REVIEW

Hormonal regulation of apolipoprotein AI

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ABSTRACT

Apolipoprotein AI (apo AI) is the major protein component of the serum high-density lipoprotein (HDL) particles. The antiatherogenic properties of apo AI alone or as part of HDL and their inverse correlation with the incidence of coronary heart disease underlie the clinical importance of the protein. A detailed understanding of the mechanisms by which apo AI is regulated will help us develop new and better ways to manipulate expression of the protein. Although there are many factors that influence apo AI expression, endogenous hormones are attractive because simple changes in abundance of these compounds will alter gene activity. Hormones belonging to the thyroid/steroid family that influence activity of the gene include

thyroid hormone, glucocorticoids, gender-specific steroids and retinoic acid. Whereas thyroid, glucocorticoid and estradiol enhance activity of the gene, retinoic acid and androgens decrease it. The mechanisms that mediate the effects of the hormones include direct effects of the ligand and nuclear receptor complex on gene activity. However, indirect means involving the participation of transcription factors other than the hormone receptors are also possible. In summary, members of the same hormone family may have different mechanisms that mediate their activities on apo AI gene activity.

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INTRODUCTION

Apolipoprotein AI (apo AI) is the major protein component of high-density lipoprotein (HDL) particles in serum. These particles have an important role in the protection against arteriosclerotic vascular disease, because they provide a shuttle mechanism that transports cholesterol from extrahepatic tissues to the liver for processing to bile salts (Miller *et al.* 1985, Franceschini *et al.* 1991). This pathway is a normal physiologic process called reverse cholesterol transport (RCT), and the end result of this process leads to lower concentrations of total cholesterol (Kashyap 1989). As hypercholesterolemia is one of the modifiable risk factors underlying ischemic heart disease, currently the most important cause of premature death in the world (Murray & Lopez 1997), new ways to lower

cholesterol are of interest to both the medical community and the general population.

The knowledge that apo AI alone or as part of HDL has antiatherogenic properties (Miller 1987, Barter & Rye 1996, Luoma 1997) provides the rationale for many investigators to examine the specific factors that regulate expression of the gene. A better understanding of how the gene is regulated will help us design new therapies to manipulate expression of the protein and thus augment RCT. The ability to control RCT is expected to benefit members of the population who suffer from arteriosclerotic vascular disease.

Well known factors that have a beneficial effect on the abundance of apo AI protein include: physical exercise (Huttunen *et al.* 1979, Ballantyne *et al.* 1982, Danner *et al.* 1984), alcohol consumption (McConnell *et al.* 1997, Manttari *et al.* 1997) and

FIGURE 1. Schematic representation of the apolipoprotein AI promoter. The *cis*-acting elements are shown by both geometric shapes and the most 5' nucleotide for each site. Listed below each of the sites, A, S, B, C, D and TATA, are the hormones or compounds and transcription factors that act at these sites. The +/- signs indicate whether the hormones, compounds or transcription factors have a positive or negative effect on the site.

being female (Heiss *et al.* 1980). These factors increase concentrations of apo AI protein. Recent studies show that pharmacologic agents such as fibrates, phenytoin and phenobarbital also modulate the concentrations of this protein (Nikkila *et al.* 1978, Kaste *et al.* 1983, Muuronen *et al.* 1985, Reddy 1985). In addition to the preceding pharmacologic agents, a host of natural hormones also influence apo AI protein concentrations. A better understanding of the mechanisms by which hormones regulate apo AI concentrations is particularly attractive, because this knowledge offers potentially useful information to help us manipulate apo AI expression by adjusting concentrations of the specific hormone or using analogs of the parent hormone. The list of hormones known to enhance or suppress apo AI protein include thyroid hormones (Apostolopoulos *et al.* 1987, Strobl *et al.* 1990), glucocorticoids (Elshourbagy *et al.* 1985, Ettinger *et al.* 1987), sex steroids (Kalin & Zumoff 1990) and retinoic acids (Zech *et al.* 1983). Most of these hormones exert effects at the gene level; a schematic diagram of the apo AI promoter and the respective *cis*-acting elements that mediate the effects of these hormones is presented below (Fig. 1). This review summarizes the knowledge of the mechanisms by which these compounds influence apo AI expression. The information provides a framework for the development of new directions in this field of research.

