thematic review

Thematic review series: Patient-Oriented Research

What we have learned about VLDL and LDL metabolism from human kinetics studies

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Abstract Lipoprotein metabolism is the result of a complex network of many individual components. Abnormal lipoprotein concentrations can result from changes in the production, conversion, or catabolism of lipoprotein particles. Studies in hypolipoproteinemia and hyperlipoproteinemia have elucidated the processes that control VLDL secretion as well as VLDL and LDL catabolism. Here, we review the current knowledge regarding apolipoprotein B (apoB) metabolism, focusing on selected clinically relevant conditions. In hypobetalipoproteinemia attributable to truncations in apoB, the rate of secretion is closely linked to the length of apoB. On the other hand, in patients with the metabolic syndrome, it appears that substrate, in the form of free fatty acids, coupled to the state of insulin resistance can induce hypersecretion of VLDL-apoB. Studies in patients with familial hypercholesterolemia, familial defective apoB, and mutant forms of proprotein convertase subtilisin/kexin type 9 show that mutations in the LDL receptor, the ligand for the receptor, or an intracellular chaperone for the receptor are the most important determinants in regulating LDL catabolism. This review also demonstrates the variance of results within similar, or even the same, phenotypic conditions. This underscores the sensitivity of metabolic studies to methodological aspects and thus the importance of the inclusion of adequate controls in studies.—Parhofer, K. G., and P. H. R. Barrett. **What we have learned about VLDL and LDL metabolism from human kinetics studies. *J. Lipid Res.* 2006.** 47: **1620–1630.**

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Disorders of lipoprotein metabolism are usually characterized by static measurements of increased or decreased plasma lipid or apolipoprotein concentrations. In addition, disorders are often characterized by describing the function of specific components of the lipoprotein metabolic system that are defective (i.e., receptor defect, enzyme defect, protein defect, etc.). However, lipoprotein metabolism represents a complex network of many components, and it is important to appreciate that this complexity is far greater than that of the individual components. Abnormal concentrations of lipids and apolipoproteins can result from changes in the production, conversion, or catabolism of lipoprotein particles. Thus, although static measurements and functional assays are important techniques that provide mechanistic clues, in the end, it is necessary to study the true unit of function (the integrated metabolic pathway) to understand the complexity of biochemical networks such as lipoprotein metabolism.

Such biochemical networks can best be studied using tracers, which allow, together with concentration measurements, the quantification of transport rates. With respect to lipoprotein metabolism, tracers can be either radioactive or stable isotopes, and lipoproteins can be labeled either endogenously or exogenously. The advantages and disadvantages of these methodological aspects are discussed elsewhere in detail (1, 2). In brief, radioactive tracers are easier to follow from a methodological point of view, but their use is limited by possible health hazards. With exogenous labeling (i.e., lipoproteins are isolated, labeled, and reinjected), the tracer may not necessarily follow the tracee, whereas endogenous labeling (i.e., a labeled amino acid is incorporated into the apolipoprotein under study) requires more sophisticated methods of analysis.

Advances in gas chromatography-mass spectrometry technology and the availability of inexpensive stable isotopes have resulted in an increasing use of endogenous labeling of apolipoproteins with labeled (stable isotopes) amino acids (1, 3–5). Furthermore, new stable isotope methodologies have been developed to study reverse cholesterol transport, fatty acid turnover, and cholesterol absorption (6–10). Therefore, in recent years, most studies evaluating aspects of human in vivo apolipoprotein B (apoB) metabolism have used amino acids labeled with

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stable isotopes as endogenously incorporated tracers and have combined this methodology with multicompartmental modeling for the analysis of enrichment data. This contrasts with early studies, which established the basic metabolic pathways using radio-iodinated lipoproteins. Notably, most findings established with radiotracers have later been confirmed using endogenous labeling with stable isotopes.

Here, we review the current knowledge of apoB metabolism using selected, clinically important conditions, with special focus on studies using stable isotope methodology. One particular goal is to demonstrate the variance of results (and thus conclusions) within similar, or even the same, phenotypic conditions. This underscores the sensitivity of metabolic studies to methodological aspects and thus the importance of the inclusion of adequate controls in any studies. We also stress that some studies include very few subjects and therefore must be interpreted cautiously.

