Editorial Commentary

# Pancreatic PCSK9 and its involvement in diabetes

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Hepatic expression of the low-density lipoprotein receptor (LDLR) is one of the main regulators of plasma low-density lipoprotein cholesterol (LDL-C) and has been an important target for the primary and secondary prevention of coronary disease. Increasing cell surface LDLR levels in the liver enhances clearance of LDL particles from the circulation, lowering plasma LDL-C levels. Statins inhibit 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), the rate-limiting enzyme for cholesterol synthesis, thereby lowering intracellular cholesterol levels. This will induce processing of sterol regulatory element-binding protein-2 (SREBP2), leading to increased surface expression of LDLR and lowering of plasma LDL levels (1). Statins have been proven to reduce coronary events in proportion to the degree to which they lower LDL cholesterol, with approximately 20% reduction in events for every 1 mmol lowering of LDL cholesterol (2).

More recently, the discovery of a key driver of LDLR degradation, pro-protein convertase subtilisin/kexin type 9 (PCSK9) has provided an alternative target for lowering high circulating LDL-C levels through LDLR. PCSK9 belongs to the family of proprotein convertases, which are secreted serine proteases that activate proteins by cleavage. PCSK9 plays a key role in cholesterol metabolism by affecting LDLR degradation although this was found to be independent of its enzymatic proteolytic activity (3). In the circulation PCSK9 binds the LDLR, initiating endocytosis and subsequent lysosomal degradation of the LDLR, thereby preventing recycling of the receptor back to the cell surface. Animal studies indicate that expression of PCSK9 is highest in the liver and that circulating PCSK9 is liver-derived (4). However, PCSK9 is also expressed in various other tissues such as brain, lung, small intestine and the pancreas. Although it is undisputed that all circulating PCSK9 originates in the liver (4) and that this drives hepatic LDLR degradation, the function of extra-hepatic produced PCSK9 is less clear. It is important to understand the function(s) of extra-hepatic PCSK9, especially as several PCSK9 inhibitory therapies are currently already in use or are in development. For now, it is unclear whether these therapies will affect PCSK9 function in organs other than the liver.

Mendelian randomization studies indicate that PCSK9 loss of function variants that lead to reduced LDL-C and cardiovascular risk were associated with an increased risk of diabetes (5-7). Others have found a positive association between plasma PCSK9 levels and the homeostasis model assessment for insulin resistance (HOMA-IR) (8). Together these findings suggesting that PCSK9 may protect against developing diabetes and that inhibiting PCSK9 function in the pancreas could lead to new onset diabetes (NODM). The mechanisms by which PCSK9 dysfunction or inhibition may lead to diabetes are unclear. In this current manuscript, Da Dalt and colleagues (9) investigate the function of pancreatic PCSK9. They elegantly show that locally produced PCSK9 controls LDLR expression in pancreatic β-cells, which in turn regulates intracellular cholesteryl ester accumulation and insulin secretion. Their study suggests an important role for pancreatic PCSK9 in glucose metabolism and the development of diabetes.

The authors observed that *Pcsk9* knockout mice (*Pcsk9*$^{-/-}$) exhibited high plasma glucose levels under basal conditions and severely delayed glucose clearance under normal chow and high fat feeding conditions, whilst not displaying changes in insulin sensitivity compared to control wild type mice. Plasma insulin levels were reduced, whilst pancreatic insulin content was increased, indicating dysfunctional insulin secretion from pancreatic β-cells. Examination of the pancreas revealed different islet size distribution in *Pcsk9*$^{-/-}$ compared to wild type mice and this was associated with cholesteryl ester accumulation within the islets of *Pcsk9*$^{-/-}$ mice. Importantly, this phenotype was completely reversed in *Pcsk9/Ldlr* double knockout mice, indicating that the effects of PCSK9 deficiency are mediated via the LDLR.

This work confirms the importance of cholesterol homeostasis in pancreatic β-cell function. High serum levels of cholesterol are associated with increased islet cholesterol content and decreased insulin secretion in mice, which could be reversed after treatment of hypercholesterolemic mice with cyclodextrin (10). Treatment of isolated islets or cultured pancreatic β-cells with cyclodextrin leading to acute depletion of cholesterol or with mevalonate to decrease cholesterol by inhibiting cholesterol synthesis, indicated that cellular cholesterol accumulation may directly affect insulin secretion. In the pancreas, uptake of lipoproteins and expression of the LDLR appears to be restricted to pancreatic β-cells (11) where it affects insulin secretion (12). Importantly, intracellular cholesterol levels in β-cells are tightly regulated by the combination of uptake of cholesterol through the LDLR and efflux of cholesterol mediated by the ATP-binding cassette transporter A1 (ABCA1) (13,14). After transplantation of wild-type islets with functional LDLR into diabetic hypercholesterolemic mice, the mice demonstrated normal β-cell function. However, when islets with impaired ABCA1 function were transplanted, abnormal β-cell function persisted, suggesting that cholesterol efflux can compensate for the harmful effects of high cholesterol uptake (14). Others found that high-density lipoprotein (HDL), which stimulates cholesterol efflux, or its main protein components can increase insulin secretion from MIN6 clonal β-cells (15) and that native HDL reverses the inhibitory effect of oxLDL on insulin secretion (16). Another ATP-binding cassette transporter, ABCG1, also has a role in insulin secretion and serves to regulate the cholesterol content of insulin granules rather than affect the efflux of excess cholesterol from the cells (17).

