Oleic Acid Modulates the Post-translational Glycosylation of Macrophage ApoE to Increase Its Secretion*

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There has been increasing interest in a potential role for fatty acids in adversely affecting organismal substrate utilization and contributing to the cardiovascular complications in insulin resistance. Fatty acids have already been implicated in regulating the expression of a number of genes in resident cells of the vessel wall. In the current studies, we evaluated a potential role for fatty acids in the regulation of macrophage apoE expression. Incubation in oleic acid increased the synthesis and secretion of apoE by human monocyte-derived macrophages. Part of this stimulation was mediated at a post-translational locus. Oleic acid increased the secretion of apoE from macrophages that constitutively expressed a human apoE3 cDNA. Incubation in palmitic acid decreased apoE secretion from these cells. The effect of oleic acid on apoE secretion could not be accounted for by the known effect of fatty acid on cellular sterol, because incubation in oleic acid did not suppress the degradation of nascent apoE. Incubation in oleic acid for at least 6 h was required to observe an effect on apoE secretion. Oleic acid altered the glycosylation pattern of cellular and secreted apoE, with a loss of the most heavily sialylated isoform. Oleic acid had no effect on the glycosylation of interleukin 6 secreted from macrophages. Elimination of apoE glycosylation, by substitution of threonine 194 with alanine, eliminated oleic acid-mediated stimulation of apoE secretion. These results indicate that oleic acid increases apoE secretion from macrophages at a locus involving post-translational glycosylation.

The level of plasma free fatty acids in normal adult humans can range from 0.3 to 0.9 mM; however, these levels can rise significantly (up to 2.5 mM) in common diseases (e.g. diabetes mellitus and insulin resistance) or with stress (1–4). In diabetes, circulating palmitic, linoleic, palmitoleic, and oleic acid levels have all been reported to be increased (5). In addition, the action of lipoprotein lipase on triglyceride-rich lipoproteins has potential to produce even higher concentrations of fatty acids in peripheral vascular beds where expression of lipoprotein lipase is high. For example, it has been estimated that only 5–10% of the long chain fatty acids released from very low density lipoproteins, or chylomicrons, by lipoprotein lipase in peripheral vascular beds equilibrate with plasma; the rest enters peripheral cells (3). Exposure of peripheral cells to fatty acids could therefore also increase in patients with fasting or post-prandial hypertriglyceridemia.

There has recently been increasing interest regarding the role of fatty acids in adversely affecting organismal substrate utilization in diseases characterized by insulin resistance (6, 7). There has therefore been increasing investigation of the role of fatty acids in regulating multiple aspects of cellular physiology. For example, fatty acids, or their metabolites, have been reported to have an important role in modulating the expression of numerous genes involved in intermediary metabolism (8–10). It has been demonstrated that such modulation may occur by direct interaction with DNA-binding proteins or the activation of cellular second messenger pathways (8–11). Fatty acids can also modulate cellular sterol balance and transport and may thereby modulate the expression of sterol-dependent genes (9, 10, 12). Moreover, as a major constituent of membrane phospholipids, fatty acids can influence the physical properties of cellular membranes including fluidity, permeability, microdomain structure, and surface potential (13). These properties in turn may regulate the transport, localization, and activity of membrane-associated proteins (14, 15).

Diseases characterized by increased circulating levels of fatty acids or by hypertriglyceridemia are also characterized by accelerated large vessel atherosclerosis (16–18). This observation has led to investigation of a role for fatty acids in regulating gene expression, membrane properties, and protein expression in vessel wall cells; including endothelial cells, arterial smooth muscle cells, and macrophages (19–21). The ability of fatty acids to modulate the expression of proteins involved in maintaining sterol homeostasis in the vessel wall would be of particular interest for further understanding of the impact of fatty acids on large vessel atherosclerosis. In this series of studies, we evaluated the effect of fatty acids on macrophage apoE expression. Macrophage infiltration and accumulation are an early response to vessel wall injury, and macrophage-derived apoE has an important role in defending sterol homeostasis in macrophages and in the vessel wall (22, 23). In line with this, macrophage-derived apoE has been shown to modulate atherogenesis in vivo, in atherosclerosis-prone mice (23, 24). Elucidation of a regulatory interaction between fatty acids and macrophage apoE expression could therefore increase our understanding of mechanisms of atherosclerosis/atheroprotection in common metabolic disorders.