Thyroid hormones

The hormones synthesized by the follicular cells of the thyroid gland were amongst the first observed to affect cholesterol metabolism. Hypercholesterole-

mia was a useful marker for the diagnosis of hypothyroidism in patients before the general availability of rapid measurements of thyroid function. This abnormality was described in studies of hypothyroid patients conducted more than 60 years ago (Mason *et al.* 1930). Since then, numerous investigators have attempted to elicit the mechanisms responsible for the changes in lipid metabolism underlying hypercholesterolemia. Both the thyroid hormones, L-thyroxine (T_4) and L-triiodothyronine (T_3), are believed to have a variety of effects on the concentration of cholesterol. For example, in the presence of thyroid hormones, hepatic synthesis of cholesterol is actually enhanced (O'Hara *et al.* 1966), adding to the concentration of total cholesterol, but these hormones also increase the fractional clearance rates of very-low-density lipoprotein and low-density lipoprotein (LDL) particles (Rossner & Rosenqvist 1974, Abrams *et al.* 1981), in addition to the hepatic excretion of cholesterol (Miettinen 1968). The net effect of the opposing actions of the hormones in the liver gives rise to greater concentrations of cholesterol in hypothyroid individuals compared with those with euthyroid or hyperthyroid levels of the hormones.

Pertinent to this review are the effects of thyroid hormone on HDL concentrations and how these changes contribute to total cholesterol. In patients with hyperthyroidism, the concentrations of HDL cholesterol are generally lower (Scottolini *et al.* 1980, Valdemarsson 1983) than those in patients with hypothyroidism (Feely *et al.* 1980, Scottolini *et al.* 1980). However, other studies show decreased concentrations of HDL cholesterol in patients with hypothyroidism (Abrams & Grundy 1981). In addition, the different classes of HDL particles

appear to be affected differently by thyroid hormone (Aviram *et al.* 1982, Kris-Etherton *et al.* 1982). This lack of a uniform effect is mirrored in the studies of hypothyroid patients treated with thyroid hormone leading to variable outcomes (Wahlqvist *et al.* 1977, Ballantyne *et al.* 1979, Brun *et al.* 1980, Engelken & Eaton 1980, Malkonen & Manninen 1981, Aviram *et al.* 1982, Raziell *et al.* 1982). Part of this lack of uniformity may arise from the fact that HDL particles are synthesized by two tissues in the body – liver and small intestine (Smith *et al.* 1978).

A better understanding of the actions of thyroid hormones on apo AI expression comes from animal studies and cell culture models. *In vivo* studies in rodents show that thyroid hormone increases the concentrations of apo AI protein and the corresponding mRNA (Romney *et al.* 1992, Mooradian *et al.* 1996). There is debate whether changes in transcription of the gene match the observed increase in the protein and mRNA after exposure to thyroid hormone (Vandenbrouck *et al.* 1995). Given this controversy, subsequent studies have used cell culture models to examine the effects of the hormones on transcription of the apo AI gene, using transient transfection experiments. The extension of promoter studies to cell culture models is based on the examination of motifs within the apo AI promoter that resemble T_3 -response elements (TRE) (Taylor *et al.* 1996b). A motif that resembles a positive TRE is located between -208 and -193 in the rat apo AI promoter, and corresponds to a similar sequence in the human gene between -214 and -192 (Rottman *et al.* 1991). This site matches the typical consensus TRE, except that the 6-bp repeats are separated by 2 bp, rather than 4 bp, of non-specific nucleotide sequence. When this site is intact, the response of the promoter is induced by T_3 and this positive effect requires the presence of the T_3 -receptor (TR) (Romney *et al.* 1992, Taylor *et al.* 1996b). Furthermore, when this T_3 -response element, TRE, was fused to the SV40 viral promoter, T_3 and its receptor caused a fourfold induction in the activity of the chimeric construct (Romney *et al.* 1992). Additional proof that the element from apo AI DNA was indeed a TRE comes from studies showing that TR binds to this sequence in the form of either a homodimer or a heterodimer, comprised of TR and retinoic acid receptor- α (RXR α) (Taylor *et al.* 1996b). The dominant actions of thyroid hormone on the apo AI promoter are mediated by the TRs, TR α and TR β . These proteins belong to a superfamily of ligand receptors that also function as nuclear transcription factors (Beato *et al.* 1995, Laudet 1997). This superfamily of receptors includes those that bind to the thyroid and steroid hormones. The actions of

