**Recent explanatory trials of the mode of action of drug therapies on
lipoprotein metabolism**

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Purpose of review

Dysregulated lipoprotein metabolism leads to increased plasma concentrations of atherogenic lipoproteins. We highlight the findings from recent studies of the effect of lipid-regulating therapies on apolipoprotein metabolism in humans employing endogenous labelling with stable isotopically labelled isotopomers.

Recent findings

Fish oil supplementation and niacin treatment both reduce fasting and postprandial triglyceride levels by decreasing the hepatic secretion of VLDL-apoB-100 (apoB) and apoB-48-containing chylomicron particles in obese and/or type 2 diabetes. Niacin also lowers plasma LDL-apoB and Lp(a) levels by increasing catabolism of LDL-apoB and decreasing secretion of Lp(a), respectively. In subjects with hypercholesterolaemia, inhibition of cholesteryl ester transfer protein raises apoA-I and lowers apoB by decreasing and increasing the catabolism of HDL-apoA-I and LDL-apoB, respectively. Antisense oligonucleotides directed at apoB mRNA lowers plasma LDL-cholesterol and apoB chiefly by increasing the catabolism and decreasing the secretion of LDL-apoB in healthy subjects. That apoB ASO treatment does not lower hepatic secretion in humans is unexpected and merits further investigation.

Summary

Kinetic studies provide mechanistic insight into the mode of action of lipid lowering therapies and lipoprotein disorders. Understanding the mode of action of new drugs in vivo is important to establish their clinical use and enable approval by regulatory authorities.

Keywords

cardiovascular disease, dyslipoproteinaemia, lipoprotein metabolism, lipid-regulating agents, stable isotope study

INTRODUCTION

Dyslipoproteinaemia, an important risk factor for atherosclerosis, is characterized by high plasma concentrations of apolipoprotein (apo) B-containing lipoproteins (i.e. apoB-48 and apoB-100-containing lipoproteins), and low concentrations of high-density lipoprotein (HDL) [1]. Recent epidemiological and genetic studies also suggest that elevated lipoprotein(a) (Lp[a]) is a causal risk factor for atherosclerotic cardiovascular disease (CVD) [2]. Dysregulation of lipoprotein metabolism may be caused by a combination of overproduction of triglyceride-rich lipoproteins (TRLs), including very-low-density lipoprotein (VLDL) and chylomicron, decreased catabolism of apoB-containing particles, and increased catabolism of HDL apoA-I particles, with elevated plasma concentrations of Lp(a) driven chiefly by hepatic oversecretion of apo(a) [3,4,5]. Pharmacological interventions regulate lipid and lipoprotein metabolism by altering the kinetics or dynamics of lipoprotein particles. Understanding the mode of action of new drugs *in vivo* is important to establish their clinical utility and to facilitate approval by regulatory authorities. We review recent findings based on tracer kinetics, which shed new light on the mechanisms of action of several lipid-regulating therapies.

Explanatory trials

Tracer kinetic studies of the effect of drugs on lipid and lipoprotein metabolism in humans fall under the remit of, so called, explanatory trials. By contrast to conventional and pragmatic trials, explanatory or mechanistic intervention trials focus on highly refined hypothesis. Eligibility criteria are necessarily usually quite specific and can be exhaustive. These trials may *ab initio* be

carried out in normal subjects; patients are also studied under conditions that not simulate clinical care. The trials are usually costly and the sample sizes accordingly comparatively small. While elucidatory and rich in explanatory information, generalizability is usually limited because of the restricted selection criteria. Nevertheless, the information furnished by these trials are useful in several respects, including provision of the rationale of combination therapy, delineation of new pathways that may be therapeutically targeted to mitigate residual CV risk, and the proffering of a mode of action for an agent that may support registration and potentially support a new clinical indication.