EXPERIMENTAL PROCEDURES

Materials—[35S]Methionine (4.3 mCi/mM) was from Amersham Biosciences. Fatty acids and fatty acid-free BSA1 were from Sigma. Goat-

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derived human apoE antisera was from International Immunology
Corporation (Madison, WI). Recombinant protein G-agarose, G418, and all
other cell culture reagents were from Invitrogen. All other materials
were from previously described sources (25, 26).

**Cell Culture**—Human monocytes were purified by elutriation. The
elutriated cell population used for these experiments was >95% mono-
cytic, as determined by differential counts of Wright-stained smears.
The human monocytes were plated and grown in DMEM with 20% FBS
and 10% human AB pooled serum for 7 days to allow their differen-
tiation into macrophages before the start of experiments. J774 macro-
phages were obtained from ATCC and grown in 10% FBS in DMEM.

**Stable Gene Expression**—J774 cells were stably transfected to ex-
press human native or mutant apoE3 in a constitutive manner under
the control of a constitutively active cytomegalovirus promoter. The apoE3
expression construct and the method for transfection have been previously de-
scribed (27). An expression vector containing a cDNA for apoE in which
threeine 194 was changed to alanine was constructed using the QuikChange
mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacture's
instructions. Mutagenic primer 1 had the sequence 5'-GGC CCG CAC G-3;
mutagenic primer 2 had the sequence: 5'-GGA GCC CAC AGC GGC
GCC CCG CAC G-3' (positions 689–689; mutagetnic primer 2 had the sequence: 5'-GGC CCG CAC GAC GGC
GCC CCG CAC G-3'). The mutant apoE cDNA was sequenced to
confirm the presence of the desired mutation. J774 cells expressing native apoE (J774-E) or the mutant apoE (J774-E194A),
along with a co-transfected neomycin resistance construct, were
selected in G418 (400 μg/ml).

**Fatty Acid/BSA Solution**—Fatty acid was dissolved in 95% ethanol
and evaporated under nitrogen at 45 °C to thin film. At that time, BSA
in phosphate-buffered saline at 45 °C was added to the tube to obtain
the desired fatty acid:BSA molar ratios. After the fatty acid was com-
pletely dissolved, the solution was cooled, filtered, and stored in aliq-
ut at 4 °C.

**Measurement of ApoE Synthesis/Secretion**—The cells were labeled
with [35S]methionine as described in the legends to the figures or tables.
After labeling, the cell medium or cell lysates were utilized for quanti-
tative immunoprecipitation as previously described (25). Equal
numbers of trichloroacetic acid precipitable counts were utilized to start
immunoprecipitations within each experiment, so the apoE signal is
already corrected for variations in cellular or medium total protein
labeling. Radioactivity in apoE was detected and measured in SDS-
PAGE of immunoprecipitates using an Amersham Biosciences Phos-
phorImage and ImageQuant software. Western blots for apoE were
performed utilizing equivalent volumes of cell culture medium after
adjusting for cellular protein. Western blot signals were localized and
quantitated using a UMAX scanner with Zero Dscan software (Scana-
lytics, Inc., Fairfax, VA).

Subcellular Fractionation and Nascent ApoE Degradation—The iso-
lation of an intermediate density nonsolysosomal cell membrane fraction
was based on previously published methods with minor modifica-
tions (28). Cells were fractionated two times and scrapped from the
culture flask with a rubber policeman in homogenizing medium
(37.5 mM Tris-HCl, 0.5 M sucrose, 1% dextran, 5 mM MgCl2, 0.1 M
phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, pH 6.5). Cells in
suspension were homogenized using 100 strokes in a Dounce homo-
genizer. The homogenate was then centrifuged at 2,000 rpm in a Beckman
SW41 rotor for 5 min, followed by 10,000 rpm for 30 min. The upper
one-half of the pellet was resuspended in 2 ml of homogenizing medium
and then layered onto 9 ml of ice-cold of 1.2 M sucrose and centrifuged
at 25,000 rpm for another 30 min in an SW41 rotor. Two closely spaced
opaque bands at the interface were recovered with a Pasteur pipette
and diluted 20-fold with 1 ml of 5% glucose solution. No lysosomes were
remained after centrifugation at 5,000 rpm for 20 min in an SW41 rotor
and discarding the supernatants. The pellet membranes were assayed
immediately for marker enzyme activity. As previously described in
detail, the specific activity for the Golgi marker in recovered mem-
branes increased ~8-fold with a 30% recovery (28). Markers for the ER,
lysosomes, or plasma membrane were reduced compared with total cell
homogenate. Overall, cellular protein recovery in the recovered mem-
brane fraction was ~5%. After isolation of the Golgi-enriched intermediate density membrane
fraction from cells that were pulse-labeled with [35S]methionine for 30
min, equal aliquots were mixed with half-volumes of 0.4 M sodium
potassium phosphate (pH 6.8) containing 8 mM dithiothreitol. Equal
potassium phosphate was then added for an additional incubation at
40 °C or kept at 4 °C. At the end of that time, total labeled protein in
each aliquot was measured by trichloroacetic acid precipitation, and
apoE was measured by quantitative immunoprecipitation. The differ-
ence between the amount of apoE present in fractions after incubation
at 40 °C versus 4 °C was taken as the amount degraded during the
3-h incubation.