NORMOLIPIDEMIC SUBJECTS

VLDL- and LDL-apoB metabolism

The main structural apolipoprotein of VLDL, intermediate density lipoprotein (IDL), and LDL is apoB-100. This apolipoprotein remains with the particle, and the lipid content changes as the particle is metabolized. Early studies demonstrated a precursor-product relationship between VLDL, IDL, and LDL particles. Subsequent studies have shown that VLDL and LDL are kinetically heterogeneous groups of particles and thus should be represented by several compartments in multicompartmental models (3, 11) (**Figs. 1, 2**). The physiological relevance of the subfractions required from a kinetics analyst's point of view remains to be determined. Furthermore, there is some controversy over whether or not there is a direct input of apoB-100 from the liver into the IDL and LDL fractions. Cell experiments, animal models, and some human studies support the concept that the liver is capable of producing apoB-100-containing lipoproteins of different densities, including LDL particles. However, human tracer data are also compatible with the existence of a fast turning-over VLDL compartment, which can shift newly secreted apoB directly to the LDL fraction (3). Demant and colleagues (12) showed that the dispute cannot be resolved by turnover studies because the rapid appearance of labeled apoB in the LDL fraction, after the bolus of labeled amino acids, may be attributable to either direct secretion or a rapidly turning-over VLDL compartment. Based on cell experiments, most researchers now assume that the liver is capable of secreting apoB-containing particles of different densities and thus include a pathway allowing for the direct production of different VLDL subfractions as well as IDL and LDL. A detailed description of apoB secretion is particularly important because perturbations such as weight loss and pharmacotherapy can change not only total apoB production but also the type of particles secreted: less VLDL-apoB secretion has been associated with more direct LDL secretion (13).

There are >30 turnover studies in which VLDL- and/or LDL-apoB kinetics parameters have been determined for normolipidemic subjects. These studies have been reviewed in detail previously (14, 15). They indicate an average VLDL-apoB-100 production rate of 14 ± 7.4 mg/kg/day, with a mean VLDL-apoB-100 fractional catabolic rate (FCR) of 9.7 ± 3.6 pools/day. IDL production rate and FCR were 11 ± 8.8 mg/kg/day and 13 ± 7.7 pools/day, respectively, whereas LDL production and LDL FCR were 12 ± 3.4 mg/kg/day and 0.46 ± 0.12 pools/day (15)

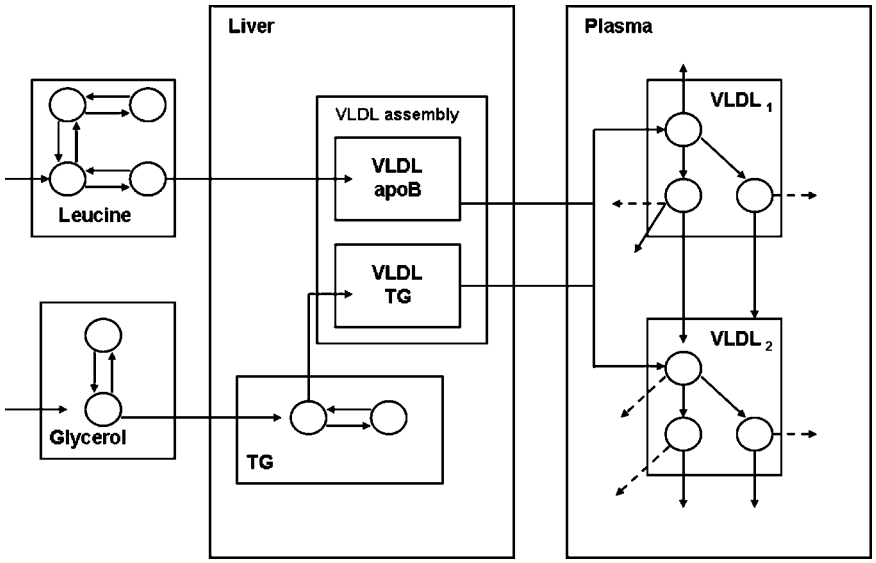

Fig. 1. Combined model of VLDL-apolipoprotein B (apoB) and VLDL-triglyceride (TG) metabolism (11). The model incorporates a leucine and a glycerol subsystem, which account for triglyceride and apoB synthesis and secretion. The model was used to quantify VLDL-apoB and VLDL-triglyceride metabolism in normolipidemic subjects and patients with the metabolic syndrome.

Fig. 2. Model of LDL subfractions. Rebound analysis of apoB mass after apheresis in different LDL subfractions indicates that small, dense LDLs are derived from larger LDLs and from LDL precursors in familial hypercholesterolemia heterozygotes (50). IDL, intermediate density lipoprotein.

