Conference: Avian Lipoprotein Metabolism: An Update

Evolution of Processes and Regulators of Lipoprotein Synthesis: From Birds to Mammals[1,2]

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ABSTRACT Mammalian lipoproteins are synthesized in the liver and secreted into the blood plasma where they are targeted to specific tissues. Through specific cell surface receptors, hepatic lipoproteins are taken up and their lipid contents are then used for anabolic and energy requirements. Because of the well-established role that plasma lipoproteins play as risk factors for the development of cardiovascular disease, a great amount of attention has been directed toward understanding their metabolism and biosynthesis. The major focus of this report is to review the evolution of gene products that are essential in regulating, synthesizing, assembling and secreting the lipid and protein components of lipoproteins. Using the primordial vitellogenin lipoprotein system as the paradigm, I show how metabolic signals derived from the sterol biosynthetic pathway provide a coordinate regulation of genes necessary to assemble and secrete mammalian apolipoprotein B–containing lipoproteins. In lower species, estrogen induces the expression of genes required for both vitellogenin synthesis and its tissue targeting (the vitellogenin receptor). This coordinate induction provides lipid to the ovaries for egg development. In mammals, a sterol-derived metabolic signal regulates the expression of genes required for lipoprotein synthesis and for the LDL receptor. This signal is not sex linked, providing an adaptive advantage to changes in nutritional status. J. Nutr. 127: 795S–800S, 1997.

KEY WORDS: • *apolipoprotein B* • *lipoproteins* • *oxysterols* • *cholesterol-7α-hydroxylase*

The ability of multicellular organisms to transport fat, in the form of lipoprotein particles, through the circulatory system is one of the most primordial functions. Although there are several distinct lipoprotein transport systems that vary with the phylogeny of the organism, they all use similar mechanisms for assembly, secretion and tissue targeting. The major emphasis of this report is to review the advances made in our knowledge concerning the development of regulatory processes involved in the assembly of apolipoprotein (apo)[3] B-containing lipoproteins by the liver of mammals.

The functional basis for why lipoprotein transport systems initially evolved provides insights into the complexity of how this pathway works and is controlled. Simply put, lipoproteins provide a means through which energy in the form of water-insoluble fat can be distributed from its site of synthesis and absorption to specific tissues and cells. The bioavailability of energy stored as fat (~37 kJ/g) is significantly greater than that which can be stored as carbohydrate glycogen (~17 kJ/g). The evolutionary advancement of storing energy in the form of fat has provided organisms with a tremendous advantage in adapting to environmental and developmental changes. For example, hibernation is successful only if the organism has had the ability to build up large stores of fat (triglycerides) during periods when food supplies are plentiful. Migrating birds and insects rely mainly on the lipoprotein transport of fat from stores to muscles, abrogating the need to stop for nourishment. When laying eggs, insects, fish, amphibians, reptiles and birds display over three orders of magnitude increases in the transport of fat, from the liver to oocytes, in order to facilitate egg development. Increases in fat transport, whether it involves lipophorin, vitellogenin or apo B–containing lipoproteins, show a common induction of the expression of genes necessary for their individual biosynthetic, assembly and secretion steps. Moreover, when physiologic requirements dictate the need for increased lipoprotein synthesis, metabolic signals activate these coordinate processes via increased transcription of specific genes. This concept has long been appreciated by those interested in dissecting the molecular events necessary for transcriptional regulation of gene expression. Hormonal induction of vitellogenin synthesis in insects and birds was one of the first model systems used to advance our knowledge of transcriptional regulation (Dolphin et al. 1971).

LIPOPROTEIN STRUCTURE AND FUNCTION

Mammalian plasma lipoproteins are stable emulsions of lipids and proteins that are arranged in spherical structures having

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[3] Abbreviations used: apo, apolipoprotein; MTP, microsomal triglyceride transfer protein; SREBP, sterol regulatory binding protein; SRE, sterol regulatory element.

densities less than that of water. The outside surface contains a single layer of phospholipids (monolayer) that surrounds a neutral lipid core consisting of reciprocally varying relative amounts of triglycerides and cholesterol esters (Davis et al. 1982). Specific proteins are embedded into the surface monolayer. These proteins function to assemble the lipoprotein and/or to direct metabolism and tissue targeting. In mammals there are three major tissues responsible for lipoprotein synthesis: liver, intestine and the yolk sac of the developing fetus. All these tissues produce triglyceride-rich VLDL. Because genetic alterations in the apo B gene, but not other apolipoprotein genes, impair the assembly of VLDL, apo B, but not other apolipoproteins, is required for VLDL assembly and secretion (Young 1990). The recent discovery that ablation of the apo B gene in mice blocks fetal development emphasizes the point that lipoproteins are essential for egg and/or fetal nutrition (Farese et al. 1995).