**Other Analyses**—Protein was measured using the DC protein assay
kit from Bio-Rad. Galactosyltransferase (Golgi marker), acid phospho-
tase (lysosomal marker), 5' nucleotidase (plasma membrane marker),
and cytochrome c reductase (ER marker) were assayed as described
previously (28). Statistical comparisons were performed using Student's
t-test.

**Results**

We first evaluated the effect of oleic acid on the synthesis and secretion of apoE by primary cultures of human monocyte-
derived macrophages. As measured by the incorporation of radiolabeled methionine during a 2-h pulse, incubation of hu-
man monocyte-derived macrophages with oleic acid resulted in a
significant increase of labeled apoE within cells and in cell
culture medium, consistent with increased synthesis and secre-
tion of apoE (Fig. 1). The effect on apoE synthesis was further confirmed by measuring apoE synthesis in human
macrophages that were pulse-labeled for 30 min (Fig. 1, inset). The effect of oleic acid on macrophage apoE expression was
observed whether oleic acid was added in the presence or ab-
sence of serum, at final fatty acid concentrations ranging from
100 μM to 500 μM and at fatty acid:BSA molar ratios ranging
from 2 to 6.8 (not shown). For the balance of the experiments,
therefore, representative results using different incubation
conditions are shown. We have previously reported that the
expression of macrophage apoE can be regulated at transcrip-
tional and post-transcriptional loci (25, 28, 29). For the current
report, we elected to focus on a series of experiments to deter-
mine whether fatty acids regulated macropage apoE expres-
sion at a post-transcriptional locus.

To evaluate post-transcriptional regulation, J774 macro-
phages (which do not express their endogenous apoE gene)
were transfected to express a physiologic level of human apoE
under the control of a constitutively active cytomegavirus
promoter. These cells constitutively secrete 0.9–1 μg of apoE/mg of cell protein over 24 h, similar to levels of apoE
produced by primary cultures of human monocyte-derived
macrophages. Incubating these cells with oleic acid signifi-
cantly increased the appearance of apoE in the medium (Fig. 2A),
similar to the results obtained with human monocyte-
derived macrophages. However, different results were obtained
in the cell lysate. Elimination of the transcriptional response in
the J774-E model led to no change, or even a small decrease,
in labeled cellular apoE (Fig. 2B). We further evaluated the poten-
tial significance of this apparent change in the partitioning
of newly synthesized apoE using a pulse-chase experimental
format. A short pulse labeling of apoE of 30 min followed by a
45-min chase period demonstrated that oleic acid significantly
increased medium apoE in conjunction with a significant
reduction of cellular apoE. The results suggest that oleic modu-
lates the partitioning of newly synthesized apoE between a
cellular and a secreted compartment by increasing the trans-
poral of nascent apoE out of the cell.

It has been previously reported that fatty acids modulate mem-
brane sterol balance in cells (12, 30). We have previously shown
that sterols increase apoE secretion from macrophages via a
post-translational mechanism (28). Sterols suppress the degra-
dation of newly synthesized apoE, thereby increasing its secre-
tion (28). However, different from the results shown in Fig. 3,
incubation with sterol does not reduce cellular apoE when eval-
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ulation (28). However, different from the results shown in Fig. 3,
Oleic acid stimulates apoE synthesis and secretion by human monocyte-derived macrophages. Human monocyte-derived macrophages were grown for 7 days, as described under "Experimental Procedures." Before the addition of BSA alone or BSA with 100 μM oleic acid at a 4:1 molar ratio. After an additional 24 h, the cells were incubated with [35S]methionine (50 μCi/ml) for 2 h. At that time, cells and media were harvested for quantitative immunoprecipitation of apoE as described under "Experimental Procedures." The values shown are the means ± S.D. of triplicate wells of cells. **, p < 0.01. OA, oleic acid. The inset shows the results of an identical experiment except cells were pulse-labeled for 30 min, and labeled apoE in cell lysates was analyzed. ***, p < 0.001.

