol/L triiodothyronine, 33  $\mu$ mol/L biotin, and 17  $\mu$ mol/L pantothenate. Cells and media were harvested 4, 10, and 17 d after plating. In *panel A*, values are means of 2 dishes at each time point. In *panels B, C* and *D*, values are means  $\pm$  SEM,  $n = 6$ . Means for a variable without a common letter differ ( $P < 0.05$ ), by one-way ANOVA and the post-hoc Tukey's test.



**FIGURE 6** Effect of insulin on SW872 production of apoC-I and apoE mRNA as determined by real-time RT-PCR and expressed relative to S14 mRNA (panel A), and cellular and medium apoC-I (panel B) and apoE levels (panel C) as determined by ELISA. Cells were grown in DMEM/F-12 (3:1, v:v) containing 10% FCS for 10 d before being incubated for an additional 7 d in medium containing 0, 10, or 1000 nmol/L insulin. Values are means  $\pm$  SEM,  $n = 6$ . Means for a variable without a common letter differ ( $P < 0.05$ ), by one-way ANOVA and the post-hoc Tukey's test.

mRNA (Fig. 6A). These data provide clear evidence for differential regulation of the apoC-I and apoE genes in SW872 cells, and suggest the presence of regulatory elements in the apoE/apoC-I/apoC-II gene cluster that are specific for individual apolipoproteins. At the same time, our data suggest that the production of these apolipoproteins was regulated post-transcriptionally, as evidenced by the increase in apoC-I protein in the absence of increased apoC-I mRNA during maturation (e.g., Fig. 5), and the decrease in secreted apoE in the absence of any change in apoE mRNA during insulin treat-

ment (Fig. 6). This is consistent with the post-transcriptional regulation of apoC-I and apoE production in human HepG2 cells (36,37) and of apoE production in macrophages (38,39). Post-translational degradation of apoC-I and apoE by specific proteases has been shown to occur in hepatocytes and macrophages (36); however, it remains to be determined whether similar proteases are responsible for regulating levels of apoC-I and apoE secretion in human adipocytes.

Insulin stimulates the uptake of glucose and FFA by adipose tissue. It can increase LPL activity, induce the translocation of fatty acid transport proteins to the surface of adipocytes, and stimulate adipocyte lipid accumulation (40,41). In view of the relation observed between lipid accumulation and increased apoC-I and apoE production in SW872 cells during maturation, we expected that insulin treatment would further stimulate apoC-I and apoE production. Surprisingly, however, long-term incubation of cells with insulin caused a decrease in apoC-I and apoE secretion, concomitant with a decrease in apoC-I mRNA, although not apoE mRNA (Fig. 6). These data suggest that insulin by itself does not induce transcription of the apoC-I or apoE gene in adipocytes, in agreement with results obtained with cultured rat hepatocytes (42) and human hepatoma cells (43,44), in which insulin had no effect on apoE mRNA levels, but a small and reproducible lowering effect on apoE secretion. From these results, it can also be deduced that the ability of the serum-free, growth-promoting medium to increase apoE and apoC-I production in SW872 cells (Fig. 5) was probably not a direct consequence of its high insulin concentration (1.25  $\mu$ mol/L).

In conclusion, the results of the present study demonstrate that SW872 liposarcoma cells synthesize and secrete both apoC-I and apoE. When these cells are incubated for an extended period of time in the presence of 10% FCS, they accumulate cholesterol and triglyceride, and produce significantly increased amounts of these apolipoproteins. Lipid accumulation and increased apoC-I and apoE production can also occur over time in the absence of extracellular lipids. In addition, evidence was obtained for the differential regulation of apoC-I and apoE production at the transcriptional as well as the post-transcriptional level, and long-term insulin treatment had an inhibitory rather than a stimulatory effect on apoC-I and apoE production. These results suggest that apoC-I and apoE play a role in the maturation of human adipocytes and may be important in mediating or regulating cell lipid accumulation. Further work is required to define this regulatory function and determine whether these apolipoproteins are essential for adipocyte maturation.

