9-cis-Retinoic acid increases apolipoprotein AI secretion and mRNA expression in HepG2 cells

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Abstract

HepG2 cells were studied as a model for regulation of hepatic apolipoprotein AI (apo AI) secretion and gene expression by 9-cis-retinoic acid. HepG2 cells cultured on plastic dishes were exposed to 9-cis-retinoic acid (9-cis-RA) for 48 h with a complete media change at 24 h. Apo AI mass in culture media was determined by ELISA, by quantitative immunoblotting and by steady-state 35S-methionine labeling. Messenger RNA levels were determined by RNase protection using probes for apo AI and the housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (G3PDH). 9-cis-RA increased secretion of apo AI by 52% at doses of 10 and 1 μM (6.3 ± 0.6 vs. 4.2 ± 0.3; P < 0.005; 6.1 ± 0.3 vs. 4.0 ± 0.7 ng of apo AI/mg cell protein, P < 0.05) and by 35% at 0.1 μM (5.5 ± 0.6 vs. 4.1 ± 0.4 ng apo AI/mg protein, P < 0.05, n = 4). Immunoblotting results were consistent with results from ELISA (70% increase at 10 μM 9-cis-RA, P < 0.001; 34% increase at 1 μM, P < 0.005, n = 3). Metabolically labeled apoAI in the medium was increased by 39% following steady-state labeling in the presence of 10 μM 9-cis-RA (597 ± 7 vs. 430 ± 13 DPM/μl media; P < 0.001; n = 4). 9-cis-RA (10 μM) also increased HepG2 cell apo AI mRNA expression by 76% (68 700 ± 400 vs. 38 900 ± 2700 DPM, P < 0.01, n = 4), whereas expression of G3PDH mRNA was slightly decreased (14%, P < 0.05). Thus, 9-cis-RA stimulates apo AI expression in HepG2 cells, suggesting a role for retinoids in activating endogenous apo AI gene expression.

Keywords: HDL cholesterol; Apo AI; Transcriptional regulation; Retinoid; Vitamin A; Hepatoma cell; Atherosclerosis

1. Introduction

Apolipoprotein AI (apo AI), the major protein component of high-density lipoproteins (HDL), plays an important role in HDL cholesterol metabolism and regulation of cholesterol transport. Apo AI promotes esterification of free cholesterol and cholesteryl ester enrichment of HDL by stimulating the enzyme lecithin: cholesteryl acyltransferase (LCAT) [1,2]. Apo AI also stimulates efflux of cholesterol from peripheral tissues and may facilitate hepatic cholesterol uptake by serving as a ligand for binding of HDL to hepatic receptors [3,4]. Apo AI thus contributes...
to the process of reverse cholesterol transport, in which cholesterol is removed from peripheral tissues and transported to the liver for excretion [5]. This process is believed to explain the strong correlation between plasma apo AI levels, HDL cholesterol levels and reduced risk of atherosclerosis [6].

Apo AI production rates can directly influence plasma concentrations of HDL-cholesterol [7-10]. Apo AI synthesized by the liver and intestine is secreted as a discoidal, nascent HDL particle which is then metabolized in plasma to mature HDL. In man, decreases in apo AI production rate correlate with and may be responsible for the decrease in HDL cholesterol levels observed when subjects are changed from a high-fat to a low-fat diet [7]. Apo AI mRNA abundance and apo AI transcription rates also appear to explain differences in plasma apo AI levels, HDL cholesterol levels and perhaps atherosclerosis susceptibility in two species of non-human primates [8]. The relationship between apo AI production and HDL cholesterol levels suggests the possibility that increasing expression of the apo AI gene could slow or prevent progression of atherosclerosis. Indeed, overproduction of human apo AI in transgenic mice raises HDL-cholesterol levels [9] and inhibits the development of early atherosclerotic lesions in this model [10].