these receptors are regulated by the binding and dissociation of specific hormones. In addition to TR, the apo AI-positive TRE may also bind two orphan members of the receptor superfamily: ARP-1 (Ladiaz & Karathanasis 1991) and HNF-4 (Sladek *et al.* 1991). The interaction of the various members of the receptor superfamily binding to the positive TRE contributes to the regulation of apo AI gene activity (Fuernkranz *et al.* 1994, Ginsburg *et al.* 1995, Murao *et al.* 1997).

The positive TRE is not the only T_3 -responsive motif within the apo AI promoter that mediates the effect of thyroid hormone. Upon removal of the positive TRE, T_3 had a suppressive effect on the deletional construct and this observation led to the identification of a negative TRE fused to the 3' end of the TATA-element in the apo AI promoter (Taylor *et al.* 1996b). This negative TRE also binds to TR. A comparison of the nucleotide sequence of the negative and positive TRE shows that, whereas the positive TRE is comprised of two 6-bp repeats (Naar *et al.* 1991, Umesono *et al.* 1991), the negative element also had the same 6-bp motif, but only a single copy (Carr & Wong 1994, Carr *et al.* 1992, Taylor *et al.* 1996b). Thus it is not surprising to find the binding of TR dimer to the positive TRE and a monomer to the negative TRE (Taylor *et al.* 1996b). The results from these studies show the dual effects of thyroid hormone on apo AI gene activity. This information has already proven useful in the research and development of thyromimetics that may eventually prove to be useful in regulating levels of apo AI expression (Taylor *et al.* 1997).

Glucocorticoids

The clue that glucocorticoids affect the expression of apo AI comes from clinical observations showing that serum concentrations of both apo AI protein and HDL are increased in patients with endogenous or exogenous glucocorticoid excess (Ettinger *et al.* 1987). This finding of a stimulatory action of glucocorticoids in humans has been extended to animals, and these models are useful for examining the mechanisms that underlie the actions of glucocorticoids. For example, rats given oral doses of the synthetic glucocorticoid, dexamethasone, on four consecutive days exhibited a twofold increase in hepatic apo AI mRNA (Elshourbagy *et al.* 1985). Similarly, rats injected with the steroid had three- to fourfold greater concentrations of apo AI in the serum and this was matched by a corresponding four- to sixfold induction of the mRNA in liver (Taylor *et al.* 1996a). Other investigators treated male rats with hydrocortisone for 20 days and found that the concentration of plasma apo AI increased to

150% of that in controls, and this finding was accompanied by a twofold increase in levels of the mRNA (Staels *et al.* 1991). In support of the data from animal models, the glucocorticoids also increase apo AI mRNA and augment synthesis of the protein in hepatoma cell lines (Masumoto *et al.* 1988, Varma *et al.* 1992). These observations clearly show that glucocorticoids increase apo AI gene expression in both *in vitro* and *in vivo* models.

