Metabolism of apolipoprotein A-IV

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INTRODUCTION

ApoA-IV was first described by Swaney, Reese, and Eder (1) as a component of rat HDL. Its presence has since been confirmed in dogs (2) as well as in man (3, 4). Apart from plasma, apoA-IV can also be demonstrated in human and rat mesenteric lymph (2, 5, 6), dog peripheral lymph (7), rat renal lymph (8), and rat bile (9).

Despite the knowledge of apoA-IV's existence for over 10 years, relatively little is known concerning its metabolism and physiological function. A distinctive feature of apoA-IV, which sets it apart from other apolipoproteins, is its occurrence in high concentrations in a lipoprotein-free form. Several studies have shown that distribution of apoA-IV between lipoprotein-free and lipoprotein-associated forms can be altered in certain disorders of lipid metabolism, clearly indicating a metabolic role for this apolipoprotein. Moreover, there are indications that apoA-IV may specifically be involved in reverse cholesterol transport, suggesting the importance of this apolipoprotein in cholesterol homeostasis. In this paper, we will present a brief review of our current knowledge of apoA-IV metabolism.

PHYSICAL PROPERTIES

Rat (1, 10) and human (3, 11) apoA-IV have been isolated and partially characterized. As determined by SDS-PAGE, apoA-IV has a molecular weight of between 44,000 and 46,000. The molecular weight of apoA-IV is not affected by reduction with mercaptoethanol, thus distinguishing it from the apoE/A-II complex (2). The amino acid compositions of human apoA-IV and rat apoA-IV are similar, with each possessing an N-terminal Glx and approximately 2 moles of Cys per mole of apoA-IV. In addition, rat apoA-IV has a C-terminal Lys. Human apoA-IV contains 6% carbohydrate, consisting of mannose (1.8%), galactose (1.55%), N-acetyl glucosamine (1.55%), and sialic acid (1.1%) (11). Both human and rat apoA-IV occur as multiple isoforms, with the predominant human isoform having a pI of between 5.12 and 5.15, while the predominant rat isoform has a pI of 4.95 (3, 11).

PLASMA CONCENTRATION AND DISTRIBUTION

Fasting human plasma apoA-IV concentrations average between 13.1 and 15.7 mg/dl (5, 11–13). However, one report places human apoA-IV levels as high as 37.4 mg/dl (14). ApoA-IV concentrations in the rat are similar to those in man (ca 24 mg/dl) (15). In both man and rat, plasma apoA-IV concentration increases during alimentary lipemia (12, 16).

In the rat, a substantial portion of apoA-IV can be found in the $d > 1.21$ g/ml fraction (8, 15–18). The remainder is primarily associated with the HDL fraction, with a small amount appearing in the $d < 1.006$ g/ml fraction. In contrast, human apoA-IV is almost exclusively in the $d > 1.21$ g/ml fraction; this distribution occurs in sera from fasted, non-fasted, normolipidemic, and hyperlipidemic subjects (3). Variable amounts of apoA-IV ranging from 1 to 10% of the plasma concentration can be found in the $d < 1.006$ g/ml fraction of human plasma (3, 11). Virtually no apoA-IV can be detected in human HDL (4, 12). ApoA-IV found in the $d > 1.21$ g/ml fraction is immunologically and electrophoretically identical to lipoprotein-associated apoA-IV (3, 11).

It should be noted, however, that the precise distribution of apoA-IV among the lipoprotein classes has been difficult to assess, owing to the ease with which

Abbreviations: apo, apolipoprotein; HDL, LDL, IDL, and VLDL, high density, low density, intermediate density, and very low density lipoproteins, respectively; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); LCAT, lecithin cholesterol acyltransferase, EC 2.3.1.43.

TABLE 1. ApoA-IV distribution in perturbed states of lipid metabolism

Condition	Reference	Plasma ApoA-IV Response
Man		
Abetalipoproteinemia	12, 14	50% decrease in plasma apoA-IV. Loss of apoA-IV from the major HDL fraction (agarose chromatography). No change in amount of apoA-IV associated with 7.8-nm particle.
LCAT deficiency	44, 45	Abnormal association of apoA-IV with a large HDL fraction containing apoE, apoA-I, and discoidal particles.
ApoE deficiency	46	Presence of apoA-IV in association with apoB-48 on LDL, IDL, and VLDL.
Rat		
Aging (96-week-old rats)	18	Increase in the proportion of lipoprotein-free apoA-IV with no change in total plasma apoA-IV.
Streptozotocin-induced diabetes	17	26% increase in plasma apoA-IV; decrease in HDL apoA-IV and increase in VLDL and d > 1.21 g/ml apoA-IV.
Cholesterol feeding	16	50% decrease of apoA-IV in the d < 1.21 g/ml fraction and a tenfold increase in the d > 1.21 g/ml fraction.

apoA-IV is removed from the lipoproteins (VLDL and HDL) during ultracentrifugation. Almost all of the apoA-IV present in the d < 1.006 g/ml fraction can be removed through repeated ultracentrifugation (3, 6).