Principles of tracer kinetics

Lipoprotein metabolism is complex and abnormal plasma concentrations result from alterations in the rates of production and/or catabolism of the various lipoprotein particles. However, static measures of either plasma lipid or lipoprotein concentrations do not adequately characterize disorders of lipoprotein metabolism. Stable isotope tracer studies using endogenous labelling of apolipoproteins with amino acid precursor molecules (isotopmers) and mathematical modelling have been employed to study lipoprotein kinetics by a core of centres expert in this technology. Such kinetic data further our understanding of metabolic disorders and the effects of therapies. Briefly, stable isotopically labelled amino acids (typically ^{13}C -leucine or D_3 -leucine) can be administered intravenously as a bolus or primed infusion with serial blood sampling over several days to assess the turnover of VLDL, intermediate density lipoprotein (IDL) and LDL-apoB-100 (apoB), HDL apoA-I

as well as apo(a). In addition to apolipoprotein turnover, stable isotopes and GCMS may also be employed to assess the kinetics of triglyceride, cholesterol and fatty acids. For examples, D₅-glycerol and ¹³C-palmitate have been used for VLDL-triglyceride metabolism whereas ¹³C₅-cholesterol and D₆-cholesterol have been used to estimate intestinal cholesterol absorption. Enrichment data are generated by gas- or liquid-chromatography mass spectrometry (GCMS or LCMS, respectively) analysis after separation of the relevant apolipoproteins. Enrichment data are subjected to modelling, typically using multicompartmental analysis to fit the tracer data and estimate fractional turnover and conversion rates of lipoproteins, from which mass transport rates may be calculated. We have detailed these methods elsewhere [6, 7].

INTERVENTIONS

Normalization of abnormal plasma lipid and lipoprotein concentrations is the major objective of therapy. Lifestyle modifications and pharmacological interventions are the cornerstone of clinical management. Several kinetic studies have been carried out to examine the effects of weight loss, increased physical activity, statin, fibrate and ezetimibe on lipoprotein metabolism in humans. Readers should refer to more detailed reviews. [3, 4, 7].

Fish oil supplementation

Fish oils are a rich source of long-chain ω -3 polyunsaturated fatty acids (PUFAs), primarily eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). There is evidence that fish oil supplementation protects against atherosclerotic CVD and this may be partly mediated by improvement in

hypertriglyceridaemia [8]. In an 8-week double-blind, randomized, placebo-controlled, cross-over intervention trial of the effect of oral supplementation with 3g/day ω -3 PUFAs on triglyceride-rich lipoprotein (TRL) kinetics in 10 men with type 2 diabetes treated with metformin [9], Tremblay et al found that ω -3 PUFA supplementation had no significant impact of postprandial apoB-48 and VLDL-apoB concentrations nor on the production or catabolic rates of these lipoproteins. This observation contrasts with our previous kinetic study showing that the addition of 12-weeks oral supplementation with 4g daily of fish oil capsules (45% EPA and 39% DHA as ethyl esters) significantly improved postprandial responses in plasma triglyceride and apoB-48 in obese subjects who were on a moderate weight loss diet [10]. In this study, fish oil supplementation also significantly decreased basal apoB-48 secretion, without significant effect on apoB-48 fractional catabolic rate (FCRs). The discrepant findings might be explained by differences in study design, subject characteristics (obesity on background weight loss diet vs type 2 diabetes on background metformin treatment), as well as the duration (8 weeks vs 12 weeks) and dose of fish oil interventions (3g/day vs 4g/day). It is noteworthy that the favourable effect of fish oil supplementation on postprandial TRL metabolism is confirmed by our recent study showing that supplementation with fish oil (4g/day) improved postprandial responses in plasma triglyceride, VLDL-apoB and apoB-48 in statin-treated patients with familial hypercholesterolaemia (FH) receiving standard cholesterol-lowering therapy [11*].

More recent research also focuses on the use of icosapent ethyl (a high-purity prescription form of the ethyl ester of the ω -3 fatty acid EPA) for treatment of hypertriglyceridaemia [12]. Several studies have demonstrated that icosapent ethyl significantly reduced triglyceride concentrations and improved other lipid parameters, including non-HDL-cholesterol, apoB and apoC-III [12, 13*]. However, the mechanism of action of icosapent ethyl on TRL metabolism, including apoC-III, has not yet been examined.

Niacin

Nicotinic acid, or niacin, is a potent agent for lowering plasma triglyceride, raising HDL cholesterol and with moderate effects on LDL-cholesterol [14]. Niacin is also one of few agents that can significantly lower plasma Lp(a) concentrations. Data from several earlier trials have supported the use of niacin with statin to treat dyslipidaemia [15]. However, recent clinical outcome trials failed to demonstrate CV benefits of niacin therapy [16, 17].