Although one of the mechanisms by which glucocorticoids exert their effect involves their binding to a nuclear receptor, the resulting complex controlling gene activity, the effect of the steroid on apo AI expression is mediated by a different pathway. Recent results of experiments in our laboratory indicate that the stimulatory effects of glucocorticoids do not appear to require the binding of the glucocorticoid receptor, GR, to apo AI DNA (Taylor *et al.* 1996a). Glucocorticoids definitely have a transcriptional effect, as demonstrated by 'run-on' studies in hepatic nuclei of rats injected with dexamethasone. These showed that transcription of the apo AI gene was two- to threefold greater in treated animals compared with controls. Additional supportive evidence that glucocorticoids have a transcriptional effect on apo AI comes from transient transfection studies in HuH7 cells, a fetal hepatoma cell line known to contain the GR (Nakabayashi *et al.* 1989). Consistent with this finding, the introduction of chloramphenicol acetyl transferase (CAT)-reporter constructs containing the apo AI promoter in the presence of dexamethasone stimulated CAT-activity 3.1-fold. Furthermore, the internal deletion of site B (-170 to 145) from the promoter completely abolished the stimulatory effects of glucocorticoids (Taylor *et al.* 1996a). The B-motif is comprised of two non-nucleotide repeats in a head-to-tail orientation separated by four base pairs (Chan *et al.* 1993, Harnish *et al.* 1994). Both of these repeats appear to be critical for the response of the motif to glucocorticoids, because disruption of either 9-bp repeat inhibited the activity of the entire motif. Next, results of electrophoretic mobility shift studies showed that the GR does not bind to site B. However, dexamethasone clearly induced site B binding activities, but these activities were not attributed to the GR. Nonetheless, the receptor has an important role in the light of recent results showing that the GR antagonist, RU486, added to rat hepatoma cells (McARH8994 cells) completely blocks the increase in apo AI mRNA that follows treatment with dexamethasone (Saladin *et al.* 1996). In addition, the presence of the receptor alone is not sufficient, as treatment of COS-1 cells, which lack endogenous GR, with dexamethasone did not

increase apo AI promoter activity in the presence of transfected GR, suggesting that the receptor acts via an indirect mechanism. Such a mechanism could involve GR activation of a factor that, in turn, binds to site B. Previous data showing that the induction of rat apo AI mRNA by dexamethasone is blocked by cycloheximide, a compound that inhibits protein synthesis, provide additional support for an indirect effect of glucocorticoids (Wong & Oppenheimer 1986). The conclusion based on these observations is that glucocorticoid-stimulated expression of apo AI is mediated by an indirect mechanism, perhaps through enhancement of the actions of transcription factor(s) that in turn activate the promoter.

Site B appears to be the mediator of glucocorticoid activity, and recent studies have shown that it has the potential to bind dimers of the transcription factor, hepatic nuclear factor-3 (HNF-3). In fact, previous studies (Harnish *et al.* 1994, 1996) have demonstrated that HNF-3 β binds to the B-motif in human apo AI DNA and increases transcription of the gene. As glucocorticoid induces site B binding activities (Taylor *et al.* 1996a), and HNF-3 β is one of the transcription factors that binds to this site in the human apo AI promoter, then the stimulatory effect of glucocorticoids on expression of the gene may act via increased abundance or activity of HNF-3 β .

Although the preceding summary focuses on the transcriptional effects of the steroid, post-transcriptional effects of glucocorticoid on apo AI gene expression are also possible. If transcription and translation alone are responsible for the steroid-induced increases in apo AI and these sequential processes have reached equilibrium, then the fold induction attributed to each process should be equal. However, apo AI transcription was increased two- to threefold, mRNA levels five- to sixfold and secreted protein three- to fourfold, which raises the possibility that glucocorticoids may have additional post-transcriptional effects that are yet to be elucidated.

Gender-specific steroid hormones

The risk of ischemic heart disease is approximately twofold greater in men than in women (Kalin & Zumoff 1990). In part, this differential in the risk of acquiring the disease is attributed to the actions of the sex steroids (Kalin & Zumoff 1990) on serum lipoprotein concentrations (Godsland *et al.* 1987). Estrogens increase the concentrations of apo AI protein in females; in contrast, testosterone decreases abundance of HDL in both sexes (Godsland *et al.* 1987, Sacks *et al.* 1995) and thus gives rise to a more atherogenic profile. Although the clinical observations

are straightforward, the mechanisms by which the sex steroids influence the plasma concentrations of apo AI are far from being simple.