(**Table 1**). In normolipidemic subjects, $VLDL_1$ and $VLDL_2$ are produced at 13 ± 10 and 8.3 mg/kg/day, respectively. The corresponding FCRs are 18 ± 8.8 pools/day for $VLDL_1$ and 11 ± 4.7 pools/day for $VLDL_2$. It is noteworthy that the kinetics parameters vary considerably more for VLDL and IDL than for LDL. This may reflect greater accuracy in measuring the plasma pool of LDL-apoB-100. Furthermore, the enrichment curves in LDL-apoB-100 display less variability relative to VLDL. The great variability for VLDL and IDL parameters may also relate to differences in subjects selected and methodology, including modeling, used in kinetics studies. Therefore, it is necessary that appropriate control subjects be studied if valid comparisons are to be made concerning the effects of an intervention.

Previous reviews have evaluated the effect of gender and age on VLDL-apoB-100 metabolism. Together, these studies indicate that the production rate of VLDL and LDL increases and the FCR of VLDL and LDL decreases with age (15). Whether there are significant gender differences for VLDL and LDL kinetics is still a matter of debate, although a meta-analysis (14) indicated that production rate is lower and FCR is higher in women than in men. The effect of gender, however, was not significant when apoE genotype was taken into account. Differences in apoB metabolism between men and women are obviously most likely related to differences in sex hormones. In support of this, a study in postmenopausal women (16) showed that oral estradiol therapy increased the production of large VLDL, which was cleared directly from the circulation and not converted to smaller lipoproteins. At the same time, LDL-apoB catabolism was increased by 36%. However, when men and women with similar apoB-100 concentrations were studied, similar VLDL- and LDL-apoB metabolic parameters were derived (17).

It is of great interest to establish whether VLDL secretion differs in the fasted and fed states and whether dietary composition can affect VLDL secretion. Marsh et al. (15) and Watts, Moroz, and Barrett (14) conclude from

TABLE 1. Metabolic parameters of apoB in different conditions

Condition	$VLDL_1$ PR	$VLDL_2$ PR	$VLDL_1$ FCR	$VLDL_2$ FRC	IDL PR	IDL FCR	LDL PR	LDL FCR	Source
Normolipidemia	13 ± 10 mg/kg/day	8.3 ± 10 mg/kg/day	18 ± 8.8 day^{-1}	11 ± 4.7 day^{-1}	11 ± 8.8 mg/kg/day	13 ± 7.7 day^{-1}	12 ± 3.4 mg/kg/day	0.46 ± 0.12 day^{-1}	
Homozygous FH null receptor	156%	168%	71%	81%	156%[a]	60%	137–330%[a]	30–39%[a]	23, 29
Homozygous FH defect receptor	75%	60%	64%	65%	101%	100%	103%	29%[a]	29
Heterozygous FH	75%			34%[a]	148	84	22%[a]	37%[a]	24, 26, 91
	149%[a]			81%			91%	50%[a]	
	547%[a]			123%[a]			173%[a]	64%[a]	
Homozygous FDB	85%			213%	ND	ND	49%	28%	33
Heterozygous FDB PCSK9 mutation	94%	212%	87%	108%	231%	176%	61%	32%	32
		293%		76%	65%	118%	192%	68%	38
Metabolic syndrome	152–164%[a]	150%[a]	60–85%[a]	87%	88%	70%[a]	76%	63%[a]	56, 102
	152%[a]		63%[a]						52, 54
Dysbetalipoproteinemia		67%[a]	30%[a]		119%	24%	66%[a]	126%[a]	88

apoB, apolipoprotein B; FCR, fractional catabolic rate; FDB, familial defective apoB-100; FH, familial hypercholesterolemia; PCSK9, proprotein convertase subtilisin/kexin type 9; PR, production rate. Values in normolipidemic subjects are mean values from a recently published meta-analysis (15). Percentage values are calculated using control subjects as the denominator.

[a]Significantly different compared with controls.

their meta-analyses that VLDL-apoB-100 production rate is higher in the fed state (25 vs. 14 mg/kg/day), whereas IDL- and LDL-apoB-100 kinetics parameters appear to be less affected by feeding, although there is considerable variation. This variation may relate to different feeding protocols used by the different groups (continuous feeding vs. bolus, fat load vs. mixed meal, etc.). When interpreting these data, it is important to keep in mind that feeding (as it occurs in real life, when larger meals are ingested in an uncontrolled setting) is difficult to study because of the non-steady-state situation induced by the meal.