Studies on the distribution of plasma apoA-IV determined by methods other than ultracentrifugation have been recently published. Beisiegel and Utermann (3), using two-dimensional crossed immunoelectrophoresis, demonstrated that uncentrifuged human plasma contains an apoA-IV particle that migrates with alpha$_2$ mobility, and thus is not associated with the major lipoprotein fractions. This is taken as evidence that the apoA-IV found in the d > 1.21 g/ml fraction is not solely an ultracentrifugational artifact. When human plasma is separated by gel filtration chromatography on 6% agarose, 20–30% of the plasma apoA-IV is associated with the major HDL fraction (12, 14). Similar separation of rat plasma on 10% agarose leads to the recovery of about 55% of plasma apoA-IV in a peak eluting after albumin (lipoprotein-free fraction[1]) (16, 18). Most of the remaining apoA-IV is evenly distributed between large and small HDL; less than 1% is associated with the VLDL and LDL fraction.

Both the concentration and distribution of plasma apoA-IV can be influenced by perturbations in lipid metabolism. **Table 1** provides a summary of some of these changes.

The precise nature of apoA-IV in the lipoprotein-free fraction has not yet been determined. Green et al. (12) reported that lipoprotein-free apoA-IV is associated with a small amount of phospholipid. The nature of the phospholipid, as well as the possible existence of other lipids associated with lipoprotein-free apoA-IV, remains to be determined. The association of lipid with lipoprotein-free apoA-IV is a potentially important determinant for its physiological behavior. This should be considered in the interpretation of data where isolated apoA-IV was used as a physiological marker for apoA-IV metabolism.

SYNTHESIS

ApoA-IV is synthesized by both the liver and the intestine, with the latter organ being quantitatively the most important (19). Aspects of the molecular biology of rat and human intestinal apoA-IV synthesis have been elucidated (20–24). The apoA-IV primary translation product (pre-apoA-IV) from rat intestine is 48,500 daltons, 2,500 daltons larger than mature plasma apoA-IV (21). Both rat and human pre-apoA-IV contain a 20 amino acid N-terminal extension which has a high degree (>59%) of homology with the pre-apoA-I signal peptide (21, 23). The N-terminal pre-peptide extension is removed co-translationally and the mature apoA-IV does not require additional post-translational proteolytic processing (23). Thus, unlike prepro-apoA-I, pre-apoA-IV does not contain a propeptide.

[1] The terms "lipoprotein-free" and "lipoprotein-associated" are used to denote those studies in which apoA-IV was fractionated by means other than ultracentrifugation. Because of the introduction of artifacts by ultracentrifugation and a reliance of different physical properties for separation by the alternative methods, "lipoprotein-free" and "lipoprotein-associated" are not necessarily physiologically or physically equivalent to "d > 1.21 g/ml" and "d < 1.21 g/ml."

Rat pre-apoA-IV is encoded by an mRNA 1780 nucleotides long, 460 nucleotides longer than the minimum size necessary to code the primary translation product (22). In vitro translation of total rat intestinal epithelial RNA indicates that both apoA-I and apoA-IV are synthesized in relative abundance, comprising 1.0% and 0.6% of translatable mRNAs, respectively (20). The value for intestinal apoA-IV mRNA obtained by in vitro translation is consistent with data obtained by mRNA-cDNA hybridization studies where mRNA encoding for apoA-IV accounted for 0.7% of total cellular mRNA (22). These data are also consistent with the in vivo observation that the rat intestine synthesizes apoA-I and apoA-IV in approximately equal amounts (24). Intestinal apoA-IV mRNA levels are about four times higher than that for hepatic apoA-IV mRNA (25).

Values for human intestinal apoA-IV mRNA appear to be somewhat lower than in the rat. Human jejunal epithelium apoA-I mRNA represents 2% of total mRNA-directed protein synthesis, while apoA-IV mRNA accounts for only 0.2% (23). Regional differences in intestinal apoA-IV mRNA may further exaggerate the differences between human and rat values. In rats, apoA-IV mRNA in the jejunum is twice as high as in the duodenum and five times higher than in the ileum (25).

The relative abundance of apoA-IV mRNA is regulated, at least in part, by nutritional status. Four hours after administration of corn oil, rat intestinal apoA-IV mRNA increases from 0.62% to 1.33% of total mRNA (22). Eight hours after corn oil administration, apoA-IV mRNA levels are still elevated (0.98%). In contrast, apoA-I mRNA levels are not significantly affected (0.87% to 0.89%), suggesting that intestinal apoA-IV and apoA-I synthesis are not regulated in concert by fat absorption.