VLDL ASSEMBLY REQUIRES APOLIPOPROTEIN B

Apolipoprotein B is one of the largest single-chain proteins known. It has structural motifs that afford unique amphipathic properties. Unlike other mammalian apolipoproteins, apo B does not exchange between lipoprotein particles; thus it behaves as an integral membrane protein. The structural motifs responsible for the association of apo B with lipids are complex and are likely to involve several domains having different secondary structures. Within the 4536 amino acids of mature apo B-100 are β-sheets having hydrophobic and hydrophilic surfaces, short stretches of amphipathic α-helices (thought to be too short to act as typical membrane-spanning domains) and β-strands that have the potential to form amphipathic domains (Segrest et al. 1994).

The functional basis for apo B's large size has recently been uncovered in elegant experiments examining the genetic basis for hypobetalipoproteinemia in humans and developing in vitro models that faithfully express the hypobetalipoproteinemic phenotype in cultured cells (reviewed in Young 1990). This disorder is characterized by a genetic alteration of the apo B gene, resulting in either the synthesis of truncated forms of apo B that are too short to assemble lipoproteins or inactivation of the gene. Mutant apo B forms having a size smaller than 31% of the N-terminus of the maximal coding region transcribed by the apo B gene show an inability to assemble lipoproteins containing a neutral lipid core. Further analysis showed that the size of apo B and the density of the lipoprotein varied inversely (Spring et al. 1992, Yao et al. 1991).

STRUCTURAL MOTIFS IN APOLIPOPROTEIN B RESEMBLE SOME OF THOSE FOUND IN VITELLOGENIN

The unique amphipathic character of apo B has been proposed as the essential feature that allows it to assemble VLDL (Davis 1991). Amphipathic β-strands present in proline-rich repeats of human apo B are similar to those that bind lipid in vitellogenin (Raag et al. 1988). These structural motifs in vitellogenin have been proposed to play a role in the assembly of the lipovitellogenin complex. It is interesting that the vitellogenin receptor of the chicken oocyte recognizes mammalian apoproteins B and E, suggesting that the lipoprotein receptor system of mammalian tissues may have evolved or co-evolved from or with the vitellogenin/oocyte system (Nimpf et al. 1994). Several structural analogies exist between apo B and vitellogenin. Intact vitellogenin, like apo B48, has a Mr of approximately 200 kDa. In the lipoprotein complex lipovitellogenin, vitellogenin exists as a dimer (Mr 500 kDa) similar in size to apo B100. Regions of the amino termini of vitellogenin and apo B are homologous (Baker 1988). The central region of vitellogenin has a long stretch of phosphorylated Ser residues; apo B is similarly phosphorylated on serine residues.

High resolution X-ray crystallographic analysis and ^{31}P-nuclear magnetic resonance studies of the lipovitellogenin complex show that β-sheets of vitellogenin surround a cavity believed to be filled with lipid (Raag et al. 1988). It has been proposed that as more lipid is added, the cavity expands by movement of the β-sheets. Apolipoprotein B might associate with lipids in a similar manner, because VLDL also consists of a cavity filled with lipid and surrounded by apo B. Furthermore, the β-sheet structures of apo B are thought to contribute to its association with lipid. If, like vitellogenin, apo B serves as the scaffolding for the lipid core, VLDL assembly might involve a process by which, as lipid is added, the surrounding apo B folds to accommodate a growing lipid core.

VLDL IS ASSEMBLED IN THE ENDOPLASMIC RETICULUM

Immuno-electron microscopic analysis of intracellular apo B show it is present at the earliest site of the secretory pathway in the rough endoplasmic reticulum unassociated with discernible VLDL particles (Alexander et al. 1976). Particles resembling VLDL containing apo B were observed in the lumen of the terminal junctions between the rough and smooth endoplasmic reticulum. These data indicate that *1*) VLDL is assembled in the endoplasmic reticulum and *2*) because the VLDL particles in the lumen of the endoplasmic reticulum have a uniform size similar to that observed in plasma, the assembly does not involve a gradual build-up of lipid core.