## ACKNOWLEDGMENTS

The authors acknowledge the help and support of Catherine Bouchard, Lucie Boulet, Jean-François Carmel, H el ene Jacques, Claudia Rodriguez, and Michel Tremblay.

## LITERATURE CITED

1. Spiegelman, B. M. & Flier, J. S. (2001) Obesity and the regulation of energy balance. *Cell* 104: 531-543.
2. Grundy, S. M. (2004) Obesity, metabolic syndrome, and cardiovascular disease. *J. Clin. Endocrinol. Metab.* 89: 2595-2600.
3. Engstrom, G., Hedblad, B., Stavenow, L., Jonsson, S., Lind, P., Janzon, L. & Lindgarde F. (2004) Incidence of obesity-associated cardiovascular disease is related to inflammation-sensitive plasma proteins. A population-based cohort study. *Arterioscler. Thromb. Vasc. Biol.* 24: 1498-1502.
4. Mahley, R. W. & Huang, Y. (1999) Apolipoprotein E: from atherosclerosis to Alzheimer's disease and beyond. *Curr. Opin. Lipidol.* 10: 207-217.
5. Shachter, N. S. (2001) Apolipoproteins C-I and C-II as important modulators of lipoprotein metabolism. *Curr. Opin. Lipidol.* 12: 297-304.
6. Cooper, A. D. (1997) Hepatic uptake of chylomicron remnants. *J. Lipid Res.* 38: 2173-2192.