The mRNA data support previous in vivo and in vitro observations. Windmueller and Wu (26), using a differential double labeling technique, could demonstrate an apparent decrease in the synthesis of apoA-IV relative to other apoproteins during acute fat deprivation. In similar studies conducted in our laboratory,[2] the intestinal contribution to total apoA-IV synthesis increased in rats during fat absorption. In fasting humans, biopsies taken 45 min after the ingestion of cream show a marked increase in apoA-IV-associated immunofluorescence in the apical portion of the cells, suggesting an increased apoA-IV content (12). These data are consistent with the increase in the concentration of apoA-IV in the urine of chyluric subjects (4, 12) and in the mesenteric lymph of rats (27) following the administration of a fat load.

SECRETION

Studies with the isolated and the in situ perfused intestine (24, 28, 29) show that the intestine can secrete apolipoproteins into both the mesenteric lymphatics and directly into the circulation. Both apoA-I and apoA-IV synthesized by the intestine are secreted in considerable amounts directly into the blood (26). The proportion of newly synthesized apoA-IV and apoA-I released directly into the blood increases as fat transport diminishes.

ApoA-IV released by the intestine into the mesenteric lymph is found associated with chylomicrons, VLDL, HDL, and the $d > 1.21$ g/ml fraction. From data reported for rats by Holt, Wu, and Bennett Clark (5) and Wu and Windmueller (24), it can be calculated that, of the lipoprotein-associated apoA-IV in the mesenteric lymph, approximately 70% of newly synthesized apoA-IV is found in chylomicrons and VLDL during fat absorption. These values are similar to those in man where, in studies of patients with chyluria, 56% of apoA-IV was found in the $d < 1.006$ g/ml fraction of chylous urine during fat absorption (12). However, when one considers the total amount of apoA-IV synthesized by the intestine, only a small fraction is associated with triglyceride-rich lipoproteins. Seventy to 80% of newly synthesized apoA-IV is found in the $d > 1.21$ g/ml fraction of mesenteric lymph (25, 26). If one considers the secretion of apoA-IV directly into the blood, an even greater proportion of apoA-IV secreted by the intestine is not associated with chylomicrons but rather is present as HDL and lipoprotein-free apoA-IV.

Rat mesenteric lymph chylomicrons (5) and human chylomicrons isolated from either thoracic duct lymph or chylous urine (12) have a lower apoA-IV content (as a percent of total chylomicron apolipoprotein) than does lymph VLDL (**Table 2**). No significant differences in apoA-IV content of chylomicrons isolated from either the proximal or distal intestine can be demonstrated (Table 2) (5). However, differences in chylomicron apoA-IV content can be induced by dietary means. Large chylomicrons obtained from the mesenteric lymph of fat-fed rats contain more apoA-IV and apoC and less apoE than do small chylomicrons obtained from glucose-fed rats (30). In addition, there is also a genetic component to chylomicron apoA-IV content. Chylomicrons isolated from the mesenteric lymph of Hooded rats, which have impaired chylomicron clearance, contain proportionately less apoA-IV than do those from albino rats, while the other apolipoproteins do not differ significantly (Table 2) (31). The physiological significance of these compositional changes remains to be established.

[2] Krause, B. R., C. H. Sloop, and P. S. Roheim. Unpublished observation.

TABLE 2. ApoA-IV content of intestinal lipoproteins

Author	Source	Lipoprotein	% ApoA-IV
Holt et al. (5)	Rat mesenteric lymph (proximal)	Chylo.	18.4
		VLDL	27.4
		LDL + HDL	13.6
	Rat mesenteric lymph (distal)	Chylo.	18.6
		VLDL	27.3
		LDL + HDL	7.6
Green et al. (12)	Human chylous urine	Chylo.	10.0
		VLDL	24.6
	Human thoracic duct lymph	Chylo.	13.0
		VLDL	30.0
Jeffrey and Redgrave (31)	Hooded rat	Chylo.	4.5
	Albino rat	Chylo.	12.1

A portion of rat mesenteric lymph apoA-IV is found associated with HDL-like particles. ApoA-IV does not appear to be associated with "nascent" apoA-I discoidal particles secreted by the intestine (32), but rather is confined to a discrete 7.8-nm HDL particle isolated at d 1.13–1.18 g/ml (33). This particle contains 12% apoA-IV and 66% apoA-I, is rich in protein and phospholipids, and appears to be a direct secretory product of the intestine. A similarly sized apoA-IV-containing particle found in human plasma has also been described (14).