MOVEMENT OF APOLIPOPROTEIN B OUT OF THE ENDOPLASMIC RETICULUM IS RATE LIMITING

In steady-state labeled rat hepatocytes, the majority of both apo B100 and apo B48 was confined to the endoplasmic reticulum, suggesting that the rate-limiting step was movement out of this organelle, the site of VLDL assembly (Borchardt and Davis 1987). Pulse-chase studies show that the first-order rate constant describing the movement of both apo B48 and apo B100 out of the rough endoplasmic reticulum determined the rate constant for the movement out of the hepatocyte (Borchardt and Davis 1987). There are two processes necessary for VLDL assembly that are localized to endoplasmic reticulum: *1*) translocation of apo B from the cytoplasmic surface into the lumen and *2*) the addition of lipid to the apo B nascent chain during its movement into the lumen.

APOLIPOPROTEIN B IS DEGRADED INTRACELLULARLY BY A PROCESS THAT CAN ACCOUNT FOR POST-TRANSCRIPTIONAL REGULATION OF SECRETION

Quantification of the portion of de novo synthesized apo B that was lost from the cellular pool of pulse-labeled rat hepatocytes (Borchardt and Davis 1987) showed that only a fraction was recovered in the culture medium. These observations were interpreted as indicating that a significant portion of apo B is degraded intracellularly, and this may be an important process regulating VLDL assembly and secretion. Intracellular degradation can explain why the secretion of VLDL apo B is varied, whereas the relative abundance of apo B mRNA remains nearly constant (reviewed in Davis 1991, Dixon and Ginsberg

1993, Vance and Vance 1990). There are several situations both in vivo and in vitro supporting this proposal. The amount of apo B48 that is secreted by hepatocytes isolated from the liver of unfed rats is decreased, whereas the relative abundance of apo B mRNA is unchanged (Leighton et al. 1990). These findings suggest that a post-transcriptional process is responsible for the decreased secretion of apo B by the liver of unfed rats (Leighton et al. 1990). In HepG2 cells, thyroid hormone and oleic acid increase the secretion of apo B100, whereas the relative abundance of apo B mRNA remains unchanged (Pullinger et al. 1989). The mechanism accounting for the post-transcriptional increase in apo B100 secretion by oleic acid–stimulated HepG2 cells has been found to be due to decreased intracellular degradation (Dixon et al. 1991). Although these cited studies suggest that degradation of apo B may be linked to the process controlling secretion, they do not necessarily indicate that degradation per se is the regulatory process. It may be a consequence of a blocked step in the secretion pathway (summarized below).

TRANSLOCATION OF APOLIPOPROTEIN B REQUIRES A UNIQUE PROCESS THAT MAY GOVERN ITS METABOLIC FATE: VLDL ASSEMBLY OR DEGRADATION

The translocation of apo B across the endoplasmic reticulum is inefficient, causing apo B to accumulate in two functionally distinct pools: one (incompletely translocated) is degraded, whereas the other (fully translocated into the lumen) is responsible for VLDL assembly and secretion (Davis et al. 1990). Thus, the translocation step is one of the major sites that determines the fate of de novo synthesized apo B: degradation or secretion as a VLDL particle. Inefficient translocation of apo B may be due to the presence of multiple "lipid-binding" domains, required for VLDL assembly. By virtue of their amphipathic structure, these domains may arrest translocation by facilitating integration into the endoplasmic reticulum membrane. This proposal may account for inefficient translocation of apo B across the endoplasmic reticulum of liver cells as demonstrated by exposure on the cytoplasmic surface (Davis et al. 1990). In vitro translation/translocation assays show that phospholipid precursor monomethylethanolamine blocks apo B15 translocation (Rusinol and Vance 1995). In HepG2 cells, translocation of de novo–synthesized apo B determines how much is degraded or secreted as a lipoprotein (Bonnardel and Davis 1995). It remains to be determined whether translocation regulates apo B secretion in mammalian livers in vivo.