7. Jong, M. C., Hofker, M. H. & Havekes, L. M. (1999) Role of apoCs in lipoprotein metabolism. Functional differences between apoC1, apoC2, and apoC3. *Arterioscler. Thromb. Vasc. Biol.* 19: 472–484.
8. Lauer, S., Walker, D., Elshourbagy, N. A., Reardon, C. A., Levy-Wilson, B. & Taylor, J. M. (1988) Two copies of the human apolipoprotein C-I gene are linked closely to the apolipoprotein E gene. *J. Biol. Chem.* 263: 7277–7286.
9. Knott, T. J., Robertson, M. E., Priestly, M., Wallis, S. & Scott, J. (1984) Characterization of mRNAs encoding the precursor for human apolipoprotein C1. *Nucleic Acids Res.* 12: 3909–3915.
10. Newman, T. C., Dawson, P. A., Rudel, L. L. & Williams, D. L. (1985) Quantitation of apolipoprotein E mRNA in the liver and peripheral tissues of nonhuman primates. *J. Biol. Chem.* 260: 2452–2457.
11. Elshourbagy, N. A., Liao, W. S., Mahley, R. W. & Taylor, J. M. (1985) Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proc. Natl. Acad. Sci. U.S.A.* 82: 203–207.
12. Zechner, R., Moser, R., Newman, T. C., Fried, S. K. & Breslow, J. L. (1991) Apolipoprotein E gene expression in mouse 3T3-L1 adipocytes and human adipose tissue and its regulation by differentiation and lipid content. *J. Biol. Chem.* 266: 10583–10588.
13. Laffitte, B. A., Repa, J. J., Joseph, S. B., Wilpitz, D. C., Kast, H. R., Mangelsdorf, D. J. & Tontonoz, P. (2001) LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc. Natl. Acad. Sci. U.S.A.* 98: 507–512.
14. Shachter, N. S., Ebara, T., Ramakrishnan, K., Steiner, G., Breslow, J. L., Ginsberg, H. N. & Smith, J. D. (1996) Combined hyperlipidemia in transgenic mice overexpressing human apolipoprotein C1. *J. Clin. Invest.* 98: 846–855.
15. Jong, M. C., Gijbels, M. J., Dahlmans, V. E., Gorp, P. J., Koopman, S. J., Ponc, M., Hofker, M. H. & Havekes, L. M. (1998) Hyperlipidemia and cutaneous abnormalities in transgenic mice overexpressing human apolipoprotein C1. *J. Clin. Invest.* 101: 145–152.
16. Koopmans, S. J., Jong, M. C., Dahlmans, V.E.H., Pijl, H., Radder, J. K., Frolich, M. & Havekes, L. M. (2001) Hyperlipidaemia is associated with increased insulin-mediated glucose metabolism, reduced fatty acid metabolism and normal blood pressure in transgenic mice overexpressing human apolipoprotein C1. *Diabetologia* 44: 437–443.
17. Jong, M. C., Voshol, P. J., Muurling, M., Dahlmans, V. E., Romijn, J. A., Pijl, H. & Havekes, L. M. (2001) Protection from obesity and insulin resistance in mice overexpressing human apolipoprotein C1. *Diabetes* 50: 2779–2785.
18. Richardson, M. A., Berg, D. T., Johnston, P. A., McClure, D. & Grinnell, B. W. (1996) Human liposarcoma cell line, SW872, secretes cholesteryl ester transfer protein in response to cholesterol. *J. Lipid Res.* 37: 1162–1166.
19. Izem, L. & Morton, R. E. (2001) Cholesteryl ester transfer protein biosynthesis and cellular cholesterol homeostasis are tightly interconnected. *J. Biol. Chem.* 276: 26534–26541.
20. Vassiliou, G., Benoit, F., Lau, P., Kavaslar, G. N., & McPherson, R. (2001) The low density lipoprotein receptor-related protein contributes to selective uptake of high density lipoprotein cholesteryl esters by SW872 liposarcoma cells and primary human adipocytes. *J. Biol. Chem.* 276: 48823–48830.
21. Von Goor, H., Gerrits, P. O. & Groud, J. (1986) The application of lipid-soluble stains in plastic-embedded sections. *Histochemistry* 85: 251–253.
22. Cohn, J. S., Tremblay, M., Amiot, M., Bouthillier, D., Roy, M., Genest, J. Jr. & Davignon, J. (1996) Plasma concentration of apolipoprotein E in intermediate-sized remnant-like lipoproteins in normolipidemic and hyperlipidemic subjects. *Arterioscler. Thromb. Vasc. Biol.* 16: 149–159.
23. Cohn, J. S., Tremblay, M., Batal, R., Jacques, H., Veilleux, L., Rodriguez, R., Bernier, L., Mamer, O. & Davignon, J. (2002) Plasma kinetics of apoC-I in VLDL and HDL in normolipidemic and hypertriglyceridemic subjects. *J. Lipid Res.* 43: 1680–1687.
24. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265–275.
25. Primer 3 Software, Whitehead Institute for Biomedical Research ([http://www-genome.wi.mit.edu/genome\\_software/other/primer3.html](http://www-genome.wi.mit.edu/genome_software/other/primer3.html)) [last accessed December 2003].