Hepatic apo AI expression is controlled by an enhancer region located between nucleotides -110 and -222 upstream of the apo AI transcription start site [11]. This enhancer region is composed of three distinct protein binding sites, designated A, B and C. Synergistic binding of nuclear factors to these three sites contributes to the maximal transcription of the apo AI gene. For example,apo AI regulatory protein-1 (ARP-1), a member of the steroid-thyroid hormone receptor superfamily, binds to site A but represses apo AI expression in cotransfection experiments using HepG2 cells [12]. A second steroid hormone receptor, the retinoid X receptor α (RXRα), binds to a retinoic acid response element within site A and can overcome repression of apo AI gene expression by ARP-1 [13]. This finding suggests that retinoids may play a crucial role in regulating apo AI gene expression. Recently, the natural ligand for RXRα has been identified as the 9-cis-stereoisomer of retinoic acid (RA), 9-cis-RA [14,15]. 9-cis-RA is postulated to activate transcription of retinoid responsive genes directly by binding to RXRα. RXRα, however, also functions as a 'promiscuous' receptor partner by forming heterodimers with other members of the thyroid/steroid hormone receptor superfamily [16]. Because of the complex interactions of RXRα, ARP-1 and possibly other steroid hormone receptors in the regulation of apo AI gene expression, we undertook studies to determine whether 9-cis-RA could regulate endogenous apo AI gene expression in HepG2 cells. We report that 9-cis-RA stimulates endogenous apo AI secretion and raises steady state levels of apo AI mRNA. We further propose that 9-cis-RA may act as an endogenous regulator of human apo AI gene transcription.

2. Materials and methods

2.1. Cell culture

HepG2 cells were obtained from ATCC at passage 76; all experiments were performed with cells maintained for an additional 4–18 passages from receipt. Cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% heat inactivated fetal bovine serum, 1 mg/ml glucose and 40 μg/ml gentamicin sulfate in a humidified, 37°C incubator aerated with 5% CO₂. Cells were cultured on plastic dishes (Costar) and allowed to attach for at least 24 h prior to each experiment. Experiments were initiated when cells were from 50–60% confluent or approximately 1.5–2 × 10⁵ cells/cm². Confluence had reached approximately 80–90% at time of harvesting of media and cells. For ELISA and immunoblotting experiments, HepG2 cells were grown in 96-well plates containing 100 μl of medium. Metabolic labeling experiments were performed in 24-well plates containing 0.5 ml of medium. mRNA isolation was performed from HepG2 cells grown on 10-cm dishes containing 10–12 ml of medium.

2.2. Assay conditions

9-cis-Retinoic acid was synthesized by Dr Jotham Coe by photoisomerization of trans-retinoic acid (Fluka) and recrystallization as de-
scribed [17]. All-trans-RA was obtained from Sigma. 9-cis-RA and all-trans-RA were dissolved in ethanol and stored at a concentration of 10 mM in the dark. A freshly dissolved batch of 9-cis-RA was used for each series of experiments, and each batch of 9-cis- or all-trans-RA was stored in −20°C freezer for no more than 3 days. Retinoids were added directly to complete medium containing 10% fetal bovine serum and pipetted gently onto cells. Cells were incubated for 24 h in the presence of retinoids and then media were replaced with fresh complete media containing newly added 9-cis- or all-trans-RA.

2.3. Apolipoprotein AI and B ELISA

Secreted apo AI and apo B levels were measured by a sandwich enzyme-linked immunosorbent assay. ELISA plates (Nunc Maxisorb) were incubated overnight with monoclonal antibodies against apo AI or apo B obtained from Chemicon Inc. Plates were blocked for 1 h with phosphate buffered saline (PBS) containing 1% bovine serum albumin (type V). HepG2 conditioned media was dispensed at 1/100 dilution for apo AI and 1/10 for apo B in blocking buffer plus 0.004% Tween-20. After an overnight incubation at room temperature for apo B and 37°C for apo AI, plates were washed three times with PBS plus 0.1% Tween-20, and 1/1000 dilution of polycolonal goat antiserum against human apo AI or apo B (Chemicon) was added for 3 h. Subsequently, plates were washed three times and incubated with a working dilution of rabbit anti-goat IgG conjugated to alkaline phosphatase (Sigma) for 1 h. Plates were then washed four times, and alkaline phosphatase substrate (Sigma) was added at 1 mg/ml in carbonate buffer at pH 9.5. The reaction was stopped with 50 μl of 0.2 N NaOH. Purified apo AI (Sigma) and isolated human LDL were used for generating standard curves in apo AI and apo B ELISAs, respectively.