To overcome the obstacles inherent in the translocation of apo B, gene products that are probably not required by other proteins are essential for translocating and secreting apo B (Du et al. 1994). Perhaps the most compelling data showing that apo B requires a unique process for translocation is the finding that CHO cells exhibit a complete inability to translocate apo B forms large enough to assemble VLDL (apo B53), but not truncated apo B15 (Thrift et al. 1992). Translocation-arrested apo B53 is by a process inhibitable by the cysteine active site protease inhibitor acetyl-leucine, leucine, nor-leucal (ALLN) (Thrift et al. 1992). Although apo B lacks any amphipathic α-helices that are sufficiently long to be predicted to span a membrane bilayer, in CHO cells treated with ALLN, apo B assumes a stable trans-membrane orientation in the endoplasmic reticulum (Du et al. 1994). The segments responsible for translocation arrest were found to reside outside of the membrane-spanning domain (i.e., on resides lying on the cytoplasmic side of the endoplasmic reticulum) (Du et al. 1994). The inability of CHO cells to translocate apo B across the endoplasmic reticulum is likely to be the lack of expression in these cells of a gene product that is present in liver and intestine. Several lines of evidence indicate that this gene product is microsomal triglyceride transfer protein (MTP).

MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN TRANSFERS LIPID TO THE NASCENT LIPOPROTEIN PARTICLE AND IS REQUIRED FOR APOLIPOPROTEIN B TRANSLOCATION: ANALOGIES TO VITELLOGENIN AND INSECT LIPOPHORIN

Microsomal triglyceride transfer protein exists as a 97-kDa protein in the lumen of the endoplasmic reticulum as a heterodimer associated with protein disulfide isomerase (55 kDa) (Wetterau and Zilversmit 1985). Microsomal triglyceride transfer protein is not highly homologous to most known lipid transfer proteins, except that it does have limited regions of similarity to vitellogenin and cholesterol ester transfer protein (Gordon et al. 1995, Shoulders et al. 1993).

Several lines of evidence indicate that MTP is essential for apo B translocation. First, in cells not expressing MTP, the translocation of apo B constructs having a size sufficient to assemble lipoproteins is completely blocked (Du et al. 1994). However, co-expression of MTP in COS and HeLa cells allows apo B to be translocated and secreted as a lipoprotein particle (Gordon et al. 1994, Leiper et al. 1994). Secondly, in the human recessive disorder abetalipoproteinemia, functional loss of MTP blocks the secretion of apo B by both the liver and intestine, the major tissue sites of MTP (Sharp et al. 1993, Shoulders et al. 1993, Wetterau et al. 1992). Recent studies suggest that the inability of the livers and intestines of abetalipoproteinemic patients to secrete apo B is due to a block in its translocation across the endoplasmic reticulum (Du et al. 1996). Variable expression of MTP and/or availability of lipid may account for the variable translocation of apo B and secretion as VLDL.

COORDINATED REGULATION OF VLDL ASSEMBLY AND SECRETION: ANALOGIES TO LIPOPHORIN AND VITELLOGENIN PATHWAYS

In lower phylogenetic species, the most fundamental role of lipoprotein assembly and transport is to provide anabolic lipid for egg development. The cascade involves three coordinated steps: *1*) hormonal activation of the fat body (insects) or liver (fish, reptiles, amphibians and birds), *2*) rapid biosynthetic induction of the gene products responsible for apolipoprotein synthesis (lipophorin and vitellogenin) and lipogenic enzymes and *3*) assembly and secretion of lipoprotein complexes. Within this perhaps oversimplified framework, a similar coordinated cascade occurs for mammalian VLDL assembly and secretion. Our current working hypothesis is that the gene products required for mammalian VLDL assembly and secretion and its regulatory signals were derived from the vitellogenin lipoprotein system. The major evolutionary advantage of the mammalian VLDL secretion pathway is that it can respond to nutritional needs, independent of reproduction processes.

Birds produce both vitellogenin and hepatic VLDL. Both lipoprotein transport systems show rapid induction by estrogen (Dolphin et al. 1971). Similar to its induction of vitellogenin synthesis, estrogen also increases the hepatic production of VLDL in birds. Recent studies in our laboratory suggest that a similar coordinated cascade of induction of the genes required for mammalian VLDL assembly and secretion occurs.

TABLE 1

Effect of cholestyramine and cholesterol-7α-hydroxylase on the expression of hepatic genes and lipogenic processes[1]

Gene	Cholestyramine[2]	Cholesterol-7α-hydroxylase[3]
Cholesterol-7α-hydroxylase	+	ND
HMG-CoA reductase	+	+
HMG-CoA synthase	+	+
Squalene synthase	+	+
Farnesyldiphosphate synthase	+	+
Cholesterol synthesis	+	+
Triglyceride synthesis	+	+
Phospholipid synthesis	+	+
Apolipoprotein B synthesis	0	0
VLDL apo B secretion	+	+

[1] Each designation indicates a statistically significant change at $P < 0.01$: + is increased; − is decreased; 0 is no change; ND is not determined.