Effect of interventions on VLDL- and LDL-apoB metabolism in normolipidemic subjects

Very few studies have examined the effect of nutrition on apoB metabolism in normolipidemic subjects. Stacpoole et al. (18) reported that in two normolipidemic subjects a high-carbohydrate, low-fat intake shifted the production of small VLDL particles to large VLDL particles. At the same time, the conversion of VLDL to LDL was reduced. The effect of fish oil supplements on VLDL-apoB-100 kinetics was investigated by Bordin et al. (19). Four weeks of feeding fish oil supplements resulted in a decreased production of VLDL-apoB-100 and a decreased triglyceride concentration. Similar findings have been reported in earlier studies using radioactive tracers (20).

Similarly, few studies have examined the effect of lipid-lowering drugs on apoB metabolism in normolipidemic subjects. Watts and colleagues (21) reported that simvastatin decreased the hepatic secretion of VLDL-apoB by 46% without altering VLDL FCR. LDL-apoB metabolism was not determined in this study. Malmendier et al. (22) evaluated the effect of simvastatin on LDL metabolism in normolipidemic subjects using radiolabeled LDL. A significant increase in LDL-apoB FCR was observed, whereas LDL production did not change. There is no study in normolipidemic subjects in which the effect of statin therapy on VLDL-, IDL-, and LDL-apoB metabolism is evaluated simultaneously. Furthermore, no kinetics studies using fibrates or nicotinic acids have been performed in normolipidemic subjects.

FAMILIAL HYPERCHOLESTEROLEMIA AND FAMILIAL DEFECTIVE APOB

VLDL- and LDL-apoB metabolism in familial hypercholesterolemia

Radiolabeling studies indicated very early that patients with familial hypercholesterolemia (FH) are characterized by a decreased clearance of LDL particles (23), which is in agreement with the underlying LDL receptor defect. Further studies have mainly focused on VLDL and IDL metabolism. Most studies report an abnormal VLDL metabolism. Cummings and colleagues (24) reported an increased secretion of VLDL, in agreement with a study by Tremblay et al. (25), who reported 50% and 100% increases in VLDL-apoB production in heterozygous and homozygous FH, respectively. On the other hand, Fisher and colleagues (26, 27) reported a decreased VLDL-apoB production rate but an increased direct production of IDL and LDL. In addition, these authors reported a dramatically altered conversion rate of VLDL to LDL. In normolipidemic subjects, between 30% and 50% of VLDL-apoB is converted to LDL; this fraction increases to 100% in FH. The discrepancies between different studies with respect to VLDL and IDL metabolism may also relate to the underlying molecular defect, because FH can be caused by a number of different mutations, as reviewed recently (28). Thus, Millar and colleagues (29) showed that receptor-defective FH patients had a total apoB production similar to controls, whereas receptor-negative FH patients had a significantly greater total apoB production than controls. Overall, in FH, the FCR of LDL-apoB (and to a lesser extent VLDL- and IDL-apoB) is decreased. Production rates also seem to be altered, with the majority of studies showing increased VLDL, IDL, and LDL production.

VLDL- and LDL-apoB metabolism in familial defective apoB-100

Several studies have addressed apoB metabolism in familial defective apoB-100 (FDB) (30–33). These studies uniformly indicate that LDL-apoB FCR is decreased to a similar extent as seen in FH. These studies also agree with respect to the production of LDL, which in all studies was found to be lower than in FH. There are, however, some differences concerning VLDL and IDL metabolism. Pietzsch and colleagues (32) reported that large $VLDL_1$ particles are produced at normal rates but smaller $VLDL_2$ particles are produced at an increased rate. At the same time, the FCR of IDL-apoB was increased (+76%) and the transfer rate to LDL was decreased, resulting in an overall reduction of LDL production of 39%. These data were confirmed, to a large extent, by Gaffney and colleagues (31) and by Schaefer et al. (33). Zulewski and colleagues (30), on the other hand, also found an increased production of VLDL but a decreased production of IDL and a nonsignificant decrease in IDL FCR. The differences between these studies with respect to VLDL and particularly IDL metabolism probably relate to the fact that the studies by Pietzsch, Gaffney, and Schaefer and colleagues (31–33) were performed in the fasting state, whereas the study by Zulewski et al. (33) was performed in the fed state. Together, these data indicate that the decreased production of LDL-apoB in FDB may originate from an enhanced removal of apoE-containing LDL precursors by LDL receptors, which may be upregulated in response to the decreased flux of LDL-derived cholesterol into hepatocytes.

