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Review

The extended abnormalities in lipoprotein metabolism in familial hypercholesterolemia: Developing a new framework for future therapies

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ABSTRACT

Familial hypercholesterolemia (FH) is a dominantly inherited disorder characterized by marked elevation of plasma low-density lipoprotein (LDL) cholesterol concentrations and premature coronary artery disease (CHD). In addition to impaired LDL receptor-mediated clearance of LDL particles, *in vitro* and *in vivo* studies suggest that hepatic oversecretion of apolipoprotein (apo) B may contribute to the hypercholesterolemia in FH. This may be due to an effect of the expanded hepatic pool of cholesterol (a consequence of increased receptor-independent uptake of LDL) and/or a direct effect of the LDL receptor on apoB secretion. Hepatic oversecretion of apoB may depend on the type and severity of the genetic mutation causing FH. FH can also increase plasma Lp(a) concentration by an undefined mechanism that may not directly involve the LDL receptor pathway. Decreased catabolism of triglyceride-rich lipoproteins could also be due to deficient LDL receptor function, accounting for postprandial dyslipidemia in FH. The metabolism of high-density lipoprotein (HDL) in FH is poorly understood, but preliminary data suggest abnormal HDL composition and functionality, as well as altered transport of apoA-I. Beyond effects related to specific genetic defects in the LDL pathway, co-existing secondary causes, particularly obesity and insulin resistance, and other genetic variants may also perturb lipoprotein metabolism in individuals with FH. Furthermore, residual risk remains high in statin-treated FH. Knowledge of an extended metabolic framework will, therefore, provide the basis for judiciously selecting new pharmacotherapies to treat FH, including apoB antisense oligonucleotides, microsomal transfer protein (MTP) inhibitors and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.

1. Introduction

Familial hypercholesterolemia (FH) is a co-dominantly inherited disorder characterized by markedly elevated plasma low-density lipoprotein (LDL) cholesterol concentrations [1,2]. Homozygous FH has a prevalence of approximately one in a million while the prevalence of heterozygous FH is estimated to be at one in 300 to 500 people. This frequency may be higher in selected populations with high consanguinity. FH is present from birth and significantly accelerates the onset of all forms of atherosclerotic cardiovascular disease (CVD), especially coronary heart disease (CHD) [1–3]. Statins and other cholesterol lowering therapies decrease the risk of CHD in FH [4], but recommended treated targets can be difficult to achieve [5,6].

A mutation in the gene encoding the LDL receptor (*LDLR*) is the underlying molecular defect in the majority of FH patients, with over more than 1000 genetic variants identified to date [7]. Consequently, the classical metabolic defect in FH is hypocatabolism of LDL particles [8–10]. This notion is well supported by experimental evidence and metabolic studies in FH patients [9,10]. Mutations in apolipoprotein (apo) B (*APOB*) and proprotein convertase subtilisin/kexin type 9 (*PCSK9*) have also been shown to result in autosomal dominant hypercholesterolemia, a phenotype and metabolic defect similar to FH [10]. A focus on the catabolic defect as the sole mechanistic basis for FH does not, however, account for an expanded hepatic cholesterol pool due to up-regulated LDL receptor-independent uptake pathways and/or a direct effect of the LDL receptor on apoB secretion. In addition, recent studies point to elevated plasma Lp(a) concentrations as an independent metabolic defect and CHD risk factor in FH and non-FH populations, although the precise mechanisms underlying this increase remain unclear. Perturbations of triglyceride-rich lipoprotein (TRL) metabolism due to deficient or absent LDL receptors may also account for postprandial dyslipidemia in FH, reviewed recently by Chan and Watts [11]. High-density lipoprotein (HDL) metabolism in FH also merits closer review, given new data that HDL may be a CHD risk factor in these subjects [12].

The purpose of this review is, therefore, to provide an integrated overview of the perturbations in lipoprotein metabolism in FH, beyond the current understanding of the LDL receptor paradigm. In addition, abnormalities in lipoprotein metabolism will be examined in the context of best current levels of care and novel therapeutic

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strategies that are in development or in clinical trials. This is particularly important given that FH individuals who are already treated with statins and/or currently available lipid-regulating therapies remain at high CHD risk [13–15].

2. ApoB-100 metabolism: LDL receptor and ApoB paradigms

The classical metabolic defect in FH is hypocatabolism of LDL particles due to decreased LDL receptor activity [8]. However, this does not account for the possibility that cholesterol overload in hepatocytes in FH and/or the diminished LDL receptor activity itself may enhance hepatic secretion of apoB, which in turn markedly elevates plasma concentrations of LDL cholesterol. This is supported by more recent studies that have challenged the LDL receptor paradigm [16–19], and details of both the experimental and human work follow.

2.1. Experimental work: cell culture and animal models

In vitro observations in perfused rat livers and in isolated hepatocytes attest to the rate-limiting effect of lipid substrate availability, in particular cholesteryl esters, in regulating the hepatic secretion of apoB. The secretion of apoB from HepG2 cell has been shown to be better correlated with the total mass of cholesteryl ester present within the cell than the rate of *de novo* cholesteryl ester synthesis [20]. In cultured human hepatocytes, the addition of cholesterol as LDL increased the cholesteryl ester content of the cells and in turn, the hepatic secretion of apoB. By analogy, in FH an expanded intrahepatic pool of cholesteryl esters due to increased non-receptor mediated transfer of cholesterol into the cell from plasma (a consequence of LDL receptor deficiency and hypocatabolism of LDL) may drive apoB secretion.