2.4. Apo AI immunoblotting

Apo AI immunoblotting was performed following SDS-polyacrylamide gel electrophoresis and electrophoretic transfer as described by Towbin et al. [18]. Briefly, proteins contained in HepG2 conditioned media were separated on 10–13% gradient SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Micron Separation Systems). The nitrocellulose membrane was blocked using 5% non-fat dry milk (in PBS) (Blotto) and then incubated with a monoclonal antibody against human apo AI (Chemicon) with constant shaking for 17 h at room temperature. Subsequently, the membrane was washed and incubated with 125I labeled goat antibodies against mouse IgG (NEN) for 4 h. Only bands specific for apo AI were detected with this method. The bands were visualized and quantified by a BAS 2000 phosphor imager (Fuji Medical Systems).

2.5. Metabolic labeling

HepG2 cells cultured in 24-well plates were preincubated for 23 h with 9-cis-RA or ethanol and washed three times with methionine-free DMEM (Gibco). Cells were then labeled with 30 μCi 35S-methionine per 16-mm well for 19 h in methionine-deficient medium in the presence or absence of 9-cis-RA. Media aliquots (210 μl) were diluted in 500 μl of NET buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% Nonidet P-40 (NP-40), 1 mM EDTA, 0.25% gelatin, 0.02% azide) and incubated with 100 μl of 5% Protein A-Sepharose (Sigma) twice for 1 h each at 4°C to remove proteins that non-specifically bind to Protein A. Supernatants were removed after centrifugation (12 000 × g for 5 min) and incubated overnight with goat anti-human apo AI, apo B or albumin at 4°C. The following morning, 80 μl of 5% Protein A-Sepharose was added for 1 h at 4°C. Subsequently, the tubes were spun for 5 min at 12 000 × g, and the pellet was washed three times with RIPA buffer (50 mM Tris, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS). Pellets were counted directly in a scintillation counter (Beckman). A control sample containing no antibody was precipitated in parallel with each sample; non-specific counts due to Protein A binding were subtracted to obtain specific counts. Immunoprecipitated apo AI, apo B and albumin were analyzed separately by SDS-PAGE and autoradiography to confirm the specificity of the immunoprecipitation procedure and to validate the direct counting method. Antibodies to apo AI, apo B and albumin were specific as
no additional bands were seen after prolonged exposures of the gels. Total trichloroacetic acid precipitable counts were determined by adding 800 µl of 20% cold trichloroacetic acid to media aliquots (20–25 µl) and incubating at 4°C overnight. Subsequently, the pellet was washed with 20% trichloroacetic acid and dissolved in 300 µl of 1 M Tris (pH 7.5). Samples were counted in a scintillation counter following addition of 10 ml of scintillation cocktail.

2.6. RNase protection assay

RNase protection was performed essentially as previously described [19]. Human apo AI riboprobe was synthesized using T7 RNA polymerase from a linearized, HindIII digested pGEM vector containing a 230-nucleotide portion of apo AI cDNA (a generous gift from Drs Jan Breslow and Neal Azrolan, Rockefeller University). Glycer-aldehyde 3-phosphate dehydrogenase (G3PDH) riboprobe was synthesized from a linear 1.1-kb DNA (Clontech) containing 1.0 kb of human G3PDH cDNA attached to the T7 promoter. Total cytoplasmic RNA was isolated using a Tri Reagent Kit (Molecular Research Center Inc.) according to the manufacturer’s suggestions. Ten micrograms and 5 µg of total RNA were hybridized with ~2–4 × 10⁶ counts of apo AI and G3PDH riboprobes, respectively, in hybridization buffer (80% formamide, 0.4 M NaCl, 1 mM EDTA and 40 mM HEPES, pH 6.7) at 63°C for 3.5 h. RNA was subsequently incubated for 45 min at 34°C with 300 µl of ice cold RNase solution containing 0.3 M NaCl, 5 mM EDTA, 10 mM Tris-HCl, pH 7.4, 4 U of T1 RNase and 36 µg RNase A. RNA-RNA hybrids were precipitated by adding 400 µl of cold 20% trichloroacetic acid and collected on glass wool filters (Whatman). Filters were extensively washed with 5% trichloroacetic acid and counted for radioactivity in a Beckman scintillation counter. The specificity of the apo AI riboprobe was verified by gel electrophoresis and the direct counting method was also previously validated [19].