Studies of hepatic secretion of apoA-IV-containing particles have been limited. In the nonrecirculating perfused rat liver, apoA-IV is found associated with HDL and accounts for 4% of the HDL apolipoprotein compared with 11% in normal rat serum (34). In the recirculating perfused liver, the perfusate lipoprotein content of apoA-IV varies, concordant with that of apoA-I (35). Most of the perfusate apoA-I is associated with the HDL_2 fraction. Addition of DTNB to the perfusate to inhibit LCAT results in increased amounts of apoA-I (33%) recovered in the d > 1.21 g/ml fraction. Under these conditions, apoA-IV is almost totally absent in both HDL_2 and HDL_3. Preliminary results from our laboratory indicate that rat hepatocytes in culture also secrete a 7.6-nm apoA-IV particle, which appears to be similar to the small HDL apoA-IV particle found in mesenteric lymph.[3] Unlike mesenteric lymph VLDL, hepatic lymph VLDL does not contain detectable amounts of apoA-IV (36).

CATABOLISM

Rat plasma apoA-IV has a $t_{1/2}$ of 10 hr, which is similar to that of rat HDL apoA-I (15). Human apoA-IV has a $t_{1/2}$ of 18–27.5 hr (13) and thus turns over more rapidly than human apoA-I (37). That the catabolism of human apoA-I and apoA-IV are not tightly linked is further demonstrated in patients with Tangier disease. In Tangier disease, apoA-I catabolism is accelerated (37), resulting in decreased plasma apoA-I concentration, yet plasma apoA-IV levels are reported to be normal (12). It is possible that the bulk of apoA-IV catabolism occurs through the lipoprotein-free form, as has been suggested for apoA-I (38).

INTRAVASCULAR METABOLISM

Chylomicrons obtained from plasma contain virtually no apoA-IV, suggesting a rapid loss of this apoprotein from chylomicrons upon exposure to the plasma (4). In vitro incubations of human chylomicrons (from chylous urine) with plasma produces a decrease in the contribution of apoA-IV to chylomicron apolipoprotein (fractional content) from 10% to 1.6%, while both apoE and apoC increase (4). This decrease in the fractional content of apoA-IV represents an absolute decrease in chylomicron apoA-IV content. The loss of apoA-IV is rapid and occurs at 4°C, suggesting that it is not an enzyme-mediated process. Addition of DTNB does not inhibit transfer, further ruling out LCAT-dependence. The exchange process is best demonstrated when HDL is present in the incubation medium; as the concentration of HDL is increased in the incubations, the loss of chylomicron apoA-I and apoA-IV is more pronounced. Incubations with the d > 1.21 g/ml fraction of plasma, while resulting in a gain of apoE, are not accompanied by a loss of chylomicron apoA-IV. Similar data are obtained when human intestinal VLDL is incubated with plasma.

Results obtained in rats appear to differ from those in humans. In vitro incubations of rat mesenteric lymph chylomicrons with rat serum leads to a decrease in the

[3] Lefevre, M., R. Davis, and P. S. Roheim. Unpublished observation.

fractional content of chylomicron apoA-IV and apoA-I, while both apoE and the C apoproteins increase (30). In this study, the amount of apoA-IV was not explicitly measured but was said to have followed that of apoA-I. The observation that the chylomicron apoA-I to triglyceride ratio was not significantly reduced by incubations with plasma when compared to the saline-incubated controls would suggest that, in contrast to humans, the absolute amount of apoA-I, and thus apoA-IV, does not change. Nonetheless, plasma chylomicrons obtained from rats do not contain apoA-IV, suggesting that additional factors may be required for the displacement of apoA-IV from intestinal chylomicrons.

Studies in vivo provide additional information as to the fate of chylomicron apoA-IV. Injection of labeled rat mesenteric lymph chylomicrons into rats results in a rapid transfer of phospholipid and protein to the HDL fraction (39). The re-isolated d < 1.02 g/ml fraction shows a marked decrease of apoA-I and apoA-IV and enrichment of apoE and apoC. Furthermore, transfer of labeled chylomicron apoA-I and apoA-IV to HDL is evident. An interesting note from this study pertains to the difference in the plasma distribution of apoA-IV between rat and man. After injection of rat mesenteric lymph chylomicrons into the rat, there is no significant change in the relative amount of apoA-IV in rat HDL. However, if human chylomicrons obtained from chylous urine are injected into rats, apoA-IV is absent from the re-isolated rat HDL. Whether this represents a species difference in the metabolism of apoA-IV or is the result of possible alterations in apoA-IV structure due to prolonged incubation with urine (carbamylation) remains to be established.

