Insights Into PCSK9, Low-Density Lipoprotein Receptor, and Low-Density Lipoprotein Cholesterol Metabolism
Of Mice and Man

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The identification of PCSK9 in 2003 has resulted in substantial revision to previous knowledge regarding cholesterol homeostasis, providing new insights into low-density lipoprotein (LDL) metabolism and LDL receptor (LDLR) function, and presented a new compelling therapeutic target to reduce LDL cholesterol (LDL-C). PCSK9 is a serine protease expressed predominantly in the liver, intestine, and kidney and regulates plasma LDL-C levels by binding to the epidermal growth factor A of the LDLR, targeting it for degradation rather than for recycling to the cell surface.2

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Plasma levels of LDL-C and PCSK9 are related because overexpression or gain-of-function mutations of PCSK9 promote degradation of mainly hepatic LDLR, whereas loss-of-function mutations promote increased LDLR activity through enhanced recycling. The relationships between PCSK9, the LDLR, and plasma LDL-C have been the subject of intense study over the last decade, helped by transgenic mouse models that can be made to express human PCSK9 and then studied in mice with and without LDLR defects. Although these studies have provided significant insights, the data have at times been inconsistent and confusing, particularly in 3 main areas that have potential clinical relevance: the role of PCSK9 in adrenal function, whether PCSK9 has an independent role in lipid metabolism other than via the LDLR, and the role of PCSK9 during statin therapy.

In a series of elegant studies reported in this issue of Circulation, Tavori et al1 use these mouse models, supplemented with additional in vitro and in vivo experiments, to further elucidate the relationship between these parameters and provide some potential applications and extrapolations to human which may answer these questions and prove useful for current therapeutics in clinical development. The LDLR−/− mouse model provides a unique system in which to study the metabolism of both LDL and PCSK9 in the absence of the LDLR activity. Earlier studies suggested that there was fairly rapid clearance of PCSK9, even in LDLR−/−, mice implying that PCSK9 must also be removed from plasma by LDLR-independent pathways.4 However, in the current study, although rapid removal of hPCSK9 was observed in wild-type and human LDL receptor transgenic mice, there was a marked delay in clearance of PCSK9 from the serum of LDLR−/− mice, consistent with a dominant role for the LDLR in PCSK9 removal. This is further supported by their studies where infused hPCSK9 resulted in reduced clearance rates, reduced intracellular PCSK9 accumulation, and increases in serum PCSK9 levels that were directly related to LDLR activity: fastest and lowest in wild type, slower and moderately elevated in LDLR−/−, and slowest and highest in LDLR−− mice. These finding are consistent with the recent report by Raal et al5 of serum PCSK9 levels in untreated patients with the equivalent clinical syndromes of homozygous (Ho) and heterozygous (He) familial hypercholesterolemia (FH) compared with a control group with normal lipid levels.

In addition Tavori et al demonstrate that overexpression of PCSK9 can, independently of the LDLR, increase serum LDL-C and its triglyceride-rich lipoprotein precursors, probably by increasing both hepatic and intestinal synthesis of apolipoprotein B (apoB). This finding is consistent with previous studies by Sun et al6 and Levy et al.7 By extrapolation of the converse situation, loss-of-function mutations or pharmaceutical inhibition of PCSK9 may therefore contribute to the reduction in apoB and LDL-C also independently of the effect on the LDLR. This may in part help explain the reduction in lipoprotein (a) seen in recent clinical trials with PCSK9 monoclonal antibodies8–10 similar to the mechanism for apoB antisense drugs.11 Perhaps even more intriguing is the potential for PCSK9 inhibition to have some effect in LDLR−/− subjects, those patients with the clinical syndrome HoFH who are truly receptor defective and thus have some residual LDLR activity there is further potential for both mechanisms to contribute to reduce LDL-C by inhibiting PCSK9.