In a randomized, cross-over study, the mechanism of action of extended-release (ER) niacin treatment on postprandial lipoprotein metabolism was studied in 11 statin-treated men with type 2 diabetes [18*]. ER niacin reduced apoB-48 concentration by lowering fasting and postprandial apoB-48 secretion. This observation is consistent with the notion that niacin inhibits diglyceride acyltransferase 2 (DGAT2), the terminal and rate-limiting enzyme in triglyceride synthesis, leading to lower rates of triglyceride production, which may decrease the lipidation of chylomicron particles and thus lower the secretion of apoB-48 containing particles [19].

In the same study, we also found that 12-week ER niacin treatment decreased Lp(a) concentrations by decreasing the production of apo(a). ER niacin decreased the concentrations of VLDL-, IDL- and LDL-apoB chiefly by decreasing the corresponding production rates [20*]. The reductions in VLDL-apoB production are consistent with the notion that niacin decreases hepatic apoB secretion while the reductions in LDL-apoB production may relate to reductions in transport of these particles down the VLDL-LDL cascade. In another 8 week, double-blind, placebo-controlled cross-over trial in the fasted state, and using LCMS method [21], Croyal et al reported that ER niacin treatment (2g/day) decreased apo(a) chiefly by a reduction in apo(a) production in 8 nondiabetic, obese and hypertriglyceridaemic men. In contrast to our study, they found that niacin treatment increased the FCR of LDL-apoB without significant effect on VLDL-apoB kinetics. Differences in study design (fasting vs postprandial studies), subject characteristics (hypertriglyceridaemia vs type 2 diabetes), laboratory methodology (GCMS vs LCMS), compartmental modeling (fasted vs nonsteady states) and especially in the context of background statin therapy [20] might have accounted for the discrepant findings in apoB kinetics.

Cholesteryl ester transfer protein (CETP) inhibitors

CETP is a glycoprotein that facilitates the transfer of cholesteryl ester from HDL to apoB-containing lipoproteins. As high plasma levels of CETP are correlated with low levels of HDL-cholesterol, the pharmacological inhibition of CETP raises HDL-cholesterol levels [22**]. In a randomized, placebo-

controlled, double-blind study [23**], Reyes-Soffer et al found, in subjects with mildly hypercholesterolaemia, that anacetrapib (a novel CETP inhibitor) increased HDL-apoA-I by decreasing the FCR of HDL-apoA-I, with no effect on HDL-apoA-I production (see Figure 1). As part of the same study, Millar et al found that anacetrapib significantly lowered plasma VLDL, IDL, and LDL apoB-100 pool sizes, chiefly owing to an increase in their catabolism [24]. The effects of anacetrapib on the kinetics of apoA-I and apoB are generally consistent with earlier studies using another CETP inhibitor, torcetrapib [25, 26]. Beyond raising apoA-I and decreasing apoB concentrations, anacetrapib was also previously shown to decrease plasma Lp(a) concentrations by 35-40%. However, the precise mechanism of action CETP inhibitor on Lp(a) metabolism remains to be elucidated. The kinetic benefits in apoA-I, apoB-100 and Lp(a) with anacetrapib could have complementary benefit on CVD. This requires to be confirmed in the ongoing REVEAL study, although earlier clinical endpoint trials failed to demonstrate clinical benefit with CETP inhibition using torcetrapib, dalcetrapib or evacetrapib [22].

Another newer CETP inhibitor TA-8995, as monotherapy or with a statin, has also been found to significantly lower LDL-cholesterol and raise HDL-cholesterol in a phase 2 randomized, parallel-group trial of more than 300 patients with dyslipidaemia [27*]. However, the translation of the antiatherogenic potential of TA-8995 remains to be investigated and warrants formal testing in a cardiovascular-outcomes trial.

Antisense oligonucleotides (ASO) of apoB

Given the central role of apoB in lipoprotein metabolism, interventions that correct apoB metabolism are clearly important. As indicated elsewhere, statins lowers LDL-cholesterol and apoB by enhancing clearance of apoB-containing particles. Hence, blocking apoB synthesis may provide a complimentary approach to reduce elevated levels of LDL-cholesterol and apoB by inhibiting the synthesis of apoB.