Interestingly, the precise origin of PCSK9 within the pancreas remains to be resolved. This current work and that of others suggest that PCSK9 expression is restricted to the somatostatin-secreting pancreatic delta-cells of mice and humans, whilst it is undetectable in the insulin-secreting (and LDLR expressing) pancreatic β-cells (9,18). It should be noted that others have suggested that PCSK9 might also be expressed in β-cells, with PCSK9 mRNA found in a β-cell enriched islet preparation of human pancreas (19) and in cell lines derived from human β-cells (20). Unfortunately, PCSK9 mRNA levels may not be an accurate reflection of PCSK9 protein levels, and assessment of PCSK9 function is further hampered by the unavailability of good antibodies that readily discriminate between active and inactive isoforms of mouse/human PCK9 in tissues (18,20).

What the above described studies do have in common is that they all clearly indicate a role for PCSK9 in regulating LDLR levels in pancreatic β-cells. The initial work by Langhi *et al.* (18) showed that exogenous recombinant PCSK9 is capable of decreasing LDLR levels in human islets and that islets cultured from *Pcsk9*$^{-/-}$ mice for 24 hours *in vitro* did not retain their elevated levels of LDLR, leading the authors to suggest that that circulating liver-derived PCSK9 rather than intra-islet PCSK9 mediated the effect on islet LDLR levels. The current paper suggests that pancreatic PCSK9 locally derived from delta cells, rather than liver-derived PCSK9, explains the findings of Langhi *et al.* in explanted beta cells. Liver specific PCSK9 knockout mice, with no circulating PCSK9, exhibited similar LDLR levels compared to mice with intact hepatic PCSK9 expression. If so, why is circulating PCSK9 unable to affect pancreatic LDLR levels? The pancreas is a highly perfused organ and circulating LDL is readily taken up from the circulation into beta-cells via the LDLR, so inadequate blood supply seems an unlikely explanation. As a much as 30–40% of circulating PCSK9 is bound to LDL (21), and the very low LDL levels in *Pcsk9*$^{-/-}$ and WT mice may mean that the biological effects of circulating PCSK9 may be underestimated in mice. Different organs may be susceptible to variable tissue penetration and/or retention of circulating PCSK9. For example, in one study circulating PCSK9 decreased LDLR expression in liver and intestine, but was unable to affect adrenal LDLR in the same animals (21). Whether a similar situation exists for the pancreas is unknown but will be important to understand the relative importance of circulating and locally-derived PCSK9 in regulating pancreatic LDLR expression. Future pancreatic (preferably pancreatic cell-type specific) PCSK9 deficient models and

 

mice exhibiting a more human-like lipoprotein profile will help to understand the contributions of intracellular, local and circulating PCSK9 in pancreatic biology.

There is good reason for expecting that targeting the LDLR could influence the development of diabetes. Clinical trials have shown that statin therapy increases the risk of new onset diabetes (22), and that acute statin therapy dose dependently increases HbA1C, insulin levels and HOMA-IR in short term studies (23). Furthermore, genetic studies indicate that variants affecting HMGCR which lower LDL cholesterol increase diabetes risk, whilst LDLR and APOB variants that lead to familiar hypercholesterolemia have a reduced risk of diabetes, further supporting a role for the LDLR in diabetes (6,7). PCSK9 loss of function variants were also associated with an increased risk of diabetes (5,7). However, so far it is unclear whether inhibitory PCSK9 therapies increase diabetes incidence. A small but significant increase in glycemia and HbA1C was concluded in a recent meta-analysis including 20 randomised clinical trials involving PCSK9 inhibitory antibodies (24), however, two other systematic reviews and meta-analysis found no effect of PCSK9 inhibition on NODM or glucose metabolism, regardless of PCSK9-mAb type, participant characteristics, treatment duration, treatment method or differences in control treatment (25,26). Based on the evidence from statin trials, however, a larger number of participants and longer treatment duration may be necessary to conclusively prove whether PCSK9 inhibition increased diabetes incidence. It also has to be noted that most of the short-term trials did not include any measurements related to diabetes. Given that prior literature showing relatively acute effects of potent statins on glucose metabolism (23), similar studies examining the short-term effects of PCSK9 inhibition may be informative.

Current PCSK9 therapy is limited to the use of the monoclonal antibodies, evolocumab and alirocumab, which bind circulating PCSK9 and inhibit the interaction between PCSK9 and the LDLR, thereby preventing PCSK9-mediated LDLR degradation. It is thought that clearance of these PCSK9 targeted antibodies is mediated by lysosomal degradation after uptake of the PCSK9-mAb complex via the LDLR (27). Tissue distribution studies have indicated that most of the antibodies are present in the circulation, however assessment of fluorescently labelled avelumab in rodents, cynomolgus monkeys and humans shows low uptake of the antibody within tissues including the pancreas (28). How this compares to the concentration achieved in plasma was not reported and it is unclear whether these tissue concentrations would affect pancreatic LDLR levels.

Other approaches to inhibit PCSK9 include the use of long acting small interfering siRNA molecules, which target intracellular PCSK9 mRNA and reduce the production of PCSK9 protein intracellularly. These molecules are targeted to the liver by conjugation to triantennary N-acetylgalactosamine carbohydrates which bind to abundant liver-expressed asialoglycoprotein receptors, leading to uptake specifically into hepatocytes. PCSK9 siRNAs show promising LDL-C lowering in animal models and in phase I and II trials in humans (29). Tissue specific targeting to the liver may have additional benefits compared to the systemic administration of mAbs and specifically preserve normal cholesterol levels in and insulin secretion from the pancreas.

Taken together, this current study provides important insights into the mechanism(s) by which pancreatic PCSK9 may affect β-cell insulin secretion and the development of diabetes and raises important issues regarding extra-hepatic PCSK9 biology and treatments designed to inhibit PCSK9 function. It is a timely reminder of how well-designed mechanistic studies can help explain important clinical observations and provide a rational basis for future clinical investigation.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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