Not all of the apoA-IV dissociated from chylomicrons is directly transferred to HDL. Injection of rat ^{125}I-apoA-IV-labeled chylomicrons into rats results in a rapid transfer (within 5 min) of 60% of the chylomicron ^{125}I-apoA-IV to the d > 1.21 g/ml fraction (15). This is followed by a slower transfer from this fraction to the HDL fraction. Transfer of d > 1.21 g/ml apoA-IV to HDL can also be demonstrated by injection of isolated ^{125}I-apoA-IV into rats. Forty to 50% of the label can be recovered in the HDL fraction, while only 2–3% is recovered in the d < 1.006 g/ml fraction.

MECHANISMS OF APOA-IV DISTRIBUTION

Those factors responsible for the redistribution of apoA-IV between lipoproteins have been the subject of recent investigations. Weinberg and Spector (40) adsorbed human apoA-IV onto model triglyceride-rich lipoprotein particles derived from Intralipid. ApoA-IV could be removed from these particles by in vitro incubation with human HDL_2. Associated with the loss of apoA-IV was the transfer of C apoproteins from HDL_2 to the triglyceride-rich particles. Furthermore, incubation of triglyceride-rich apoA-IV particles with purified apoC-III_1 alone was sufficient to displace the apoA-IV, suggesting that the transfer of the C apoproteins from HDL to chylomicrons is the primary mechanism for apoA-IV displacement.

The transfer of apoA-IV from the lipoprotein-free fraction to the lipoprotein-associated fraction has also been studied (41). Incubation of rat plasma or whole blood for 4 hr at 37°C results in the recovery of greater than 90% of apoA-IV in the lipoprotein-associated fraction, the majority of which is associated with the HDL peak on 10% agarose columns (**Fig. 1**). This compares with only 45% recovery of apoA-IV in this fraction when unincubated samples are applied immediately to the agarose column. Addition of DTNB or heat treatment (56°C) of the plasma reverses the distribution such that greater than 90% of apoA-IV elutes in the lipoprotein-free fraction. The data strongly suggest a role for LCAT in transfer of apoA-IV from the lipoprotein-free fraction to the lipoprotein-associated fraction. Confirmation of this came when the addition of a purified LCAT preparation to heat-inactivated plasma resulted in a 92% recovery of apoA-IV in the lipoprotein-associated fraction. We have since confirmed an LCAT-dependent redistribution of apoA-IV during in vitro incubation of human plasma,[4] indicating that this reaction is not specific to the rat.

Studies on the redistribution of apoA-IV from HDL to the lipoprotein-free fraction have been further investigated.[5] In these studies, apoA-IV was separated into lipoprotein-free and HDL fractions by crossed-immunoelectrophoresis of plasma. In vitro incubation of rat plasma with either postheparin plasma or lipoprotein lipase causes a rapid redistribution of apoA-IV from HDL to the lipoprotein-free fraction. The extent of redistribution is dependent upon the availability of substrate for lipoprotein lipase; very little redistribution occurs in the plasma of fasted rats. Furthermore, as lipase activity diminishes, the LCAT-dependent redistribution of apoA-IV from the lipoprotein-free fraction to the HDL fraction becomes dominant, suggesting that a dynamic relationship exists between the LCAT-dependent and lipoprotein lipase-dependent redistribution of plasma apoA-IV. As with model triglyceride-rich particles, the displacement of apoA-IV from HDL appears to be related to the C apoprotein content. Addition of

[4] Lefevre, M., M-Y. Chuang, R. Weinberg, and P. S. Roheim. Unpublished observation.

[5] Lefevre, M., M-Y. Chuang, and P. S. Roheim. Manuscript in preparation.

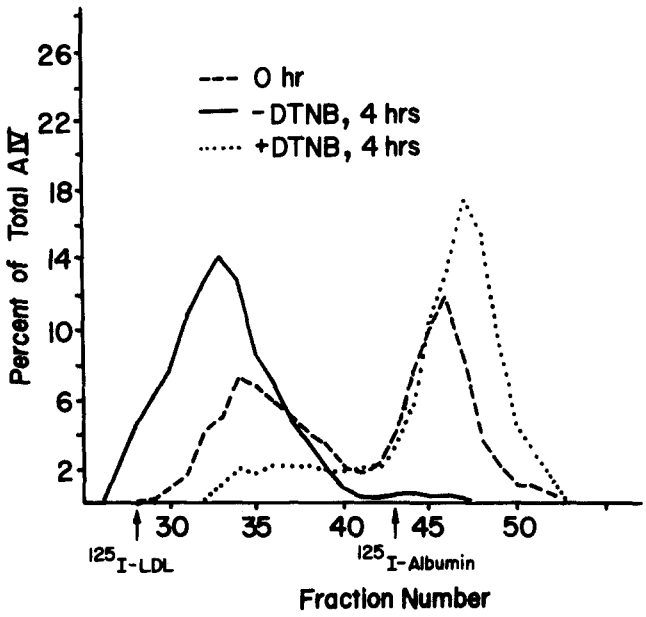

Fig. 1. Plasma was fractionated on 10% agarose immediately after bleeding (0 hr), after 4 hr of incubation (−DTNB), or after 4 hr of incubation in the presence of 1.5 mM DTNB (+DTNB).

