The Journal of Biomedical Research, 2015, 29(6):429-436

Review Article

PCSK9 and triglyceride-rich lipoprotein metabolism

Irena Druce¹, Hussein Abujrad¹, Teik Chye Ooi¹,²*

¹Clinical Research Laboratory, Division of Endocrinology and Metabolism, Department of Medicine, University of Ottawa, Ottawa, Ontario K1H 8L6, Canada; ²Chronic Disease Program, Ottawa Hospital Research Institute, The Ottawa Hospital, Ottawa, Ontario K1H 7W9, Canada.

Abstract

Pro-protein convertase subtilisin-kexin 9 (PCSK9) is known to affect low-density lipoprotein (LDL) metabolism, but there are indications from several lines of research that it may also influence the metabolism of other lipoproteins, especially triglyceride-rich lipoproteins (TRL). This review summarizes the current data on this possible role of PCSK9. A link between PCSK9 and TRL has been suggested through the demonstration of (1) a correlation between plasma PCSK9 and triglyceride (TG) levels in health and disease, (2) a correlation between plasma PCSK9 and markers of carbohydrate metabolism, which is closely related to TG metabolism, (3) an effect of TG-lowering fibrate therapy on plasma PCSK9 levels, (4) an effect of PCSK9 on postprandial lipemia, (5) an effect of PCSK9 on adipose tissue biology, (6) an effect of PCSK9 on apolipoprotein B production from the liver and intestines, (7) an effect of PCSK9 on receptors other than low density lipoprotein receptor (LDLR) that are involved in TRL metabolism, and (8) an effect of anti-PCSK9 therapy on serum TG levels. The underlying mechanisms are unclear but starting to emerge.

Keywords: hyperlipidemia, hypercholesterolemia, molecular biology

Introduction

Pro-protein convertase subtilisin-kexin 9 (PCSK9) belongs to a family of intracellular enzymes that convert inactive precursor proteins to active products. These products comprise a broad array of molecules, including hormones, receptors, growth factors and even other enzymes.¹ Yet, PCSK9 itself is not known to have an enzymatic role, other than to auto-catalytically cleave a pro-domain off itself. PCSK9 plays a role in liver regeneration², viral infection³ and neuronal differentiation⁴, but since its discovery in 2003⁵ and the demonstration of familial hypercholesterolemia (FH) in subjects with gain-of-function (GOF) PCSK9 mutations⁶, the majority of research has been done to elucidate its role in lipoprotein metabolism.

PCSK9 is most highly expressed in the liver, intestine, brain and kidneys⁷. It is a secreted protein, mostly from the liver, and its levels are measurable in human plasma or serum⁸. After reaching the circulation, it is delivered to the cell surface of tissues, notably the liver, where it binds to the low density lipoprotein receptor (LDLR), prevents its recycling and instead promotes its lysosomal degradation⁹. This results in diminished uptake of LDL particles and elevation in LDL cholesterol (LDLC) levels. Thus, PCSK9 can be seen as a pro-atherogenic molecule.

The PCSK9 precursor protein has a molecular weight of -74 kDa. Autocatalytic cleavage in the endoplasmic

*Corresponding author: Dr. T C Ooi, The Ottawa Hospital - Riverside Campus, 1967 Riverside Drive, Ottawa, Ontario K1H 7W9, Canada, Tel: (613) 738 8400 Ext. 81950, E-mail: tcooi@toh.on.ca.

© 2015 by the Journal of Biomedical Research. All rights reserved. doi: 10.7555/JBR.29.20150052
reticulum separates a -14 kDa prodomain from the -60 kDa mature PCSK9 protein. The prodomain remains associated with the rest of PCSK9 by non-covalent bonds and is essential for its role as a modulator of lipoprotein metabolism\(^{[12]}\). The cleaved complex is transported through the Golgi apparatus and secreted\(^{[2]}\).

Circulating PCSK9 binds to the EGF-A-like domain of hepatic LDLR\(^{[19]}\) and escorts it from the endosomal recycling pathway to the lysosome for degradation\(^{[9]}\), independent of its catalytic activity\(^{[13]}\). There is also evidence for furin cleavage of a -7kDa segment, Ser\(^{153}\)-Arg\(^{218}\), from the N-terminus of the catalytic domain. The -53kDa furin-cleaved PCSK9 has less LDLR degradation activity, possibly because of the loss of a region of PCSK9 required for LDLR binding as well as the concomitant loss of the -14kDa prodomain\(^{[14]}\). Another study, however, has shown that furin cleavage did not result in dissociation of the pro-domain\(^{[15]}\).