Several *in vitro* studies also suggest a direct effect of the LDL receptor on hepatic apoB secretion [21–24]. In mice that overexpress the sterol regulatory element binding protein (SREBP)-1a, cholesterol and fatty acid synthesis is increased 3-fold [21]. Despite the increase in hepatic lipids, plasma cholesterol and triglyceride concentrations were not different to wild-type mice. However, in transgenics that are also LDL receptor negative (null mutation), plasma cholesterol and triglyceride concentrations were elevated 15- and 7-fold, respectively. Studies in mouse primary hepatocytes have also described a similar association between the LDL receptor and apoB secretion. Despite having similar intracellular lipid, microsomal triglyceride transfer protein (MTP) and apoB mRNA levels, LDL receptor negative hepatocytes exhibited almost 2-fold higher apoB secretion rates compared with wild-type cells [22]. Overexpression of the human *LDLR* gene in the receptor negative hepatocytes decreased apoB secretion to normal levels. The increase in apoB secretion was, therefore, due to newly synthesized apoB escaping intracellular degradation and to diminished re-uptake of secreted apoB by the LDL receptor. Further evidence that LDL receptor deficiency results in enhanced very low-density lipoprotein (VLDL) or LDL production comes from a recent study using human induced pluripotent stem cell (iPSC) derived hepatocytes from a patient with homozygous loss of LDL receptor function [25]. The iPSC derived hepatocytes had an 8-fold increase in the level of secreted apoB compared with hepatocytes derived from control stem cell lines. No difference in apoB mRNA expression between these cell lines was observed, however. This concurs with findings from earlier studies that increase in apoB secretion are determined by the amount of apoB escaping intracellular degradation rather than altered apoB gene expression *per se* [25]. Of note, inhibition of apoB secretion by the LDL receptor requires interaction of its ligand binding domain with apoB in the endoplasmic reticulum (ER), as well as re-uptake of secreted particles via apoB and apoE ligand-dependent endocytosis [23,24].

By contrast to the above, apoB secretion in the Watanabe Heritable Hyperlipidemic (WHHL) rabbit, an animal model of FH, is not different to that in wild-type rabbits [26]. WHHL rabbits synthesize LDL receptors that have ligand binding affinity, but which have delayed transport from the ER to Golgi. LDL receptors trapped within the secretory pathway may still regulate apoB secretion [26]. More recent kinetic studies in *LDLR* knockout mice, however, have questioned the potential for LDL receptors to regulate the secretion of apoB [27]. In summary, cell culture and animal model data appear to disagree as the role of defective LDL receptors on hepatic secretion of apoB.

2.2. Human studies

The early findings from radiokinetic studies of apoB metabolism in FH were inconclusive [28,29], but the results from stable isotope tracer studies have been more uniform. Cummings et al. found that VLDL apoB secretion was almost 2-fold higher in phenotypic heterozygous FH patients compared with controls [30]. Zulewski et al. confirmed the increase in VLDL apoB secretion in a group of patients with *LDLR* mutations [31]. This finding is consistent with the report of Tremblay et al. in FH patients with the same null *LDLR* mutation [32]. Millar et al. investigated the turnover of apoB using endogenous labelling in 7 genetically defined patients with homozygous FH and 4 controls [33]. As anticipated, LDL apoB fractional catabolic rate (FCR) was significantly lower in the FH patients compared with controls, with no significant group differences in the FCR of $VLDL_1$, $VLDL_2$ and intermediate-density lipoprotein (IDL) apoB. However, when divided by type of *LDLR* mutation (defective *vs.* null), receptor null FH patients had significantly higher total apoB production rate compared with controls. This was attributed to increased production rates of $VLDL_2$, IDL and LDL apoB.

By contrast to predictions from the aforementioned findings, studies employing plasmapheresis to reduced LDL cholesterol concentrations in FH patients and normal subjects have not confirmed a reduction in the hepatic output of VLDL apoB [34–36]. This suggests that acute changes in LDL concentrations do not regulate apoB secretion. The determination of apoB kinetics in such studies is challenging, however, due to the non-steady state conditions [37]. Moreover, the time to induction of LDL receptor activity or reduction in apoB secretion may lag behind acute changes in the plasma cholesteryl ester pools.

By contrast to FH due to *LDLR* mutations, hepatic VLDL apoB secretion does not appear to be increased in patients with familial defective apoB (FDB) [38]. FDB patients also exhibit decreased conversion of IDL to LDL and hence reduced production of LDL apoB [39]. Accordingly, the increase in plasma LDL concentrations in FDB appears to be exclusively due to hypocatabolism of LDL apoB. Given that plasma LDL cholesterol concentration is generally lower in FDB than in FH, the rate of non-receptor mediated uptake of cholesterol and hence, the extent of hepatic cholesterol overload is likely to be lower in FDB patients, accounting for the lower hepatic secretion rate of apoB compared with those with classical FH.

A rarer cause of autosomal dominant (AD) hypercholesterolemia involves gain-of-function mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9). PCSK9 has an active role in regulating the expression of LDL receptors through reducing their recycling by binding with the LDL receptor and targeting it for lysosomal degradation. The production of VLDL, IDL and LDL apoB has been reported to be higher in AD hypercholesterolemia due to *PCSK9* compared with *LDLR* mutations [40]. This suggests that either increased cholesterol substrate availability and/or PCSK9 may be more important than a direct effect of the LDL receptor in regulating hepatic secretion of apoB in FH. By contrast to AD hypercholesterolemia, the hepatic secretion of apoB is not increased in autosomal recessive hypercholesterolemia (ARH), suggesting that the LDL receptor adaptor protein may not regulate apoB secretion [41].

3. Lipoprotein (a)

Elevated plasma concentrations of Lp(a) independently predict risk of coronary artery disease (CAD) [42,43]. In FH subjects, elevated Lp(a) concentrations have been reported to be associated with CHD,