Mipomersen is a second-generation antisense oligonucleotide (ASO) designed to directly inhibit the synthesis of apoB by targeting its mRNA [28]. Clinical studies in hypercholesterolaemic subjects, including FH, have shown that mipomersen (dose range 50-300 mg) significantly lowers plasma LDL-cholesterol and apoB by up to 50% [29]. In a single blind, two-period, linear, placebo-controlled study in 17 healthy subjects [30**], Reyes-Soffer et al reported that mipomersen (200 mg/week, 7-9 week intervention) significantly lowered apoB in VLDL, IDL and LDL by increasing the FCRs of VLDL and LDL-apoB, as well as decreasing the production rates of IDL and LDL-apoB (see Figure 2). The increases in apoB catabolism may have resulted from the mipomersen-mediated decrease in apoC-III concentration. Surprisingly, mipomersen had no significant effect on the production of either VLDL-apoB or triglycerides. These results are unexpected given the current understanding of the inhibitory effect of mipomersen on apoB synthesis. To further elucidate the lack of effect of mipomersen on the secretion of VLDL-apoB and triglycerides in vivo, the authors found that ASO knockdown of apoB mRNA in chow-fed mice preserved both apoB and triglyceride secretion, consistent with

their findings in healthy volunteers. Interestingly, similar experiments was carried out in high-fat-fed mice showing stepwise reductions in both apoB and triglyceride secretion with titrated ASO knockdown of apoB mRNA. This suggests that the effect of mipomersen on VLDL metabolism might have been different in subjects with hypercholesterolaemia but this remains to be demonstrated.

Several studies have also consistently demonstrated that mipomersen effectively reduced Lp(a) levels in patients with FH or severe hypercholesterolaemia [31]. Falls in Lp(a) may be consequent on lower apoB concentrations, which limits the pool of apoB available to link to apo(a), but kinetic studies are required to test this hypothesis.

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) inhibitors

PCSK9 is a key regulator of the LDL receptor and hence the metabolism of LDL [32]. Gain-of-function mutations of PCSK9 cause autosomal dominant hypercholesterolaemia, whereas PCSK9 deficiency results in low LDL-cholesterol and importantly, protection against CVD [33, 34]. Beyond LDL metabolism, PCSK9 may play an important role in TRL metabolism [35]. We have reported, in obese individuals, that plasma PCSK9 concentration is directly associated with postprandial TRL metabolism in response to a fat load [36]. Recent data also suggest that PCSK9 is associated with hepatic steatosis and expression of genes involved in lipogenesis, such as sterol regulatory element binding protein 1C, acetyl-CoA carboxylase and fatty acid synthase [37]. Hence, PCSK9 is a new and essential target for correcting

both hypercholesterolaemia and possibly hypertriglyceridaemia in high-risk patients. Recent trials with monoclonal antibodies (mAbs) to PCSK9 have demonstrated significant reductions in LDL, Lp(a) and TRLs [38, 39*]. The clinical benefit of PCSK9 has been recently demonstrated in two long-term studies of patients at high risk of CVD or with FH reporting approximately 50% reductions in composite cardiovascular events at 12 to 18 months with anti-PCSK9 therapy (alirocumab and evolocumab) [40*, 41*]. The mechanisms for PCSK9 inhibition on lipoprotein metabolism, including LDL, Lp(a) and TRLs, remains to be fully demonstrated.

Antisense oligonucleotides (ASO) of apolipoprotein C-III

ApoC-III is strongly associated with hypertriglyceridemia and progression of CVD [42*]. ApoC-III impairs the lipolysis of TRLs by inhibiting lipoprotein lipase and the hepatic uptake of TRLs by the LDL receptor [43]. Two recent reports have demonstrated that apoC-III is an important determinant of TRL catabolism [44, 45*]. Hence, strategies to inhibit apoC-III may be effective to correct TRL metabolism in clinical practice [42].