Nascent apoE is post-translationally modified in the ER and Golgi by the addition of O-linked carbohydrate residues at threonine 194 (34). The addition of a variable number of sialic acid residues in the Golgi results in the synthesis and secretion of heterogeneous forms of macrophage apoE that can be distinguished by isoelectric focusing or SDS-PAGE (32, 34–36). Analysis of the SDS-PAGE pattern of newly synthesized cellular and secreted apoE suggested that fatty acid modulated the post-translational sialylation of apoE (for example see Fig. 2). This issue was further examined by analyzing the isoform distribution of cellular and secreted apoE after labeling cells with high specific activity methionine. In Fig. 5, the multiple bands of apoE that are evident result from the addition of variable numbers of sialic acid residues, as we have previously demonstrated by digestion with neuraminidase (32), i.e., after exhaustive digestion of labeled cellular and secreted apoE with neuraminidase, the multiple bands shown in Fig. 5A collapse into the lowest band, which represents asialo apoE (32, 34, 36). In Fig. 5A, the SDS-PAGE pattern of apoE isolated from control cells demonstrates three clearly identifiable bands, and the two lowest bands are designated Band 1 and Band 2. Incubation with fatty acid leads to the disappearance of the uppermost band (most highly sialylated), with increased label in Bands 1 and 2. This altered pattern may result from the failure of the more highly sialylated forms of apoE (with lowest mobility on SDS-PAGE) to be synthesized, or alternatively, to be retained by the cell. Analysis of the distribution of medium apoE distinguished these possibilities (Fig. 5B). Labeled cellular apoE isolated from control cells is included in Fig. 5B to facilitate comparison with Fig. 5A. Band 1, which contains asialo-apoE, is largely absent from the medium as has been reported by others (35). The abundance of Band 2 is markedly increased by the incubation with oleic acid and accounts for most of the increase in medium apoE. In multiple experiments, the intensity of discrete bands above Band 2 in the medium were either unchanged, or even decreased, by incubation in oleic acid.
Thus, fatty acids appeared to selectively increase the secretion of an apoE isoform with an intermediate level of sialylation. The results of a similar experiment on apoE isoform distribution in medium obtained from human monocyte-derived macrophages are shown in Fig. 5C. As in the J774-E model, the increase in secreted apoE after incubation with oleic acid is predominantly found in Band 2. We further demonstrated that the effect of oleic acid on the sialylation pattern of apoE did not represent a generalized change in the post-translational glycosylation of all proteins by examining the glycosylation/sialylation...
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**TABLE I**

Oleic acid does not suppress the degradation of nascent apoE.

ApoE-secreting J774 cells were incubated for 24 h in 10% FBS when BSA was added alone or with 175 μM oleic acid in a 6.8:1 molar ratio. The cells were incubated for an additional 24 h, labeled with [35S]methionine for 30 min, and then harvested for subcellular fractionation, as described under “Experimental Procedures.” ApoE degradation was measured in an intermediate density membrane fraction as described under “Experimental Procedures.” The percentage of nascent apoE degraded is given as the mean ± S.D. from triplicate wells of cells.

| Nascent apoE  | % degradation |
|---------------|---------------|
| Control       | 58.7 ± 0.1    |
| Oleic acid    | 62.6 ± 0.1    |

**TABLE II**

Time course for oleic acid stimulation of apoE secretion by macrophages

ApoE-secreting J774 cells were grown for 24 h in 10% FBS when BSA was added alone or with 400 μM oleic acid in a 4:1 molar ratio. At the times indicated, the medium was collected for analysis of apoE secretion by Western blot. The fold change is based on values from triplicate wells of cells. The difference between control and fatty acid-treated cells is significant at 12 and 18 h at the p < 0.05 level.

| Fold increase |
|---------------|
| 3 h           | 1.2 ± 0.1    |
| 6 h           | 1.2 ± 0.1    |
| 12 h          | 1.7 ± 0.4    |
| 18 h          | 1.7 ± 0.4    |

Fig. 4. Palmitic acid reduces apoE secretion from macrophages. J774 macrophages constitutively expressing a physiologic level of human apoE were grown for 24 h in 10% FBS before the addition of BSA alone or BSA with 100 μM palmitic acid at a 5:1 molar ratio. After an additional 24 h, the cells were pulse-labeled with [35S]methionine (100 μCi/ml) for 30 min before the chase period was stated. DMEM containing 500 μM unlabeled methionine was added, and after an additional 45 min, the media were harvested for quantitative immunoprecipitation of apoE. The values shown are the means ± S.D. from triplicate wells of cells. **, p < 0.01. PA, palmitic acid.