The effect on the LDLR is the best known action of PCSK9, but there are indications from several lines of research that PCSK9 may have other effects on lipoprotein metabolism. Here, we provide a review of the positive as well as contradictory data on the possible involvement of PCSK9 in the metabolism of triglyceride-rich lipoproteins (TRL), namely chylomicrons (CM), very low density lipoproteins (VLDL) and their remnants.

**Data on a possible role of PCSK9 in TRL metabolism**

**Link between plasma PCSK9 and parameters of triglyceride metabolism**

One of the first hints at a link between PCSK9 and TRL metabolism was the demonstration of a positive correlation between plasma PCSK9 and plasma triglyceride (TG) levels\(^{[16-21]}\). This, however, is akin to the earlier finding of a correlation between plasma PCSK9 and HDL levels, which prompted the subsequent elucidation of a mechanism for PCSK9’s role in regulating LDLR-mediated clearance of LDL. Regarding the link to TG, investigation into possible mechanisms for PCSK9’s role is currently underway. A cohesive model has not yet been arrived at. It is noteworthy that there are some studies that do not show this correlation\(^{[17,24]}\). The reason for the discrepancy among studies is unclear.

An important recent study went beyond TG levels to look at the relationship between plasma PCSK9 and sub-fractions of VLDL and LDL. Plasma PCSK9 was shown to predominantly relate to intermediate density lipoproteins (IDL), a TG-rich sub-fraction, which represents primarily VLDL remnants. This finding suggests the possibility of PCSK9 having an effect on plasma TG via effects on the metabolism of VLDL and their remnants\(^{[22]}\).

Plasma PCSK9 concentration has also been evaluated via VLDL-TG kinetic studies using stable isotopes in non-diabetic but obese subjects. Here, the results provide no indication that PCSK9 has an impact on TRL metabolism as plasma PCSK9 concentrations did not correlate with VLDL-TG secretion or clearance\(^{[25]}\).

**Link between plasma PCSK9 and parameters of triglyceride metabolism in disease states**

The positive correlation between plasma PCSK9 and TG levels has been found not just in the general population but also in some specific disease states.

In patients with proteinuria and chronic kidney disease (CKD) stages 2 and 3\(^{[26]}\), and in those with CKD 5 on hemodialysis\(^{[27]}\), PCSK9 levels correlate positively with TG levels\(^{[26]}\).

An intriguing observation regarding the relationship of PCSK9 and TG was the finding that in members of four British families with the D374Y gain-of-function (GOF) PCSK9 mutation, which is linked to FH, TG levels were significantly higher (but still within reference range), as compared to controls\(^{[20]}\). This suggests that a PCSK9 perturbation may have an impact on TRL metabolism. However, in a study on heterozygous FH (HeFH) and homozygous FH (HoFH) with identified LDLR mutations, TG levels were similar to those in controls\(^{[29]}\). This discrepancy in findings may be due to a difference in underlying genetic defect and to the fact that plasma PCSK9 levels in the D374Y patients are low\(^{[29]}\), while those in patients with LDLR mutations are high\(^{[29]}\).

**Link between PCSK9 and diabetes and glucose metabolism**

Given the close association between glucose and TG metabolism, further support for a link between PCSK9 and TG metabolism is emerging in the form of evidence that PCSK9 may have a role in glucose homeostasis. In one of the largest cohorts with plasma PCSK9 measurement, in addition to a positive correlation between plasma PCSK9 and TG, Lakoski et al. identified a significant correlation between plasma PCSK9 and fasting glucose, insulin and homeostasis model assessment of insulin resistance (HOMA-IR), a marker of insulin sensitivity\(^{[23]}\). The same correlations were found in a healthy adult population\(^{[21]}\), and a cohort of children...
and adolescents aged 9-16 years in a French Canadian population[17]. Another very large population also demonstrated an association between plasma PCSK9 and plasma glucose and insulin levels[33].