2.7. Northern blot hybridization

RNA isolated from HepG2 cells treated with or without retinoic acid for 48 h was separated by agarose gel electrophoresis and transferred to nitrocellulose membranes by Northern blotting. Apo AI and G3PDH riboprobes were synthesized as described above and used for hybridization overnight at 65°C. Nitrocellulose membranes were washed three times with 2 × SSC plus 0.2% SDS and once with 0.2 × SSC plus 0.2% SDS at 65°C. Bands were visualized and quantified in a phosphor imager.

2.8. Statistics

Unpaired Student’s t-tests were performed using Statview statistical software package for Macintosh. Results are reported as means ± 1 S.D.

3. Results

3.1. Effect of 9-cis-retinoic acid on apo AI secretion from HepG2 cells

9-cis-RA stimulated apo AI secretion from HepG2 cells in a dose-responsive manner (Fig. 1, upper panel). Incubation of HepG2 cells with 9-cis-RA for 48 h increased media accumulation of apo AI during the second 24 h by about 50% at 10 and 1 µM (P < 0.005 and P < 0.01, respectively) and by ~35% at 0.1 µM (P < 0.05). Smaller increases in apo AI accumulation were observed following treatment with 0.01 and 0.001 µM 9-cis-RA (not significant). Apo AI in media from cells incubated for the first 24 h with 9-cis-RA was not elevated (data not shown), indicating a prerequisite for the 24-h preincubation period. Increasing concentrations of the diluent (ethanol) had no effect on apo AI secretion (Fig. 1, upper panel). In the same cells and series of experiments, 9-cis-RA consistently did not affect secretion of another protein involved in lipoprotein metabolism, apolipoprotein B (lower panel).

Secretion of apo AI from HepG2 cells was also assessed by quantitative immunoblotting (Fig. 2). Relative to control (ethanol treated) HepG2 cells, 9-cis-RA increased apo AI secretion into the media by 75% at 10 µM (P < 0.001) and by 33% at 1 µM (P < 0.01). These results confirm the increase in apo AI mass observed in the ELISA.

Incubation of HepG2 cells with all-trans-RA under the same conditions stimulated apo AI secretion to a lesser degree (data not shown).
Because a greater response was obtained for 9-cis-RA than for all-trans-RA and the possibility of isomerization of all-trans-RA to 9-cis RA under our experimental conditions, only 9-cis-RA was used in subsequent experiments.

Metabolic labeling studies were performed to examine the specificity of 9-cis-RA in stimulating apo AI secretion. HepG2 cells were incubated with 9-cis-RA (10 μM) for 23 h and then metabolically labeled with 35S-methionine for 19 h in the presence of 10 μM 9-cis-RA. Apo AI immunoprecipitated from the media of 9-cis-RA treated cells was significantly increased (40%).

Fig. 1. Effect of 9-cis-RA on apo AI secretion by HepG2 cells. HepG2 cells in 96-well plates were incubated for 24 h in complete medium containing 9-cis-RA or vehicle (ethanol) as described in the experimental procedures. Media were replaced with fresh media containing 9-cis-RA or vehicle and incubated for an additional 24 h. Media collected at the end of the second 24 h were analyzed by ELISA for apo AI and apo B as described in the experimental procedures. Data is expressed per mg of total cellular protein (n = 4 ± S.D.). Media concentrations of apo AI were significantly greater for cells incubated with 0.1, 1.0 and 10 μM 9-cis-RA (upper panel). Concentrations of apo B were not significantly different (lower panel). Results shown in this figure are representative of three separate experiments. ● 9-cis-RA; ○ ethanol (control). *P < 0.05; **P < 0.01; ***P < 0.005.

Fig. 2. Immunoblot analysis of apo AI in medium from HepG2 cells incubated with 9-cis-RA. HepG2 cells were incubated for 48 h with ethanol 1 μM 9-cis-RA or 10 μM 9-cis-RA as described in the legend for Fig. 1. Media proteins were separated by SDS-PAGE, immunoblotted using a monoclonal antibody directed against apo AI. Visualization and quantitation of radioactivity was performed on a Fuji BAS 2000 Phosphor imager. Upper panel: Bands from a representative immunoblot. Lower panel: Quantitation of bands shown in upper panel. *P < 0.01; **P < 0.001, n = 3 ± S.D.