The complex nature of estrogen effects arises from the involvement of the hormone in multiple steps of lipoprotein metabolism. For example, clinical studies show that estrogen replacement therapy in post-menopausal women increases apo AI concentrations by suppressing the activity of hepatic triglyceride lipase (HTGL) (Hazzard *et al.* 1984, Applebaum-Bowden *et al.* 1989, Colvin *et al.* 1991, Quintao *et al.* 1991). In addition, other clinical studies showed that the hormone also increases synthesis of the lipoprotein (Schaefer *et al.* 1983, Sacks *et al.* 1995). Unlike clinical studies, with their restrictions, animals models make it possible to examine specific steps in apo AI gene expression that are influenced by the hormone. Thus, in support of the stimulatory effects of estrogen on apo AI expression, rodents treated with pharmacologic doses of estrogen were found to have greater concentrations of apo AI mRNA and gene transcription. Although these actions should give rise to higher levels of apo AI protein, the hormone also had post-transcriptional effects that decreased the abundance of the protein (Seishima *et al.* 1991, Tang *et al.* 1991, Srivastava *et al.* 1993). For example, pharmacologic doses of estrogens enhance LDL receptor (LDLR) activity, thereby decreasing apo AI (Windler *et al.* 1980, Srivastava *et al.* 1993, Croston *et al.* 1997). Thus it is evident that estrogens regulate many aspects of apo AI gene expression.

Given the possibility that estrogens affect several steps of apo AI expression, investigators have turned to the use of cell lines to address specific questions. Consistent with the observations *in vivo*, the exposure of Hep G2 cells to estrogen increases the concentrations of apo AI protein, and this effect is dependent on the amount of hormone (Archer *et al.* 1986, Tam *et al.* 1985). In addition, increases in apo AI mRNA correlate well with the increase in the concentrations of the protein (Archer *et al.* 1986). Further studies in Hep G2 cells showed that this cell line contained two estrogen-binding activities in the nucleus, with different affinities for the hormone. Estrogen induction of apo AI protein and mRNA correlated with the higher-affinity site. The addition of estrogen concentrations beyond those required to saturate the nuclear binding activity had minimal effects on induction of apo AI (Archer *et al.* 1986, Tam *et al.* 1986). These results show that estrogen affects apo AI expression via the estrogen receptor by regulating either gene transcription or mRNA stability. Results of recent studies suggest that the steroid influences transcrip-

tion of the gene (Jin *et al.* 1998). However, the apo AI promoter does not contain a binding motif for the nuclear estrogen receptor.

The preceding reports suggest that the actions of the hormone on apo AI involve a receptor for estrogen; however, the lack of a recognizable estrogen receptor binding site in apo AI promoter points to an indirect effect of the hormone. In support of the latter idea, recent studies have examined the *cis*-acting sites and the respective nuclear factors that participate in the actions of the hormone. Results showed that estradiol potentiated the synergistic interactions between two transcription factors, HNF-4 and HNF-3 β (Harnish *et al.* 1996), that bind to tandem *cis*-acting elements, sites A (Malik & Karathanasis 1996, Murao *et al.* 1997) and B (Harnish *et al.* 1994) respectively. The synergism between HNF-4 and HNF-3 β is believed to be mediated by factor(s) that influence protein-protein interactions between the two proteins. Recent studies show that the co-repressor, RIP140, could fulfil such a role, and the activity of this factor is modulated by estradiol (Harnish *et al.* 1998). The current belief is that estradiol-activated endoplasmic reticulum somehow sequesters RIP140 protein, leading to the relief of repression and thus greater levels of apo AI gene transcription.

This section can deal only briefly with the effects of androgens on apo AI expression, because there is less information on this topic compared with that of estrogens. In general, androgens act in a fashion opposite to that of estrogen. For example, androgens upregulate HTGL activity (Larosa 1995) and antagonize the estrogen induction of (low density lipoprotein receptor (LDLR) activity (Croston *et al.* 1997). Although experiments in animals show that testosterone influences apo AI gene activity (Tang *et al.* 1991), the results of cell culture studies suggest that the hormone acts by antagonizing the actions of estrogen, and that androgens have no direct effect on gene transcription (Tam *et al.* 1986).