VLDL- and LDL-apoB metabolism in proprotein convertase subtilisin/kexin type 9 mutations

In recent years, mutations in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene have also been reported as possible causes of hypercholesterolemia. Overexpression of PCSK9 in the livers of mice results in a marked reduction in LDL receptors in this organ (34–37)

and an inhibition of LDL clearance in mice and cultured cells (36). Thus, an increased LDL-apoB FCR would be the expected primary abnormality in kinetics studies. However, Ouguerram and colleagues (38) reported that PCSK9 mutations dramatically increase the production of apoB-100 compared with controls or FH patients with an LDL receptor mutation. These patients also showed a decrease in VLDL and IDL conversion and a decreased LDL FCR (−30%) that was still higher than that observed in LDL receptor-mutated FH. These findings again highlight the close functional relationship between apoB secretion and LDL catabolism (39). Interestingly, several nonsense mutations of PCSK9 result in hypobetalipoproteinemia (40), which is associated with longevity (41). Although no kinetics studies have been performed in these subjects, one could imagine that these forms of hypolipoproteinemia are characterized by both low production of VLDL-apoB and increased catabolism of LDL.

Effect of interventions on VLDL- and LDL-apoB metabolism in FH or FDB

Given the impact of statin therapy on LDL concentrations in patients with FH or FDB, it is surprising that few studies have examined the effect of these drugs on apoB metabolism in such patients. Most studies were performed using radiolabeled lipoproteins as tracers. In 1983, Bilheimer and colleagues (42) demonstrated that mevinolin (lovastatin) and colestipol decreased LDL-cholesterol by 27% in patients with heterozygous FH. This modest decrease in LDL-cholesterol was associated with a 37% increase in LDL-apoB FCR, whereas LDL production did not change. Later, the same group reported that the same combination (mevinolin and colestipol) decreased LDL-cholesterol by 52%, increased LDL FCR by 40%, and decreased production by 26% (43). Thus, in heterozygous FH patients, statins primarily affect LDL FCR, whereas production is only decreased with higher doses, resulting in more pronounced LDL reduction. In children with homozygous FH, lovastatin did not affect the plasma concentration or metabolism of LDL, but it decreased VLDL production by 74% (44). In more recent studies performed using stable isotope methodology in six heterozygous FH patients, simvastatin decreased VLDL-apoB production by 83% with no significant effect on VLDL FCR (45). IDL and LDL kinetics were not studied. However, in another study with mild hypercholesterolemia, it was shown that pravastatin significantly increased LDL FCR by 24% with no significant effect on production (46). In a further study, Winkler and colleagues (47) demonstrated that lifibrol increased LDL-apoB FCR by 75% and increased LDL-apoB production by 33% without affecting VLDL or IDL kinetics.

A number of studies have evaluated the effect of LDL apheresis on kinetics parameters in FH patients. Regular LDL apheresis (weekly reduction of LDL-cholesterol concentration by ~60%) did not affect VLDL-, IDL-, or LDL-apoB kinetics parameters. ApoB metabolism was studied with stable isotopes before the first apheresis and then again after 3–6 months of weekly apheresis. Both studies revealed the same kinetics parameters (48). However, when apoB metabolism before and immediately after LDL apheresis was compared, it was shown that VLDL-to-LDL conversion decreased temporarily from 76% to 51%. Furthermore, the tracer data indicated that in some patients LDL-apoB FCR increased transiently by 50% after apheresis (49).

Because LDL apheresis has little effect on endogenous apoB metabolism, it can also be used as a tool to study certain aspects of apoB metabolism. After apheresis, the concentration of apoB in LDL subfractions was analyzed. The resulting rebound curves were used to determine whether small, dense LDL particles are the metabolic product of LDL precursors or of larger LDL particles. In statin-treated heterozygous FH patients, small, dense LDL appeared to originate from both sources (50) (Fig. 2). Whether this is also true in other forms of hyperlipoproteinemia remains to be determined. This study also demonstrates that non-steady-state kinetics can be used to determine metabolic parameters in the absence of a tracer (51), although additional assumptions with respect to modeling are required (51a).

PATIENTS WITH THE METABOLIC SYNDROME AND/OR TYPE 2 DIABETES

VLDL- and LDL-apoB metabolism

ApoB metabolism in the metabolic syndrome and related disorders was recently reviewed by Chan, Barrett, and Watts (52). Individuals with insulin resistance have increased secretion rates of VLDL-apoB and triglycerides. The FCRs of VLDL-apoB and VLDL-triglycerides as well as of LDL-apoB are reduced compared with lean individuals (53–56). Visceral fat (57), hepatic fat (58), insulin resistance (59), and plasma adiponectin (60) seem to be the primary regulators of abnormal apoB and triglyceride kinetics in this condition. An increased amount of peritoneal fat results in an alteration of portal free fatty acid flux (61), an altered pattern of adipocytokines (62), and a disturbed ratio of proinflammatory to anti-inflammatory processes (63). It is currently unknown how these factors (FFA, adipocytokines, inflammation) affect each other, but they seem to be intimately linked. Although each factor may increase VLDL secretion by itself, it is the altered flow of free fatty acids that probably has the greatest impact on driving hepatic VLDL overproduction.