C apoproteins to plasma leads to an enrichment of HDL C apoprotein content and the displacement of apoA-IV from HDL to the lipoprotein-free fraction. Conversely, depletion of HDL C apoproteins by incubation with Intralipid leads to an increase in HDL apoA-IV content and a decrease of apoA-IV in the lipoprotein-free fraction. Thus, it appears that the C apoproteins modulate the binding of apoA-IV to both chylomicrons and HDL particles.

INTERSTITIAL FLUID METABOLISM

A potentially important feature of apoA-IV metabolism centers on its presence in substantial quantities in peripheral lymph. Dog peripheral lymph, which is accepted to be representative of interstitial fluid, contains apoB, apoE, apoA-I, and apoA-IV (7). Peripheral lymph apoA-IV is associated with LDL, HDL, and the d > 1.21 g/ml fraction, with the majority of the apoA-IV being present in the latter fraction (42). Relative to apoA-I, there is a considerable enrichment of both apoE and apoA-IV in the HDL fraction of peripheral lymph. This enrichment is increased severalfold upon cholesterol feeding such that the lymph HDL apoA-IV concentration is over twice that of plasma HDL apoA-IV. Furthermore, the increase in peripheral lymph HDL apoA-IV coincides with a marked increase in the appearance of discoidal particles in the peripheral lymph (40). Subfractionation of the peripheral lymph lipoproteins shows that apoA-IV is associated with a population of discoidal particles that do not contain apoE and may or may not contain apoA-I.[6]

The fact that the concentration of peripheral lymph HDL apoA-IV increases more than fourfold upon cholesterol feeding and is in association with discoidal particles argues for the participation of apoA-IV in reverse cholesterol transport. It is envisioned that the majority of peripheral lymph apoA-IV represents filtered plasma lipoprotein-free apoA-IV. Within the interstitial fluid compartment, lipoprotein-free apoA-IV either participates in the peripheral synthesis of these particles or redistributes to a population of preformed HDL particles. The fate of the peripheral lymph HDL apoA-IV particles, once they reach the circulation, remains to be established. However, in LCAT-deficient patients (Table 1), the appearance of apoA-IV in association with plasma HDL containing apoE, apoA-I, and discoidal particles would suggest that LCAT may be involved in their metabolism.

CONCLUSION

Our understanding of apoA-IV metabolism has increased greatly within the past few years. It has been recognized that apoA-IV is synthesized in relative abundance by the intestine and that its synthesis is regulated by fat absorption. In addition to its presence in the lipoprotein-free fraction, apoA-IV is also a component of chylomicrons, HDL, and a distinct 7.8-nm lipoprotein particle. Recent studies have begun to establish the mechanisms responsible for the redistribution of apoA-IV between the lipoprotein and lipoprotein-free fractions. However, the physiological significance of apoA-IV redistribution has not been elucidated. A number of questions remain to be answered. Does apoA-IV transport specific lipids as it transfers from one fraction to another? Does the presence of apoA-IV alter the metabolism of the lipoprotein particle in a significant manner?

A specific functional role for apoA-IV in lipoprotein metabolism has yet to be demonstrated. Recent evidence would suggest that apoA-IV can activate LCAT (43). If indeed it does serve as an LCAT activator, how does its physiological role differ from that of apoA-I? Its presence in association with discoidal particles in the peripheral lymph of cholesterol-fed dogs argues for a potential role in reverse cholesterol transport. Does apoA-IV participate in the formation of peripheral lymph discoidal particles? Does it direct their metabolism? Are there specific hepatic or extrahepatic receptors for apoA-IV? With further research, the answers to these questions

[6] Dory, L., L. M. Boquet, R. L. Hamilton, C. H. Sloop, and P. S. Roheim. Manuscript in preparation.

will be forthcoming and will further our understanding of lipoprotein metabolism.