The mechanism of how PCSK9 affects glucose metabolism is still unclear. One study found that rat hepatocytes incubated with insulin for 24 hours had an increase in PCSK9 messenger RNA (mRNA)[31]. In human studies, a 24-hour insulin infusion in a hyperinsulimemic clamp experiment in healthy and diabetic (type 2) individuals had no effect on plasma PCSK9 levels[32], while a 3-hour euglycemic-hyperinsulimemic clamp study in non-diabetic postmenopausal obese patients actually decreased plasma concentrations of PCSK9[33]. In addition, human liver-derived cell lines produced less PCSK9 protein and PCSK9 mRNA and secreted less PCSK9 into their culture medium in response to the presence of insulin[33).

Other research found that plasma PCSK9 levels were not different among patients with normal glucose metabolism, impaired glucose metabolism and type-2 diabetes mellitus[41]. Also, no association was found between plasma PCSK9 levels and body mass index (BMI), waist circumference, fat mass and fat-free mass, or visceral and subcutaneous adipose tissue measured by computed tomography in abdominally obese men[41].

Thus, there are differing data on the effect of insulin on the regulation of PCSK9 and on how insulin resistance affects PCSK9 status. Insulin action has major effects on TRL metabolism and more studies are needed to clearly determine its effect on PCSK9. It is possible that the putative link between PCSK9 and TG is the result of the influence of insulin action on PCSK9.

Conversely, the impact of PCSK9 deficiency on glucose metabolism also remains uncertain. In a study comparing wild-type to PCSK9 knockout mice, Mbikay et al. showed that knockout mice had higher fasting plasma glucose, lower plasma insulin and higher glycemia on an oral glucose tolerance test[35]. However, another study failed to detect any alteration in glucose homeostasis in PCSK9-deficient mice[36].

PCSK9 and fibrate therapy

Fibrates are a class of medications used to treat hypertriglyceridemia, and they exert their action via the peroxisome proliferator-activated receptor α (PPAR-α) to increase peripheral TRL lipolysis via lipoprotein lipase, decrease intracellular lipolysis in adipose tissue and decrease secretion of VLDL[37]. The demonstration of fibrate therapy having an effect on plasma PCSK9 levels raises the question of whether PCSK9 mediates some of the effects of fibrates on TRL metabolism. The picture, however, is unclear. Some studies have demonstrated a decrease in PCSK9 with fibrate use[7,38] while others have demonstrated an increase[8,39,40]. A recent meta-analysis looked at 6 studies and a total of 218 patients treated with fibrates for a period of 6 to 24 weeks. The conclusion was that fibrate treatment, especially when compared to controls, significantly raised PCSK9 levels[42]. In cell culture experiments, various fibrates were shown to repress PCSK9 expression in immortalized human hepatocytes[43]. Beyond that, it is not known how the fibrate-induced changes in PCSK9 influence TRL metabolism. It is also plausible, but unproven, that plasma PCSK9 changes are secondary to fibrate-induced changes in TRL metabolism. Further research is needed to clearly elucidate the link between fibrate treatment and plasma PCSK9; the implications are relevant and interesting.

PCSK9 and postprandial lipemia

Postprandial lipemia (PPL) refers to the status of lipids and lipoproteins in blood following a fat load or a meal. Le May and his colleagues fed PCSK9 knockout mice and their wild-type littermates, a bolus of olive-oil. At time zero, levels of TG were comparable; however, after 2 hours, the knockout mice showed a significantly attenuated TG response. In addition, this study demonstrated that PCSK9 was highly expressed throughout the digestive tract and colon at levels equivalent to that in the liver. Intestinal lymph analysis revealed reduction in apoB, but not TG output, resulting in larger TG-rich CM in knockout mice compared to wild-type littermates. In addition, kinetic studies showed that PCSK9-deficient mice had an increased ability to clear CM compared to wild-type littermates. The study further demonstrated that the difference in observed TG levels in the knockout mice was not due to alterations in fat absorption, gastric emptying or intestinal transit[40].