Fig. 5. Oleic acid modifies the isoform pattern of cellular and secreted apoE. ApoE-secreting J774 cells (A and B) or human monocyte-derived macrophages (C) were incubated for 24 h in 10% FBS when BSA alone or BSA with 500 μM oleic acid in a 5:1 molar ratio was added. After an additional 2 h, the cells were pulse-labeled with [35S]methionine (100 μCi/ml) for 2 h. Media and/or cells were collected at that time for immunoprecipitation of apoE. A, cell apoE; B, medium apoE; the migration pattern of apoE isolated from control cells is included for comparison. C, medium apoE from human monocyte-derived macrophages; the migration pattern of apoE isolated from control human macrophages is included for comparison. OA, oleic acid.

**TABLE III**

Oleic acid does not enhance the secretion of a nonglycosylated form of apoE

J774 macrophages expressing the threonine 194 → alanine mutant of apoE were grown for 24 h in 10% FBS before the addition of BSA alone or with oleic acid at the concentration (and molar ratio) indicated. After 24 h, the cells were pulse-labeled with [35S]methionine (50 μCi/ml) for 2 h. At that time, media were harvested for the quantitative immunoprecipitation of apoE. The values shown are the means ± S.D. from triplicate wells of cells.

| Addition | Medium apoE |
|----------|-------------|
| None     | 8.4 ± 0.2   |
| 300 μM oleic acid (3:1) | 6.5 ± 0.3 |
| 500 μM oleic acid (5:1) | 6.0 ± 0.1 |

This hypothesis was directly tested by evaluating the effect of oleic acid on the secretion of an apoE mutant that is not a substrate for glycosylation. It has previously been shown that threonine 194 is the sole site for the addition of sugar residues to apoE (34). We replaced the threonine at 194 with alanine to eliminate this site of post-translational glycosylation. We transfected J774 cells to secrete this mutant at a physiologic level, similar to the level measured in J774 cells expressing native apoE. It has been previously reported that the alanine 194 mutant is secreted free of sialic acid (34). We confirmed that the apoE194A secreted by the J774 macrophages contained no covalently bound sugar residues by confirming its resistance to O-glycosidase and neuraminidase digestions (not shown). The effect of oleic acid on secretion of apoE194A is shown in Table III. Utilizing incubation conditions identical to those that increased the secretion of native apoE, oleic acid did not increase the secretion of the mutant apoE and, in fact, decreased its secretion.

**DISCUSSION**

We have found that treatment of human monocyte-derived macrophages with oleic acid increases the synthesis and secretion of apoE. A major finding of this manuscript is that one locus for this stimulatory effect of oleic acid is at the post-translational glycosylation of apoE. In macrophages constitutively expressing an apoE3 cDNA oleic acid, but not palmitic...
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acids, increases apoE secretion. The effect of oleic acid on apoE secretion can be observed whether fatty acid is added in serum-free medium or with serum and over a range of final concentrations and molar ratios of fatty acid to BSA. Elimination of threonine 194, the sole site of apoE glycosylation, eliminates the enhanced apoE secretion after incubation in oleic acid.

In investigating the mechanism for the effect of oleic acid on apoE secretion, we considered the well established role for fatty acids in modulating cellular sterol balance and trafficking (9, 10–12). We have previously shown that changes in cellular sterol trafficking can modulate apoE secretion by altering the stability of the nascent protein (28, 39). However, utilizing the same assay in which we demonstrated the stabilization of nascent apoE by changes in macrophage sterol balance, we could not demonstrate the stabilization of nascent apoE by treatment with oleic acid. Therefore, it is unlikely that the effect of oleic acid on apoE secretion is mediated via changes in cellular sterol trafficking. We have also previously reported that apoE can be sequestered on the macrophage cell surface prior to secretion and may, in fact, undergo recycling through intracellular compartments prior to secretion (31, 32). We therefore considered the possibility that exogenous fatty acids could displace apoE from the cell surface and thus produce the simultaneous increase in medium apoE and decrease in cellular apoE that we observed in pulse-chase experiments. However, the time course data shown in Table II makes simple displacement of cell surface apoE by oleic acid unlikely. The effect of oleic acid required at least 6 h of incubation before any effect on apoE secretion could be measured.