Volanesorsen, an ASO to apoC-III, is the most advanced pharmacological inhibitor of apoC-III. Clinical studies showed that volanesorsen significantly lowered plasma apoC-III and triglyceride concentration in patients with familial chylomicronaemia syndrome or type 2 diabetes [46*, 47*]. The underlying mechanism of action of volanesorsen in lowering plasma triglycerides is not fully understood, but may be due to an improvement in hepatic TRL clearance via the LDL receptor and LDL receptor-related protein-1 pathway. However,

the mechanism of action of volanesorsen on TRL and glucose metabolism merits further investigation.

CONCLUSION

Kinetic studies provide mechanistic insight into the therapy of lipid and lipoprotein disorders. Table 1 summarises the potential mechanisms of several pharmacological interventions in regulating lipid and lipoprotein metabolism. These interventions are effective for treating dyslipoproteinaemia via different mechanisms, including lower secretion of TRLs and Lp(a), enhanced LDL-apoB clearance and lower rates of HDL-apoA-I catabolism. Several agents are in development for regulating lipoprotein metabolism that are relevant to the future management of atherogenic dyslipidaemia. These include selective peroxisome proliferator-activated receptors (PPARs)- α modulators; PPAR- α/δ agonists; inhibitors of DGAT-1 and angiopoietin like protein-3, as well as ASO of apo(a) [4, 48, 49, 50*]. Although these newer agents appear to be promising for use in the treatment of atherogenic dyslipidaemia, safety issues, in particular increased risk of hepatic steatosis and/or thrombocytopenia with ASO therapy (e.g. mipomersen and volanesorsen) requires caution. Nevertheless, PCSK9 inhibition appears to be a safe and promising therapeutic approach for regulating lipoprotein metabolism. However, the precise mechanism of action of these agents on lipoprotein metabolism remains to be demonstrated.

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Conflicts of interest

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KEY POINT

- Kinetic studies provide mechanistic insight into the mode of action of therapies of lipid and lipoprotein disorders.
- Pharmacological interventions improve lipoprotein disorders via different mechanisms, including lower secretion of TRLs (VLDL-apoB-100 and apoB-48) and Lp(a), enhanced LDL-apoB-100 clearance and lower rates of HDL-apoA-I catabolism.
- The mode of action of newer agents on lipoprotein kinetics, including Lp(a) metabolism, merits further investigation.

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Legend

Table 1. Mechanisms of action of omega-3 fatty acid, niacin, cholesteryl ether transfer protein (CETP) inhibitor and antisense oligonucleotides (ASO) to apoB on lipid and lipoprotein metabolism.

Agents	Plasma lipid and lipoprotein concentrations						Lipoprotein kinetics									
	LDL-C	ApoB-100	Triglyceride	ApoB-48	HDL-C	Lp(a)	VLDL-apoB		LDL-apoB		apoB-48		HDL-apoA-I		Lp(a)	
							PR	FCR	PR	FCR	PR	FCR	PR	FCR	PR	FCR
ω-3 FAs	↑5-10%	↓5-20%	↓25-30%	↓25-30%	↑0-5%	↓0-10%	↓↓	→	→	→	↓↓	→	↓	↓	?	?
Niacin	↓10-15%	↓10-15%	↓5-35%	↓25-30%	↑10-25%	↓20-30%	↓↓	→	↓↓	↑/↓	↓↓	→	→/↑	↓	↓↓	→
CETP inhibitor	↓15-35%	↓10-35%	↓5-15%	↓25-50%	↑50-125%	↓35-40%	→	↓↓	→	↓↓	↓↓	→	↑↑	→	?	?
ApoB-ASO	↓10-35%	↓10-35%	↓5-25%	?	↑0-10%	↓5-25%	→	↑↑	↓	↑↑	?	?	?	?	?	?

Apo: apolipoprotein; ASO: antisense oligonucleotides; CETP: cholesteryl ether transfer protein; FAs: fatty acids; FCR: fractional catabolic rate; HDL: high-density lipoprotein; HDL-C: HDL-cholesterol;; LDL: low density lipoprotein; Lp(a): lipoprotein(a); PR: production rate; VLDL: very-low-density lipoprotein
↑: mild increase; ↑↑: marked increase; ↓: mild decrease; ↓↓: marked decrease; →: no change; ? : not investigated