A few studies have looked at PCSK9’s relation to PPL in humans. The first, by Cariou et al., found no change in PCSK9 levels following an oral fat load in a small 10-patient sample. They also demonstrated that postprandial triglyceride excursion was not altered in 2 carriers of a PCSK9 loss-of-function (LOF) mutation compared with non-carriers[19]. The second by Chan et al. looked at 17 obese subjects who were given an oral fat load. They found that in the postprandial period (total of 24 hours post), PCSK9 was significantly associated with the area-under-the-curve for apoB-48 and inversely with the TG-apoB-48 fractional catabolic rate. They interpreted their findings as indicating that
catabolism of TG and apoB-48-containing CM may be coordinated by PCSK9 in the postprandial state in obese individuals.\(^ {20}\)

Overall, data on the effect of PCSK9 on PPL, and of PPL on PCSK9, are very limited. Since the postprandial period is when TRL metabolism is most active, a demonstration of an effect of PCSK9 on indices of PPL would be an indication of an involvement of PCSK9 in TRL metabolism.

**PCSK9 and fat deposition**

Animal studies have shown a link between PCSK9 and fat-deposition. Roubtsova and colleagues demonstrated that *PCSK9* knockout mice accumulated 80% more visceral body fat (perigonadal and perirenal deposits) than wild-type mice. They subsequently showed that this was the result of adipocyte hypertrophy and increased fat uptake into adipose tissue via higher cell surface levels of very-low-density lipoprotein receptor (VLDLR; see below). Further ex-vivo models showed that adipose, muscle and liver tissue from knockout mice had a higher rate of TG synthesis. Finally, they demonstrated the same effect in mice that were knockouts for LDLR (VLDLR; see below). Further ex-vivo models showed that adipose, muscle and liver tissue from knockout mice had a higher rate of TG synthesis. Finally, they observed the same effect in mice that were knockouts for *PCSK9* and LDLR, demonstrating that the effect they observed was LDLR-independent.\(^ {44}\)

A recent study by Mbikay et al. supported these findings; they also found that PCSK9 knockout mice accumulated more perigonadal fat. Interestingly, they noted a greater effect in female mice fed a “Western” high fat diet.\(^ {45}\)

These findings provide evidence for a role of PCSK9 in adipose tissue biology, which in turn could have a strong influence on overall TRL metabolism.

**PCSK9 effect on apolipoprotein B**

**Effect of PCSK9 on ApoB in the liver**

Various cell culture and animal studies have demonstrated a link between PCSK9 and apolipoprotein B (apoB) secretion from the liver. Rat hepatoma cells (McArdle-7777) overexpressing the GOF D374Y-PCSK9 mutant demonstrated increased secretion of apoB-containing lipoproteins.\(^ {46}\) Similarly, mice overexpressing PCSK9 from a transgenic vector had higher plasma levels of both apoB100 and apoB48, and TG, and the effect was independent of the LDLR.\(^ {47}\)

Interestingly, this study also provided data on the lack of effect of PCSK9 expression on genes governing cholesterol and TG biosynthesis,\(^ {47}\) suggesting that PCSK9’s effect on TRL metabolism may be mediated mainly through an effect on apoB. Furthermore, studies showed that apoB100 secretion from primary hepatocytes of *PCSK9* knockout mice was reduced.\(^ {48}\)

There have also been some studies in human subjects on the effect of PCSK9 on apoB. It was shown in a stable isotope kinetic study that FH patients carrying the GOF S127R mutation in PCSK9 over-produce apoB-100 (3-fold) along with overproduction of VLDL (3-fold), IDL (3-fold), and LDL (5-fold).\(^ {49}\) Furthermore, Chernogubova et al. looked at a sample of almost 6000 middle-aged subjects and found a significant correlation between serum PCSK9 and apoB.\(^ {50}\)

The link between PCSK9 and secretion of TRL has also been studied, although data are limited. Mice over-expressing PCSK9 were found to have increased hepatic VLDL production during fasting and increased secretion of TRL into the serum.\(^ {31}\)

A possible mechanism by which PCSK9 may regulate apoB levels is via the LDLR. It has been shown that intracellular LDLR protein can bind nascent apoB-containing lipoproteins and direct them for degradation.\(^ {42}\) It is therefore possible that when LDLR is depleted through the action of PCSK9, apoB is degraded at a lesser rate.