Although initial studies indicated an overall overproduction of VLDL particles, recent studies indicate a more complex abnormality. Adiels and colleagues (11, 54) developed a multicompartmental model that allows the kinetics of triglyceride and apoB-100 in different VLDL factions to be assessed simultaneously (Fig. 1). They found a significant positive correlation between triglyceride and apoB production in $VLDL_1$ and $VLDL_2$. Insulin resistance and type 2 diabetes were associated with excess hepatic production of $VLDL_1$ but not $VLDL_2$ particles. The direct secretion of $VLDL_2$-apoB and triglycerides was compa-

rable in insulin-resistant and insulin-sensitive subjects, consistent with previous studies (11, 54, 64), indicating that $VLDL_1$ and $VLDL_2$ production are independently regulated. The overproduction of $VLDL_1$ particles may result in a number of subsequent metabolic abnormalities, such as abnormal postprandial lipoprotein metabolism, the generation of small, dense LDL particles, and the increased catabolism of HDL particles (65).

An increased concentration of $VLDL_1$ results in an increased exchange of triglycerides for cholesteryl ester in HDL particles. This process, which is mediated by cholesteryl ester transfer protein, results in the formation of short-lived triglyceride-rich HDL particles and explains why hypertriglyceridemia is associated with low HDL-cholesterol. A similar process is probably responsible for the formation of small, dense LDL particles, although the formation of small, dense LDL may be the result of a more complex interaction.

Effect of interventions on VLDL- and LDL-apoB metabolism in patients with the metabolic syndrome and/or type 2 diabetes mellitus

The effect of nutritional interventions on apoB metabolism in patients with the metabolic syndrome or type 2 diabetes mellitus was recently reviewed by Barrett and Watts (66). Riches et al. (67) demonstrated that weight reduction decreases hepatic apoB secretion and increases LDL catabolism, although in another study reduction of abdominal fat resulted in a decreased rate of VLDL-triglyceride secretion that was not accompanied by decreased VLDL-apoB secretion (68). In another study in hypertriglyceridemic, obese patients, weight reduction induced complex changes in VLDL and LDL metabolism (13). VLDL secretion and LDL FCR were decreased, whereas LDL production remained unchanged. However, weight reduction was associated with changes in the source of LDL-apoB. Before weight loss, 73% of LDL was derived from VLDL and 27% from direct secretion, whereas after weight loss, only 47% originated from VLDL and 53% was secreted directly.

Fish oil supplements decrease VLDL-apoB secretion, do not alter VLDL-apoB FCR, and increase VLDL- to LDL-apoB conversion, accounting for the absence of any effect on plasma LDL-apoB (69). Plant sterols decrease the hepatic secretion of VLDL-apoB in hypercholesterolemic diabetics resulting from a reduced hepatic cholesteryl ester pool. Somewhat surprisingly, LDL-apoB catabolism was not changed by sitostanol in this condition (70). The effect of plant sterols or stanols on apoB kinetics in subjects with the metabolic syndrome has yet to be tested.

The effects of statins and other lipid-lowering drugs on apoB metabolism in patients with the metabolic syndrome were recently reviewed by Chan, Barrett, and Watts (52). A large body of evidence suggests that statins significantly increase the catabolism of all apoB-100-containing lipoproteins, such as VLDL, IDL, and LDL. With respect to VLDL-apoB secretion, the results are less consistent, with some studies showing no effect on apoB secretion (71–74) and others demonstrating a decrease in VLDL secretion during lovastatin or simvastatin therapy (75, 76). A recently published study further questions whether statin therapy can indeed affect VLDL-triglyceride secretion (77). In this study, the triglyceride-lowering effect of statins was solely attributable to increased triglyceride clearance.