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REFERENCES

1. Swaney, J. B., H. Reese, and H. A. Eder. 1974. Polypeptide composition of rat high density lipoprotein: characterization by SDS electrophoresis. *Biochem. Biophys. Res. Commun.* **59:** 513–519.
2. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apoprotein from the d < 1.006 lipoproteins of human and canine lymph homologous with the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.
3. Beisiegel, U., and G. Utermann. 1979. An apolipoprotein homolog of rat apolipoprotein A-IV in human plasma. *Eur. J. Biochem.* **93:** 601–608.
4. Green, P. H. R., and R. M. Glickman. 1979. Human intestinal lipoproteins. Studies in chyluric subjects. *J. Clin. Invest.* **64:** 233–242.
5. Holt, P. R., A-L. Wu, and S. Bennett Clark. 1979. Apoprotein composition and turnover in rat intestinal lymph during steady-state triglyceride absorption. *J. Lipid Res.* **20:** 494–502.
6. Utermann, G., and U. Beisiegel. 1979. Apolipoprotein A-IV: a protein occurring in human mesenteric lymph chylomicrons and free in plasma. *Eur. J. Biochem.* **99:** 333–343.
7. Sloop, C. H., L. Dory, B. R. Krause, C. Castle, and P. S. Roheim. 1983. Lipoproteins and apolipoproteins in peripheral lymph of normal and cholesterol-fed dogs. *Atherosclerosis.* **49:** 9–21.
8. Roheim, P. S., D. Edelstein, and G. G. Pinter. 1976. Apolipoproteins in rat serum and renal lymph. *Proc. Natl. Acad. Sci. USA.* **73:** 1757–1760.
9. Casu, A., D. Cottalassu, and M. A. Pronzato. 1981. Lipids and proteins of rat bile during chronic drainage. *IRCS Med. Sci.* **9:** 951.
10. Swaney, J. B., F. Braithwaite, and H. A. Eder. 1977. Characterization of the apolipoproteins of rat plasma lipoproteins. *Biochemistry.* **16:** 271–278.
11. Weinberg, R. B., and A. M. Scanu. 1983. Isolation and characterization of human apolipoprotein A-IV from lipoprotein-depleted serum. *J. Lipid Res.* **24:** 52–59.
12. Green, P. H. R., R. M. Glickman, J. W. Riley, and E. Quinet. 1980. Human apolipoprotein A-IV. Intestinal origin and distribution in plasma. *J. Clin. Invest.* **65:** 911–919.
13. Fidge, N., and P. Nestel. 1981. Studies on the in vivo metabolism of A-IV apoprotein in human subjects. *Circulation.* **64:** 159A.
14. Bisgaier, C., O. Sachdev, L. Megna, and R. Glickman. 1983. Plasma distribution of human apoA-IV. *Circulation.* **68:** 859A.
15. Fidge, N. H. 1980. The redistribution and metabolism of iodinated apolipoprotein A-IV in rats. *Biochim. Biophys. Acta.* **619:** 129–141.
16. DeLamatre, J. G., and P. S. Roheim. 1983. The response of apolipoprotein A-IV to cholesterol feeding in rats. *Biochim. Biophys. Acta.* **751:** 210–217.
17. Bar-On, H., P. S. Roheim, and H. A. Eder. 1976. Serum lipoproteins and apolipoproteins in rats with streptozotocin-induced diabetes. *J. Clin. Invest.* **57:** 714–721.
18. Van Lenten, B. J., and P. S. Roheim. 1982. Changes in the concentrations and distributions of apolipoproteins of the aging rat. *J. Lipid Res.* **23:** 1187–1195.
19. Wu, A-L., and H. G. Windmueller. 1979. Relative contribution by liver and intestine to individual plasma apolipoproteins in the rat. *J. Biol. Chem.* **254:** 7316–7322.
20. Gordon, J. I., D. P. Smith, R. Andy, D. H. Alpers, G. Schonfeld, and A. W. Strauss. 1982. The primary translation product of rat intestinal apolipoprotein A-I mRNA is an unusual preproprotein. *J. Biol. Chem.* **257:** 971–978.
21. Gordon, J. I., D. P. Smith, D. H. Alpers, and A. W. Strauss. 1982. Proteolytic processing of the primary translation product of rat intestinal apolipoprotein A-IV mRNA. *J. Biol. Chem.* **257:** 8418–8423.
22. Gordon, J. I., D. P. Smith, D. H. Alpers, and A. W. Strauss. 1982. Cloning of a complementary deoxyribonucleic acid encoding a portion of rat intestinal preapolipoprotein A-IV messenger ribonucleic acid. *Biochemistry.* **21:** 5424–5431.
23. Gordon, J. I., C. L. Bisgaier, H. F. Sims, O. P. Sachdev, R. M. Glickman, and A. W. Strauss. 1984. Biosynthesis of human preapolipoprotein A-IV. *J. Biol. Chem.* **259:** 468–474.
24. Wu, A-L., and H. G. Windmueller. 1978. Identification of circulating apolipoproteins synthesized by rat small intestine in vivo. *J. Biol. Chem.* **253:** 2525–2528.
25. Elshourbagy, N. A., M. A. Gholson, K. H. Weisgraber, R. W. Mahley, and J. M. Taylor. 1983. Cloning of rat apolipoprotein A-IV complementary DNA and examination of mRNA levels. *Federation Proc.* **42:** 358A.
26. Windmueller, H. G., and A-L. Wu. 1981. Biosynthesis of plasma apolipoproteins by rat small intestine without dietary or biliary fat. *J. Biol. Chem.* **256:** 3012–3016.
27. Krause, B. R., C. H. Sloop, C. K. Castle, and P. S. Roheim. 1981. Mesenteric lymph apolipoproteins in control and ethinyl estradiol-treated rats: a model for studying apolipoproteins of intestinal origin. *J. Lipid Res.* **22:** 610–619.
28. Windmueller, H. G., and A. E. Spaeth. 1972. Fat transport and lymph and plasma lipoprotein biosynthesis by isolated intestine. *J. Lipid Res.* **13:** 92–105.
29. Windmueller, H. G., P. N. Herbert, and R. I. Levy. 1973. Biosynthesis of lymph and plasma lipoprotein apoproteins by isolated perfused rat liver and intestine. *J. Lipid Res.* **14:** 215–223.
30. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure to blood serum and serum proteins. *J. Lipid Res.* **19:** 712–722.
31. Jeffery, F., and T. G. Redgrave. 1982. Chylomicron catabolism differs between Hooded and albino laboratory rats. *J. Lipid Res.* **23:** 154–160.
32. Green, P. H. R., A. R. Tall, and R. M. Glickman. 1978. Rat intestine secretes discoid high density lipoproteins. *J. Clin. Invest.* **61:** 528–534.
33. Forester, G. P., A. R. Tall, C. L. Bisgaier, and R. M. Glickman. 1983. Rat intestine secretes spherical high density lipoprotein. *J. Biol. Chem.* **258:** 5938–5943.
34. Marsh, J. B. 1976. Apoproteins of the lipoproteins in a