3.2. 9-cis-Retinoic acid increases steady state levels of apo AI mRNA

The effect of 9-cis-RA on steady state apo AI mRNA levels was assessed by RNase protection assay and Northern blot analysis. Incubation of HepG2 cells with 10 μM 9-cis-RA for 48 h increased apo AI RNase resistant hybrids by 76%, indicating a significant increase in apo AI mRNA abundance (Fig. 3; P < 0.0001). By contrast, mRNA levels for a housekeeping gene, G3PDH, were slightly decreased (14%, P < 0.05). Levels of apo B mRNA were unaffected by 9-cis-RA (data not shown). Results of Northern blot hybridization were similar to those for RNase pro-
Incubation of HepG2 cells for 48 h with 10 μM 9-cis-RA increased apo AI mRNA abundance by 51% (P < 0.05) and slightly decreased levels of G3PDH mRNA (not significant). These results are consistent with increased expression of apo AI mRNA in response to 9-cis-RA.

4. Discussion

We have demonstrated that 9-cis-RA stimulates apo AI secretion and increases endogenous apo AI mRNA abundance in HepG2 cells. Secretion of apo AI was stimulated by about 50% using 1 μM and 10 μM 9-cis-RA, and a similar increase was found for apo AI mRNA abundance using both RNase protection assay and Northern blot hybridization. These results suggest a physiological role for retinoids, particularly 9-cis-RA, in regulating human apo AI gene expression and HDL cholesterol metabolism.

Our results are similar to those obtained for primary cynomolgus monkey hepatocytes incubated with stereoisomers of retinoic acid, including 9-cis-RA [20]. In these experiments, incubation with all-trans- or 9-cis-RA for 48 and 72 h stimulated apo AI secretion up to 2.7-fold and increased mRNA abundance threefold. A twofold increase in apo AI transcriptional activity was also observed, indicating that retinoids stimulate hepatic apo AI expression at the transcriptional level. In contrast with our results, however, Kaptein et al. [20] were unable to detect increases in endogenous apo AI expression using HepG2 cells. The reason for the disparity of results using HepG2 cells is unclear, although differences in the strains of HepG2 cells or subtle differences in the culture conditions could be responsible. A recent report has confirmed our observation that endogenous apo AI mRNA expression is stimulated by 9-cis-RA in HepG2 cells [21]. In their study, Berthou et al. demonstrate a doubling of apo AI mRNA following a 24-h incubation with 10 μM 9-cis-RA and all-trans-RA. Incubation with actinomycin D abolished apo AI mRNA induction by all-trans-RA, indicating that all-trans-RA acts at the transcriptional level [21]. Furthermore, unpublished observations based on nuclear run-on
experiments using HepG2 cells confirm that 9-cis-RA also activates endogenous apo AI gene expression at the transcriptional level (S. Karathanasis, personal communication).

A physiological role for retinoids in the regulation of apo AI gene expression was first proposed by Karathanasis and coworkers following the identification of a selective retinoic acid response element (RARE) in the apo AI promoter [22]. This response element (site A) interacts with several orphan steroid/thyroid hormone receptors, including ARP-1 and RXRα [12,13]. ARP-1 represses apo AI transcription while RXRα, expressed in the presence of RA, alleviates ARP-1 repression. ARP-1/RXRα heterodimers are known to bind to site A with high affinity [13], and ARP-1 may sensitize the apo AI promoter to RXRα and RA or act as a negative regulator to restrict RA signaling [23]. RXRα can form homodimers with itself or can heterodimerize with other steroid hormone receptors that are expressed in liver, including receptors for retinoic acid [14], thyroid hormone [24], vitamin D₃ [25], the peroxisomal proliferator activated receptors (PPAR) [26], and possibly the orphan receptor COUP-TF [27]. Interaction of either of these receptors with RXRα could potentially affect the equilibrium of RXRα at site A and thus affect transcriptional regulation of the apo AI gene. The action of the natural ligands for these receptors in modulating heterodimer formation and transactivation of gene expression adds complexity to the interactions occurring at site A. The simplest model suggested by published data [13,20–23] is that binding of 9-cis-RA to its endogenous receptor, RXRα, transactivates apo AI transcription at site A or alleviates repression of apo AI transcription mediated by binding of other receptors such as ARP-1 to site A. HepG2 cell extracts display specific binding to [³H]9-cis-RA that can be competed with excess unlabeled LG100268, a specific ligand for RXRα [28]. These data, along with immunoprecipitation results using subtype specific antibodies to the retinoic acid and retinoid X receptors, indicate the presence of RXRα in HepG2 cells. The effect of 9-cis-RA in mediating RXRα homodimer formation, heterodimer formation and apo AI gene transactivation in HepG2 cells, however, remains to be determined.