Thus, in patients with the metabolic syndrome, the effect of statins on apoB metabolism seems to be more complex than in FH, in which statins primarily increase LDL FCR and decrease production, but only at higher doses. A recently published review (78) indicates that the effect of statins is dependent upon the lipoprotein phenotype before treatment. In patients with low LDL FCR at baseline, statins increase the FCRs of all apoB-containing lipoproteins, whereas in patients with normal LDL FCR, statins primarily decrease apoB production. In the metabolic syndrome, however, in which the state of insulin resistance and increased fatty acid flux drive VLDL-apoB secretion, statins increase rates of catabolism but do not appear to reduce VLDL secretion, even at high doses (79).

Statins also affect the metabolism of triglyceride-rich lipoproteins, presumably via LDL receptor-independent mechanisms. Atorvastatin reduces apoC-III concentrations (80), which may result in increased LPL activity, allowing more VLDL-apoB to be converted to LDL, thus minimizing the effect on LDL-apoB concentration. A similar mechanism has been proposed to account for the effect of statins on postprandial lipoprotein metabolism (81).

Peroxisome proliferator-activated receptor α agonists, such as fibrates, primarily increase the catabolism of VLDL-, IDL-, and LDL-apoB, whereas they seem to have little effect on the hepatic secretion of lipoproteins (79), although earlier studies performed with exogenously radiolabeled lipoproteins indicated that fibrates increase the FCRs of VLDL and IDL but decrease LDL FCR in hypertriglyceridemia (82). This discrepancy may relate to different fibrates and to differences in patient groups. Because fibrates are agonists for a nuclear receptor and thus affect a master regulator, the observed effects on apoB metabolism may depend more on the underlying condition than in the statins. This may also explain why the effect of fibrates appears broader than the rather specific effect of statins on LDL concentration via the regulation of HMG-CoA reductase and hence the LDL receptor. In diabetic patients, fibrates result in a more pronounced shift in LDL subtype distribution compared with statins (83).

The dyslipidemia in diabetic patients is the result of both insulin resistance and hyperglycemia. Therefore, it is not surprising that the lipid profile during pioglitazone therapy is more favorable than during sulfonylurea treatment, despite achieving similar levels of glucose control (84). Because insulin resistance is characterized by an overproduction of $VLDL_1$, it could be expected that the beneficial effect of pioglitazone on lipids is primarily the result of decreased VLDL secretion. In a recent study, Nagashima and colleagues (85) evaluated the effect of pioglitazone on the kinetics of VLDL-, IDL-, and LDL-apoB as well as on VLDL-triglycerides. Surprisingly, they found that pioglitazone treatment reduced VLDL-triglyc-

eride concentration primarily by increasing the lipolytic removal of VLDL-triglycerides without affecting VLDL-triglyceride and -apoB production. Conversion of VLDL to LDL was unaffected as well. It is unclear why weight reduction and pioglitazone therapy, both of which decrease insulin resistance, supposedly have such different effects on apoB metabolism. This is particularly disturbing because the effect on triglyceride concentration and LDL subtype distribution is similar.

There are no studies evaluating the effect of nicotinic acid or ezetimibe on apoB metabolism in the metabolic syndrome or type 2 diabetes, although both drugs were shown to have effects on lipid concentrations in such patients.

In a recently published study, the effect of the cholesteryl ester transfer protein inhibitor torcetrapib on apoB metabolism was evaluated (86). Although there is no evidence that the subjects studied were insulin-resistant, they exhibited characteristics of the metabolic syndrome (low HDL-cholesterol, high VLDL-apoB production). In this study, torcetrapib reduced VLDL-, IDL-, and LDL-apoB-100 levels primarily by increasing the rate of clearance. In contrast, when torcetrapib was added to atorvastatin, it enhanced the clearance of VLDL particles, whereas the effects on IDL- and LDL-apoB concentration were primarily mediated by reduced production (86).

DYSBETALIPOPROTEINEMIA (TYPE III HYPERLIPIDEMIA)

Dysbetalipoproteinemia, which is usually characterized by homozygosity of apoE2, is characterized by a delayed catabolism of apoB-100- and apoB-48-containing lipoproteins with little conversion to IDL and LDL (87). Turner and colleagues (88) showed that the production of IDL is increased in dysbetalipoproteinemia and that the time for conversion of IDL- to LDL-apoB is delayed 5-fold. More recent studies focusing on chylomicron metabolism have shown that dysbetalipoproteinemia is associated with delayed chylomicron metabolism (89, 90).

In the study by Turner et al. (88), gemfibrozil therapy reduced VLDL and IDL production in dysbetalipoproteinemia. A more recent study in patients with coexisting dysbetalipoproteinemia and heterozygous FH (91) demonstrated that VLDL and IDL FCRs were decreased, whereas LDL FCR was increased, compared with FH heterozygotes and normolipidemic control subjects. Furthermore, fenofibrate treatment increased VLDL and IDL FCRs but also increased LDL production.