[2] Values represent either a change in enzyme activity, synthesis or secretion from rats treated with cholestyramine for 2 wk.

[3] Cholesterol-7α-hydroxylase was stably expressed in cultured hepatoma cells. Values represent either a change in enzyme activity, synthesis or secretion.

We propose that nutritional and metabolic needs for hepatic VLDL secretion are signaled by a steroid that is not involved in the signaling required for reproduction. As a result, the mammalian VLDL secretory pathway can respond in a more versatile manner, independent of sex-linked processes. Although the identity of this signal remains to be elucidated, it—like estrogen—seems to be derived from the isoprenoid/steroid biosynthetic pathway. It is possible that this steroid signal has evolved from the estrogen signal.

CHOLESTYRAMINE INDUCES HEPATIC LIPOGENESIS AND VLDL ASSEMBLY AND SECRETION

Cholestyramine is a synthetic anionic resin that binds bile acids in the intestinal lumen and prevents their reabsorption. It was developed as a therapeutic agent for the treatment of hypercholesterolemia (high plasma concentration of LDL, a major risk factor in cardiovascular disease). By preventing the reabsorption of bile acids, cholestyramine increases the activity of cholesterol-7α-hydroxylase, the liver-specific enzyme responsible for bile acid synthesis (reviewed in Myant and Mitropoulos 1977). The bile acid synthetic pathway is the major pathway through which cholesterol is degraded and removed from the body. Thus, plasma LDL concentrations are modestly reduced in some animals and subjects treated with cholestyramine. The reduction in plasma LDL concentrations is the result of increased levels of expression of the LDL receptor in liver (Angelin et al. 1983). However, cholestyramine treatment also induces the expression of cholesterol biosynthetic enzymes, causing more cholesterol to be synthesized and limiting the hypocholesterolemic efficacy of this drug.

Another unexpected feature of cholestyramine treatment is that it increases hepatic VLDL production (Einarsson et al. 1991). In some hyperlipidemic patients, there is a direct relationship between hepatic bile acid synthesis and VLDL triglyceride secretion (Angelin et al. 1978). Clearly, cholestyramine causes multiple changes in hepatic lipid and lipoprotein metabolism (summarized in **Table 1**): *1*) induction of cholesterol-7α-hydroxylase, *2*) increased expression of the LDL receptor, *3*) induction of cholesterol biosynthesis by increasing the transcription of the some of the genes regulating the isoprenoid/sterol biosynthetic pathway, *4*) increased synthesis of all VLDL lipids (i.e., cholesterol, cholesterol esters, triglycerides and phospholipids; R. A. Davis, unpublished observation) and *5*) increased secretion of VLDL. All of these events can be ascribed to the activation of a single transcription factor that has the ability to coordinately induce the genes responsible for hepatic VLDL assembly: sterol regulatory binding protein (SREBP). To explain how this might occur, I will review how cholesterol metabolism, lipogenesis, and VLDL assembly and secretion are linked together.

The amount of cholesterol in a cell is precisely regulated (reviewed in (Brown and Goldstein 1986). When cellular cholesterol levels are sufficient to meet the anabolic and catabolic requirements, there is a coordinate repression of the transcription of most, if not all, gene products involved in the cholesterol biosynthetic pathway: HMG-CoA synthase (Gil et al. 1986), HMG-CoA reductase (Gil et al. 1986), farnesyldiphosphate synthase (Ashby and Edwards 1989) and squalene synthase (Jiang et al. 1993). There are additional post-transcriptional mechanisms regulating the activity of many of these enzymes, including phosphorylation, protein degradation and rate of mRNA translation. In mammals, the major pathway for cholesterol uptake is via the LDL receptor (Brown and Goldstein 1986). Like the cholesterol biosynthetic enzymes, expression of the LDL receptor is regulated transcriptionally by negative feedback signaled by cellular cholesterol levels. Recent findings suggest that distinct transcription factors, whose activity is sensitive to the "regulatory" pool of cholesterol, control the transcription of genes that produce enzymes required for sterol biosynthesis and the LDL receptor (Briggs et al. 1993, Wang et al. 1994). Initiation of de-repression of conditionally positive sterol regulatory element (SRE) containing promoters begins with the proteolytic cleavage of the cytosolic domain of SREBP, a unique class of transcription factors whose initial translation produces an integral membrane protein of the endoplasmic reticulum. Once cleaved into its mature form, the N-terminal domain of SREBP, in concert with other transcription factors, activates gene transcription.