The stimulation of apo AI expression in HepG2 cells and primary monkey hepatocytes appears to require an induction period of at least 24 h and relatively high concentrations of retinoids [20,21]. This long induction period is consistent with regu-

![Fig. 4. Northern blot analysis of apo AI and G3PDH mRNA. Total RNA (10 μg) from HepG2 cells (treated with or without 10 μM 9-cis-RA) was separated on a 1% agarose gel and analyzed by Northern blotting using ³²P-labeled riboprobes. Two bands hybridized with the apo AI riboprobe: a ~ 5.0-kb band (not shown) and a ~ 1-kb band (designated on the gel as apo AI). The 5-kb band was caused by cross hybridization of the apo AI riboprobe with 28S ribosomal RNA. Quantitation of Northern blot results is shown in the lower panel. Radioactive bands shown in the upper panel were quantitated using Fuji BAS 2000 Phosphoimager. Data is expressed as photostimulated luminescence units (PSL) per μg of RNA. N = 4 ± S.D. *P < 0.05.](image-url)
lation at the transcriptional level but may also indicate that activation or suppression of other retinoid responsive genes is required before apo AI transcription can be activated. In addition, high concentrations of retinoids may be required to overcome degradation or metabolic isomerization. Kaptein et al. [29] have recently presented evidence that activation of apo AI gene transcription follows a rise in the mRNA for RARβ and suggest that this induction may be required for the subsequent apo AI response. Kaptein et al. also report that all-trans-RA undergoes extensive degradation in cynomolgus monkey hepatocytes. Thus, induction of retinoid receptors (or retinoid binding proteins) and degradation or isomerization of retinoids could contribute to the need for a lengthy induction period and relatively high concentrations needed to stimulate apo AI expression.

Regulation of apo AI expression by steroid hormones has been previously reported both in vivo and in vitro. Thyroid hormone was shown to stimulate apo AI production in rats, although much of this effect appears to be due to post-transcriptional stabilization of apo AI mRNA [30]. Estrogen was reported to stimulate apo AI secretion and increase apo AI mRNA abundance in HepG2 cells by > 50% at a concentration of only 20 nM [31]. The hypolipidemic drug and peroxisome proliferator gemfibrozil was also able to stimulate apo AI expression in HepG2 cells [32], suggesting a role for the PPAR orphan steroid receptor in apo AI gene expression. Recently, another orphan steroid receptor, HNF-4, was reported to enhance apo AI promoter activity when expressed in conjunction with apo AI promoter-reporter constructs in transfected hepatoma cell lines [33]. These findings suggest that hepatic apo AI expression and production may be controlled by a number of steroid hormones that are known to affect lipid metabolism as well as by steroid hormone receptors whose physiological role and specific ligand have not yet been determined.

The physiological significance of stimulation of hepatic apo AI secretion by 9-cis-RA or other retinoids remains unclear. Retinoids affect expression of numerous genes and may regulate a variety of physiological processes in vivo [34]. A physiological link between retinoids and lipid metabolism is conceivable, since dietary retinoids are transported by lipoprotein particles that contain apo AI (i.e. chylomicrons). Unexpectedly, however, administration of RA to rats (10 mg/kg) significantly reduced hepatic apo AI mRNA and did not elicit a change in plasma apo AI levels [21]. Moreover, vitamin A-deficient rats demonstrate two- to six-fold increases in hepatic apo AI mRNA levels without significant changes in hepatic or plasma apo AI protein levels [35]. Further studies are underway to clarify the physiological role of 9-cis-RA in regulation of apo AI gene expression and to address whether stimulating apo AI expression using 9-cis-RA or a chemical analog of 9-cis-RA could potentially elevate plasma levels of HDL cholesterol and reduce the severity of premature atherosclerosis and coronary heart disease.

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References