Other research has suggested that PCSK9 may affect apoB by stabilizing it and preventing its degradation. Using pulse chase experiments on primary hepatocytes from these mice over-expressing apoB, Sun et al. showed that PCSK9 binds to apoB via its N-terminus and protects it from undergoing autophagy. This effect was found to be independent of the LDLR.\(^ {57}\) Autophagy has been previously demonstrated to be a means of apoB degradation in hepatocytes, in addition to the lysosome pathway.\(^ {47}\)

**Effect of PCSK9 on ApoB in the intestine**

Several studies have shown that human enterocytes treated with recombinant human PCSK9 demonstrated increased cellular and secreted apoB-48 and apoB-100.\(^ {50,52,53,54}\) Levy et al. demonstrated in a human enterocyte cell line (Caco2/15 cells) that GOF D374Y-PCSK9 enhanced cholesterol uptake was associated with increased expression of cholesterol transporters NPC1L1 and CD36, and increased CM secretion through increased lipid and apoB48 biogenesis. PCSK9 silencing had the opposite effects. These responses were independent of the LDLR.\(^ {53}\)

Rashid et al. showed that the same cells, treated with recombinant human PCSK9, had increased production of TRL through both LDLR-dependent and LDLR-independent mechanism. Cellular and secreted apoB48 and apoB100 were enhanced. The increase in apoB was due to increased apoB mRNA transcription and enhanced apoB stability. In line with findings that intracellular neutral lipids inhibit degradation and enhance stability of apoB, they demonstrated that
PCSK9 treatment increased cellular neutral lipids via augmentation of levels of lipid-generating enzymes. They also demonstrated in mice that the levels and activity of intestinal microsomal triglyceride transfer protein (MTP), which transfers neutral lipids to apoB, is increased by PCSK9, again resulting in greater apoB stability. All of the demonstrated effects were reversed by short-term inhibition of PSCK9 via small interfering RNA (siRNA)\(^{[54]}\).

Taken together, these findings indicate that PCSK9 may be linked with production of apoB48 necessary for intestinal CM assembly.

**PCSK9 effect on other receptors in the LDLR family**

*Effect of PCSK9 on VLDLR, apoE2 receptor and LRP-1*

Other research has suggested that PCSK9 may affect TRL metabolism via its effects on receptors other than LDLR. It has been shown that PCSK9 binds to 3 other members of the LDLR family; the VLDL receptor (VLDLR), apoE2 receptor (apoER2)\(^{[12,44,55]}\), and LRP-1\(^{[56]}\). These receptors show high homology with the LDLR; the VLDLR has 59% identity, and the apoE2 receptor has 46% identity with the LDLR\(^{[55]}\) and the LRP-1, 40%\(^{[14,56]}\). When HEK293 cells (human embryonic kidney cell line) expressing VLDLR and apoER2 were incubated with conditioned media containing PCSK9, cellular levels of these receptors were decreased\(^{[55]}\). Thus VLDLR and apoER2 are downregulated by PCSK9 and the mechanism is the same as for the LDLR, namely, redirection of receptors to the lysosomal compartment for degradation\(^{[55]}\).

Both the VLDLR and apoER2 bind VLDL, but whether these receptors play a significant role in TG metabolism in humans is still unclear. Homozygous *VLDLR* knockout mice had normal plasma lipoproteins but they had lower body weight and adipose tissue mass, indicating a possible role for VLDLR in storage of lipids in adipose tissue\(^{[57]}\). This was further supported by the finding that when PCSK9 was added back to the knockout mice, the expression of VLDLR protein in adipose tissue significantly decreased\(^{[44]}\).

LRP-1, on the other hand, is known to play a major role in clearance of CM and VLDL remnants. While early studies suggested little link between LRP1 and PCSK9\(^{[54,14]}\), subsequent research by Canuel *et al.* showed that PCSK9 could degrade LRP-1 and that it competes with the LDLR for PCSK9 activity\(^{[56]}\). A positive effect of PCSK9 on LRP-1 function would lend further support for a role of PCSK9 in TRL metabolism.