HYPOBETALIPOPROTEINEMIA

Hypobetalipoproteinemia is characterized by low concentrations of LDL-cholesterol and apoB (92, 93). Familial hypobetalipoproteinemia is primarily caused by mutations in the apoB gene. Almost 60 different mutations have been described that interfere with the translation of the full-length apoB. Many of these mutations cause the production of truncated apoB. Hooper, van Bockxmeer, and Burnett (94) recently reviewed this field.

The metabolism of several truncated apoBs has been studied using stable isotope technique (**Table 2**). These studies have provided important information on the structure-function relationships of normal apoB. Both decreased production rates and increased clearance rates are responsible for the low plasma concentrations of apoB. Truncations that retain the LDL receptor binding domain, such as apoB-89, apoB-87, and apoB-75, have increased FCRs (95–97). On the other hand, truncated forms of apoB are also secreted at lower rates. VLDL-apoB secretion was reduced by ~1.4% for each 1% of apoB truncated (98). Truncated apoBs smaller than apoB-30 are not detectable in plasma; thus, this appears to be the minimum length of apoB that is required for microsomal triglyceride transfer protein (MTP)-dependent lipoprotein assembly. In vitro data indicate that the lipid content of secreted apoB-containing lipoproteins is decreased as apoB is shortened (99).

When heterozygotes for truncated apoBs are studied with an endogenous labeling technique, the metabolism of apoB-100 and of the truncated apoB can be determined simultaneously. Aguilar-Salinas and colleagues (100) evaluated apoB-100 metabolism in eight heterozygote subjects, characterized by truncations of apoB ranging from apoB-31 to apoB-89. The production of apoB-100 was decreased in all eight subjects compared with control subjects, whereas VLDL FCRs were variable.

Some forms of hypobetalipoproteinemia are not attributable to truncated forms of apoB. Latour and colleagues

TABLE 2. Metabolic parameters of truncated apoB

Truncation	VLDL PR	VLDL FCR	LDL PR	LDL FCR	Comment	Source
ApoB-31	4%	5%	—	—	ApoB-31 only detectable in HDL	98
ApoB-50	Normal	↑↑↑	—	—	Exogenous labeling study; no quantification of parameters	103
ApoB-54.8	37%	158%	—	—	Not enough apoB-54.8 in intermediate density lipoprotein/LDL fractions to determine metabolic parameters	98
ApoB-67	6.5%	65%	3%	91%		104
ApoB-75	64%	215%	11%	147%		97
ApoB-87	—	—	6%	190%		96
ApoB-89	92%	144%	18%	192%		95

PR, production rate. Percentage values are calculated using apoB-100 parameters determined in the same subjects as the denominator.

(101) performed a stable isotope study in such a kindred and observed 2- to 3-fold increases in VLDL- and LDL-apoB-100 FCRs. The underlying mechanisms are unclear and remain to be elucidated (93).

CONCLUSION

Human lipoprotein kinetics studies provide insight into the factors that control the secretion and catabolism of apoB-containing lipoproteins. Studies in hypolipoproteinemia and hyperlipoproteinemia have elucidated the processes that control VLDL secretion as well as VLDL and LDL catabolism. In hypobetalipoproteinemia attributable to a truncation in apoB, the rate of secretion is closely linked to the length of apoB. On the other hand, in patients with the metabolic syndrome, it appears that substrate, in the form of free fatty acids, coupled to the state of insulin resistance can induce the hypersecretion of VLDL-apoB. Studies in patients with FH, FDB, and PCSK9 mutations show that these mutations are the most important determinants in regulating LDL catabolism.

The overall importance of these studies relates to our need to understand the factors that link abnormalities of lipoprotein metabolism with an increased risk of atherosclerotic disease. Although apoB metabolism is a central component of lipoprotein metabolism, it is only one component. Therefore, apoB metabolism must be seen in context together with postprandial lipoprotein metabolism and reverse cholesterol transport. Although such interactions are of great interest and relevance, few kinetics data are available to date. Future studies should focus on the interaction between different pathways of lipoprotein metabolism and how lipoprotein metabolism is linked to protein and carbohydrate metabolism at the whole body level.

This review also demonstrates that metabolic studies may provide contradictory data even under similar or identical conditions. This further highlights the sensitivity of the methodology used for kinetics studies and underscores the need to design studies carefully and include appropriate controls.

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