nonrecirculating perfusate of rat liver. *J. Lipid Res.* **17:** 85–90.
35. Felker, T. E., M. Fainaru, R. L. Hamilton, and R. J. Havel. 1977. Secretion of the arginine-rich and A-I apolipoproteins by the isolated perfused rat liver. *J. Lipid Res.* **18:** 465–473.
36. Tso, P., J. B. Ragland, and S. M. Sabesin. 1983. Isolation and characterization of lipoprotein of density < 1.006 g/ml from rat hepatic lymph. *J. Lipid Res.* **24:** 810–820.
37. Schaeffer, E. J., L. L. Kay, L. A. Zech, and H. B. Brewer. 1982. Tangier disease. High density lipoprotein deficiency due to defective metabolism of an abnormal apolipoprotein A-I (apoA-$I_{Tangier}$). *J. Clin. Invest.* **70:** 934–945.
38. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol. Chem.* **258:** 7161–7167.
39. Tall, A. R., P. H. R. Green, and R. M. Glickman. 1979. Metabolic fate of chylomicron phospholipids and apoproteins in the rat. *J. Clin. Invest.* **64:** 977–989.
40. Weinberg, R. B., and M. S. Spector. 1983. Human apolipoprotein A-IV: displacement from the surface of triglyceride-rich particles by HDL_2 associated C-apoproteins. *Circulation.* **101:** 464A.
41. DeLamatre, J. G., C. A. Hoffmeier, A. G. Lacko, and P. S. Roheim. 1983. Distribution of apolipoprotein A-IV between the lipoprotein and lipoprotein-free fractions of rat plasma: possible role of lecithin:cholesterol acyltransferase. *J. Lipid Res.* **24:** 1578–1585.
42. Sloop, C. H., L. Dory, R. L. Hamilton, B. R. Krause, and P. S. Roheim. 1983. Characterization of dog peripheral lymph lipoproteins: the presence of a disc-shaped "nascent" high density lipoprotein. *J. Lipid Res.* **24:** 1429–1440.
43. Steinmetz, A., and G. Utermann. 1983. Human apolipoprotein A-IV activates the enzyme lecithin:cholesterol acyltransferase. *Arteriosclerosis.* **3:** 495A.
44. Soutar, A. K., B. L. Knight, and N. B. Myant. 1982. The characterization of lipoproteins in the high density fraction obtained from patients with familial lecithin:cholesterol acyltransferase deficiency and their interaction with cultured human fibroblasts. *J. Lipid Res.* **23:** 380–390.
45. Kodama, T., Y. Akanuma, M. Okazaki, H. Aburatani, H. Itakura, K. Takahashi, M. Sakuma, F. Takaku, and I. Hara. 1983. Abnormalities in plasma lipoprotein in familial partial lecithin:cholesterol acyltransferase deficiency. *Biochim. Biophys. Acta.* **752:** 407–415.
46. Ghiselli, G., E. J. Schaefer, P. Gascon, and H. B. Brewer. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science.* **214:** 1239–1241.