26. Ross, S. E., Erickson, R. L., Gerin, I., DeRose, P. M., Bajnok, L., Longo, K. A., Misek, D. E., Kuick, R., Hanash, S. M., Atkins, K. B., Andresen, S. M., Nebb, H. I., Madsen, L., Kristiansen, K. & MacDougald, O. A. (2002) Microarray analyses during adipogenesis: understanding the effects of Wnt signaling on adipogenesis and the roles of liver X receptor  $\alpha$  in adipocyte metabolism. *Mol. Cell Biol.* 22: 5989–5999.
27. Seo, J. B., Moon, H. M., Kim, W. S., Lee, Y. S., Jeong, H. W., Yoo, E. J., Ham, J., Kang, H., Park, M.-G., Steffensen, K. R., Stulnig, T. M., Gustafsson, J.-A., Park, S. D. & Kim, J. B. (2004) Activated liver X receptors stimulate adipocyte differentiation through induction of peroxisome proliferator-activated receptor  $\gamma$  expression. *Mol. Cell Biol.* 24: 3430–3444.
28. Chiba, T., Nakazawa, T., Yui, K., Kaneko, E. & Shimokado, K. (2003) VLDL induces adipocyte differentiation in apoE-dependent manner. *Arterioscler. Thromb. Vasc. Biol.* 23: 1423–1429.
29. Lin, C. Y., Duan, H. & Mazzone, T. (1999) Apolipoprotein E-dependent cholesterol efflux from macrophages: kinetic study and divergent mechanisms for endogenous versus exogenous apolipoprotein E. *J. Lipid Res.* 40: 1618–1627.
30. Lin, C. Y., Huang, Z. H. & Mazzone, T. (2001) Interaction with proteoglycans enhances the sterol efflux produced by endogenous expression of macrophage apoE. *J. Lipid Res.* 42: 1125–1133.
31. Simonet, W. S., Bucay, N., Pitas, R. E., Lauer, S. J. & Taylor, J. M. (1991) Multiple tissue-specific elements control the apolipoprotein E/C-I gene locus in transgenic mice. *J. Biol. Chem.* 266: 8651–8654.
32. Simonet, W. S., Bucay, N., Lauer, S. J. & Taylor, J. M. (1993) A far-downstream hepatocyte-specific control region directs expression of the linked human apolipoprotein E and C-I genes in transgenic mice. *J. Biol. Chem.* 268: 8221–8229.
33. Allan, C. M., Taylor, S. & Taylor, J. M. (1997) Two hepatic enhancers, HCR. 1 and HCR. 2, coordinate the liver expression of the entire human apolipoprotein E/C-I/C-IV/C-II gene cluster. *J. Biol. Chem.* 272: 29113–29119.
34. Shih, S. J., Allan, C., Grehan, S., Tse, E., Moran, C. & Taylor, J. M. (2000) Duplicated downstream enhancers control expression of the human apolipoprotein E gene in macrophages and adipose tissue. *J. Biol. Chem.* 275: 31567–31572.
35. Mak, P. A., Laffitte, B. A., Desrumaux, C., Joseph, S. B., Curtiss, L. K., Mangelsdorf, D. J., Tontonoz, P. & Edwards, P. A. (2002) Regulated expression of the apolipoprotein E/C-I/C-IV/C-II gene cluster in murine and human macrophages. A critical role for nuclear liver X receptors alpha and beta. *J. Biol. Chem.* 277: 31900–31908.
36. Wenner, C., Lorkowski, S., Engel, T. & Cullen, P. (2001) Apolipoprotein E in macrophages and hepatocytes is degraded via the proteasomal pathway. *Biochem. Biophys. Res. Commun.* 282: 608–614.
37. Bouchard, C., Dubuc, G., Davignon, J., Bernier, L. & Cohn, J. S. Post-transcriptional regulation of apoC-I synthesis and secretion in human HepG2 cells. *Atherosclerosis* (in press).
38. Duan, H., Lin, C. Y. & Mazzone, T. (1997) Degradation of macrophage apoE in a nonlysosomal compartment. Regulation by sterols. *J. Biol. Chem.* 272: 31156–31162.
39. Duan, H., Gu, D. & Mazzone, T. (2000) Sterols and inhibitors of sterol transport modulate the degradation and secretion of macrophage ApoE: requirement for the C-terminal domain. *Biochim. Biophys. Acta* 1484: 142–150.
40. Stahl, A., Evans, J. G., Pattel, S., Hirsch, D. & Lodish, H. F. (2002) Insulin caused fatty acid transport protein translocation and enhanced fatty acid uptake in adipocytes. *Dev. Cell* 2: 477–488.
41. Rondinone, C. M., Trevillyan, J. M., Clampit, J., Gum, R. J., Berg, C., Kroeger, P., Frost, L., Zinker, B. A., Reilly, R., Ulrich, R., Butler, M., Monia, B. P., Jirousek, M. R. & Waring, J. F. (2002) Protein tyrosine phosphatase 1B reduction regulates adiposity and expression of genes involved in lipogenesis. *Diabetes* 51: 2405–2411.
42. Kim, M. H., Nakayama, R., Manos, P., Tomlinson, J. E., Choi, E., Ng, J. D. & Holten, D. (1989) Regulation of apolipoprotein E synthesis and mRNA by diet and hormones. *J. Lipid Res.* 30: 663–671.
43. Dashti, N., Williams, D. L. & Alaupovic, P. (1989) Effects of oleate and insulin on the production rates and cellular mRNA concentrations of apolipoproteins in HepG2 cells. *J. Lipid Res.* 30: 1365–1373.
44. Ogbonna, G., Theriault, A. & Adeli, K. (1993) Hormonal regulation of human apolipoprotein E gene expression in HepG2 cells. *Int. J. Biochem.* 25: 635–640.