There does appear to be a divergence of the results between mouse and man in terms of relative increases in serum PCSK9 and LDL-C related to LDLR function. In the current study the absence of the LDLR had a greater effect on the half-life of PCSK9 with a nearly 10-fold increase, compared with a 5-fold increase of LDL-C, suggesting that LDLR-dependent clearance of PCSK9 is more important to PCSK9 than to LDL. The most recent human studies show that PCSK9 levels are elevated ≈2-fold in untreated subjects with HeFH and 2- to 3-fold in those with HoFH compared with 3- and 6-fold increases in LDL-C, respectively.5 The defective LDLR function seen in human subjects with He- or HoFH

DOI: 10.1161/CIRCULATIONAHA.113.003360

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Circulation is available at http://circ.ahajournals.org

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thus appears to have a greater impact on the serum levels of LDL-C than on the level of PCSK9. How does one then explain the relatively poor correlation between serum PCSK9 and LDL-C seen in humans? This is probably because the majority of HoFH patients are receptor defective, rather than receptor negative, and have some residual LDLR activity, which is able to bind PCSK9. In addition, because PCSK9 binds to a different domain of the LDLR, receptors that are dysfunctional in binding LDL may still bind PCSK9 normally.

The levels of PCSK9 and LDL-C are not invariably coupled, as treatment with statins reduces plasma levels of LDL-C as a result of increased LDLR expression but also increases levels of circulating PCSK9 as a result of increased PCSK9 synthesis via SREBP-2. As discussed by Tavori et al this counter-regulatory effect has been proposed to explain why greater LDL-C reduction occurs with the starting dose of a statin, whereas further doubling of the dose reduces LDL-C by only another 6%. However, a potential alternative explanation could be the paradoxical effect whereby 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase inhibition initially decreases intracellular cholesterol, resulting in increased expression of SREBP-2, and upregulating both LDLR and PCSK9 synthesis. However, despite the resultant higher levels of PCSK9, the increased LDLR activity still stimulates greater uptake of LDL-C reduction occurs with the starting dose of a statin, whereas further doubling of the dose reduces LDL-C by only another 6%. However, a potential alternative explanation could be the paradoxical effect whereby 3-hydroxy-3-methyl-glutaryl-Coenzyme A reductase inhibition initially decreases intracellular cholesterol, resulting in increased expression of SREBP-2, and upregulating both LDLR and PCSK9 synthesis. However, despite the resultant higher levels of PCSK9, the increased LDLR activity still stimulates greater uptake of LDL-C whereas further doubling of the dose reduces LDL-C by only another 6%.

In the current study, despite a number of different approaches to test the impact of PCSK9 on the adrenal, there was little effect of hPCSK9 overexpression on LDLR activity in the adrenal glands other than in extreme circumstances. LDLR activity was also not increased in the adrenal glands of PCSK9−/− mice, implying that PCSK9 has little effect on LDLR function in the adrenal. This is reassuring and should alleviate fears of high-dose statin therapy, which elevates PCSK9 levels, or inactivation of plasma PCSK9 with monoclonal antibody therapy, compromising adrenal function.

A number of other findings by Tavori et al are still unclear as to their potential implications in human, such as why expression of hPCSK9 would be highest in the kidney and not the liver whereas LDLR reduction was greater in the liver, and that whereas 30% of serum PCSK9 is associated with LDL it appears the non–lipid-associated PCSK9 is the most active form in terms of suppressing LDLR activity.

In summary, this study provides additional evidence to support that the LDLR is the main route of elimination of PCSK9, that PCSK9 likely plays an independent role in apoB-related lipoprotein synthesis, and that there is a reciprocal regulation between serum PCSK9 and hepatic LDLR levels which together determine LDL-C levels. They confirm that the role of PCSK9 appears confined predominantly to the liver and neither overexpression nor inhibition is likely to have any impact on adrenal function.

Disclosures
Dr Stein has received consulting fees from Amgen Inc, Adnexus Therapeutics/BMS, Genentech/Roche, and Regeneron/Sanofi related to PCSK9 inhibitors. Dr Raal has received consulting fees from Amgen Inc and Sanofi related to PCSK9 inhibitors, and his institution has received research funding related to PCSK9 inhibitor clinical trials from Regeneron/Sanofi and Amgen Inc.

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**Key Words:** Editorials ■ lipid metabolism ■ low-density lipoprotein cholesterol ■ PCSK9 protein, human
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Circulation. 2013;127:2372-2374; originally published online May 20, 2013;
doi: 10.1161/CIRCULATIONAHA.113.003360

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