In evaluating the results of our experiments, we made the surprising observation that the sialylation pattern of apoE was altered by the incubation with oleic acid. Specifically, we detected the loss of the most heavily sialylated isoform of apoE in both the cellular and secreted compartments. This observation indicated that oleic acid modulated the post-translational glycosylation of apoE in the Golgi and suggested this as a potential locus for the effect of oleic acid on apoE secretion. The subsequent experiment utilizing a mutant of apoE in which threonine 194 (the only glycosylation site for apoE) was changed to alanine (which is not a substrate for glycosylation) is consistent with this mechanism, i.e. treatment with oleic acid did not enhance the secretion of the threonine 194 → alanine mutant. Another example of lipid-mediated effects on glycosylation regulating protein secretion has been reported (40), i.e. incubation of neuronal cells with sterol has been shown to influence both the glycosylation and secretion of amyloid precursor protein. The results of an experiment in which we evaluated the effect of oleic acid on the secretion of IL-6, another major protein product of macrophages that is secreted with heterogeneous degrees of O-glycosylation/sialylation (37, 38), indicated that the effect of oleic acid on apoE did not reflect a generalized change in the post-translational glycosylation of proteins in the Golgi. Treatment of macrophages with oleic acid did not alter the secretion rate nor the sialylation pattern of IL-6.

Several mechanisms for the effect of oleic acid on apoE glycosylation can be considered. The modulation of second messenger pathway activity by fatty acids has been described and could be involved. For example, it has been reported that other aspects of the post-transcriptional processing of apoE can be regulated by protein kinase C (41). Alternatively, changes in cellular phospholipid metabolism could be involved. ApoE is secreted from macrophages in conjunction with sterol and phospholipid (42). The cellular site where apoE and phospholipids are first associated has not been determined but could be the Golgi where the synthesis of both apoE and phospholipids is completed. Other reports in the literature demonstrate that phospholipids can act as intracellular chaperones for proteins (43). It has also been shown that fatty acids influence cellular phospholipid metabolism (15, 44), and this could impact either the composition or the amount of phospholipid associated with apoE in intracellular membranes, thereby altering its conformation, its availability to be a substrate for post-translational glycosylation, and its retention time in the Golgi. Third, we have previously shown that newly synthesized apoE is retained in a cell surface pool and can be recycled to the Golgi where additional sialic acid residues are added prior to secretion (32). Although the time course data rule out simple displacement of apoE from this cell surface pool as an explanation for our findings, changes in cellular phospholipid synthesis/transport could alter the conformation of apoE or the properties of cellular membranes so as to modulate apoE recycling prior to secretion. Treatment with fatty acid has already been shown to influence membrane lipid composition, and membrane lipid composition has been implicated in membrane trafficking (9, 10, 12, 15, 43). Reports in the literature suggest that changes in Golgi lipid composition and architecture, subsequent to the expression of cytosolic phospholipase A2, in cells, influence the trafficking of proteins through the Golgi (45).

The regulation of apoE expression by macrophages is unexpectedly complex and there are several important post-transcriptional loci of regulation. Nascent apoE is a highly unstable protein in macrophages, and its stability can be modulated by sterols (28, 39). In addition, nascent apoE is retained at the plasma membrane and can be recycled prior to its secretion (31, 32). ApoE is secreted from macrophages in a heterogeneous form because of varying degrees of sialylation, and it is now clear that the degree of sialylation of apoE by macrophages can be regulated and is importantly linked to the secretion of apoE. Altered biologic function of apoE with different levels of sialic acid residues has already been demonstrated (46). Ethanol feeding of rats has recently been reported to decrease the sialylation state of circulating apoE, and this decrease in sialylation was associated with a reduction of apoE binding to high density lipoprotein. Macrophage-derived apoE exerts important regulatory effects on vessel wall cells (22, 23), and potential regulatory changes in the vessel wall related to the degree of apoE sialylation remain to be investigated. Further, the observations in this report provide a basis for further study of the relationship between apoE post-translational processing and cellular fatty acid metabolism.

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