Sterol regulatory binding protein also regulates the transcription of genes controlling fatty acid synthesis (Kim et al. 1995). Because the availability of all of the lipid components required for VLDL consists of either sterols or fatty acid esters (triglycerides and phospholipids), the cellular level of mature SREBP will coordinately regulate the availability of the individual lipids required for its assembly. Previous studies showed that adding oleic acid to the medium of cultured rat hepatocytes increases both triglyceride and phospholipid synthesis and secretion (Davis and Boogaerts 1982). The combined data suggest that the availability of fatty acids, produced in response to SREBP induction of acetyl-CoA carboxylase and fatty acid synthase may be all that is needed to increase VLDL glycerolipid synthesis.

THE SIGNAL MEDIATING SREBP PROCESSING

Although it is clear that, in nonhepatic cells, cellular cholesterol initiates transcriptional repression of SRE-governed genes, it may not be the direct effector of SREBP processing. Characterization of the ability of different sterols to alter SREBP processing showed that synthetic oxysterols decrease this site-specific regulatory cleavage, whereas cholesterol itself has little, if any, effect (Wang et al. 1994). An attractive, but as yet unproved, hypothesis that may explain these results is that a hydroxylated metabolite of cholesterol, or a sterol pre-

cursor of cholesterol, is the signal responsible for "cholesterol feedback regulation" (Kandutsch et al. 1978). These hydroxylated sterols derivatives, commonly referred to as "oxysterols," may regulate SREBP processing in vivo in a manner similar to that which occurs in cultured cells (described above).

CHOLESTEROL-7-α-HYDROXYLASE DECREASES THE ABILITY OF CHOLESTEROL TO REPRESS SRE-GOVERNED GENES AND INCREASES THE SYNTHESIS OF ALL VLDL LIPIDS AND THE SECRETION OF VLDL APOLIPOPROTEIN B

One unique characteristic of the liver is a resistance to repression of SRE-governed gene expression by cholesterol (Dueland et al. 1992). This resistance toward cholesterol repression could be recapitulated by expressing cholesterol-7α-hydroxylase in nonhepatic cells (Dueland et al. 1992). These results are consistent with the proposal that metabolic signal controlling SREBP processing may be metabolized by 7α-hydroxylase or its synthesis decreased by 7α-hydroxylase. We have extended these studies to examine whether this metabolic signal, decreased by 7α-hydroxylase, may also govern VLDL assembly and secretion.

7α-Hydroxylase was stably expressed in two different cultured cell lines that do not normally express it: CHO cells expressing apo B and McArdle rat hepatoma cells. The results (Table 1) show that 7α-hydroxylase increased the cellular abundance of mature SREBP, induced SRE-governed gene transcription, increased the synthesis of all VLDL lipid components and the secretion of apo B (data not shown). Furthermore, this increased expression of 7α-hydroxylase also decreased the intracellular degradation of apo B (data not shown). These changes can be reversed by adding two "oxysterols": 7-oxo-cholesterol together with 25-hydroxycholesterol (data not shown).

The combined data suggest that a common metabolic signal, whose activity is attenuated by 7α-hydroxylase, may control SREBP–governed gene transcription and VLDL assembly. This signal, like estrogen in lower species, can induce all of the processes required for lipoprotein secretion and tissue targeting. The major difference is that this metabolic signal is related to nutritional state and is not limited to reproduction, as is estrogen. This versatile metabolic signaling provides mammals a means to control lipid metabolism and transport (i.e., VLDL secretion) in a manner that is responsive to both nutritional status and reproduction needs. Identification of the signal regulating SREBP-governed gene transcription and VLDL secretion will provide important clues as to the evolutionary divergence of the vitellogenin lipid transport system. Moreover, if this regulator is found to be derived from the same sterol precursor as estrogen, this finding would provide new insights into the co-evolution of ancestral metabolic regulators and genes of a common primordial pathway.

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