In summary, the sex hormones influence both catabolic rates and hepatic synthesis of apo AI. The effects on hepatic synthesis are complex and involve the regulation of gene expression at several levels. Estrogen upregulates apo AI promoter activity by influencing the synergy between HNF-3 β and HNF-4 at sites B and A respectively. Androgens are believed to block the actions of estrogens.

Retinoic acids

Retinoids are commonly used in the clinical setting to treat a wide range of dermatologic disorders, including nodulocystic acne, rosacea, severe psoriasis, disorders of keratinization, and the prevention

of skin cancers. In humans, the synthetic retinoids, isotretinoin (13-*cis* retinoic acid) and etretinate (aromatic retinoid) induce significant increases in triglycerides, total and LDL cholesterol, but they have an opposite effect on HDL, leading to concentrations decreased by 10–24% (Zech *et al.* 1983, Melnik *et al.* 1987). These retinoid-induced alterations are felt to be detrimental because of a more atherogenic lipid profile that thus exposes the patients to the risk for developing premature cardiovascular disease. However, the changes associated with retinoids were ameliorated by administering fish oil that contained omega-3 fatty acids in conjunction with retinoid therapy. In fact, the combined therapy led to an 11% increase in HDL concentrations (Ashley *et al.* 1988).

The mechanism by which retinoids regulate the apo AI component of HDL has been examined using both *in vitro* and *in vivo* model systems. When normal rats are treated with retinoic acid (RA), the effects on hepatic concentrations of apo AI mRNA are variable, with decreases ranging from 60 to -70%, but protein concentrations in the serum remain unchanged (Berthou *et al.* 1994). In contrast, other studies showed that RA increased concentrations of apo AI mRNA in small intestine, but there was no effect on abundance of hepatic mRNA (Nagasaki *et al.* 1994). However, vitamin A-deficient rats given excess RA had increased hepatic concentrations of apo AI mRNA (Nagasaki *et al.* 1994). Other investigators have assessed the actions of retinoids on the biosynthesis of apo AI *in vitro* using human intestinal and liver cancer cell lines, Caco-2 and HepG2 respectively. Exposure of Caco-2 cells to either all-*trans* or 9-*cis* RA increased both the abundance of apo AI mRNA and protein secretion by 40–80% above control. In Hep G2 cells or primary cultured hepatocytes, retinoids had a similar effect, leading to an induction of apo AI from 25% to 30% that matched secretion of the protein (Berthou *et al.* 1994, Kaptein *et al.* 1994, Giller *et al.* 1995). Furthermore, the stimulatory effect of RA on apo AI mRNA was completely blocked by actinomycin D, thus pointing to an underlying transcriptional effect. These findings suggest that the actions of retinoids on synthesis of apo AI protein are opposite to those on the levels of HDL in serum, but that retinoids have a stimulatory or inhibitory effect on hepatic mRNA levels, depending on the model studied.

Evidence in support of the stimulatory effect of retinoids on apo AI gene expression comes from transient transfection studies of the promoter. Others (Rottman *et al.* 1991) showed that site A (bp -214 to -192) within the human apo AI promoter is a highly selective retinoic-acid-responsive element

that responds preferentially to one member of the retinoic acid receptor family, RXR α . Overexpression of RXR α exposed to 10^{-6} M RA activated a reporter construct containing four copies of site A to over 300-fold above baseline. In addition, the authors showed that RXR α binds to radiolabeled site A. These findings appear to contradict the clinical data, in that effects of RA on apo AI transcription should, theoretically, increase the abundance of the protein and, hence, result in greater HDL concentrations. The explanation underlying the discordant effects of RA in clinical studies compared with those observed in animal and *in vitro* models is not clear. As retinoids are morphogens, the functions of which are most pronounced during development and cellular differentiation, it is conceivable that their stimulating effects on apo AI gene expression are restricted to the fetal or early neonatal period. This may explain why RA has a stimulatory effect in the fetal Hep G2 cells. Alternatively, the *in vitro* data may reflect accurately the apo AI gene activity at the cellular level in human/animal liver, but increased catabolism of the protein that surpasses the synthesis rate may result in a net reduction in serum apo AI protein and HDL levels. Regardless of the mechanism, it is clear that further studies are needed to test these hypotheses.

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