**Effect of PCSK9 on CD36**

Finally, there is preliminary evidence that PCSK9 may target CD36, a scavenger receptor with multiple ligands and cellular functions, including facilitating cellular uptake of free fatty acids, though the results are contrasting. Roubtsova and her colleagues noted that in PCSK9 knockout mice, there was no effect on CD36 expression in perigonadal fat deposits of male mice, but that in females CD36 mRNA expression was increased by almost 80% in response to the loss of PCSK9\(^{[144]}\). Similarly, Levy *et al.* showed that human enterocytes made to overexpress normal PCSK9, and the D374Y-PCSK9 GOF mutant, produce increased CD36\(^{[153]}\). On the other hand, recent research by Demers noted that overexpression of PCSK9 led to the downregulation of CD36 in several cell lines, including adipocytes\(^{[54]}\). Whether PCSK9 targets CD36 for degradation or regulates it via a different mechanism requires further study; however, as CD36 has been linked to CM remnant clearance\(^{[159]}\), its link to PCSK9 is relevant to further support PCSK9’s role in TRL metabolism.

**Effect of pharmacologic targeting of PCSK9 on TG**

LDL cholesterol is a well-established risk factor for cardiovascular disease and it is a primary target in lipid-lowering therapy. TRL have also been implicated in atherosclerosis\(^{[4]}\).

In light of the discovery of PCSK9’s effect on LDLc, it has recently become a target for pharmaceutical intervention. Several different approaches have been explored to inhibit or reduce PCSK9 action. In human clinical trials, there has been most interest in antibodies against PCSK9 and two have been extensively studied; evolocumab and alirocumab are fully human monoclonal antibodies against PCSK9. The trials were designed to examine the effects of these novel medications on LDLc, but several of them have reported on the effect on TG and VLDL.

Trials have been done on patients without any known genetic predisposition towards dyslipidemia\(^{[61-69]}\), and those with FH, both HeFH\(^{[70,71]}\) and HoFH\(^{[72-74]}\). The reported effects on TRL have been mixed; several trials report no change in serum TG or VLDL cholesterol (VLDLC) level with treatment\(^{[61,65,66,70,72,73]}\), while others demonstrate a significant lowering of these measured parameters\(^{[62,64,66,71,74]}\). Interestingly, the majority of the positive trials tended to be larger, their study population being generally between 300 and 900 patients\(^{[62,64,66,71,74]}\), while the negative ones were smaller, with patient numbers ranging from eight to 183\(^{[61,65,67,66,70,72,73]}\), suggesting...
that perhaps the negative trials lacked power to demonstrate an effect.

Stein et al. recently published a pooled analysis of a total of 1359 patients from 4 of the above-discussed trials and they showed that treatment with anti-PCSK9 antibodies significantly lowered TG\(^{175}\). Overall, these findings further suggest a link between PCSK9 and TRL metabolism and hint at the possibility of a novel therapy against hypertriglyceridemia.

**Conclusions**

The effect of PCSK9 on LDL metabolism through promotion of LDLR degradation is well described. As more data are gathered, there are indications that PCSK9 may have additional roles in other aspects of lipoprotein metabolism. This review focuses specifically on TRL metabolism. The pieces of evidence available are mostly observational and piecemeal. In some cases, the data are contradictory and uncertain. The available data seem also to suggest that the effect of PCSK9 on TRL metabolism is modest. No cohesive mechanistic model has been thus far been described. However, the existing data for a putative role of PCSK9 in TRL metabolism should be recognized and pursued further. It is encouraging that evidence to support a mechanism for the effect of PCSK9 on TRL metabolism is starting to emerge, especially in the area of PCSK9’s effect on intestinal production of TRL. As with the current development of treatment for elevated LDLC with anti-PCSK9 agents, it is possible that similar PCSK9-related strategies could be found for the treatment of hypertriglyceridemia.

**References**

[1] Seidah NG, Sadr MS, Chrétien M, et al. The multifaceted proprotein convertases: their unique, redundant, complementary, and opposite functions[J]. *J Bio Chem*, 2013, 288(30):21473-21481.

[2] Seidah NG, Benjannet S, Wickham L, et al. The secretory proprotein convertase neural Liver regeneration and neuronal differentiation[J]. *Proc Natl Acad Sci USA*, 2002, 100(3):928-933.

[3] Labonte P, Begley S, Güevin C, et al. PCSK9 impedes hepatitis C virus infection in vitro and modulates liver CD81 expression[J]. *Hepatology*, 2009,50(1):17-24.

[4] Abifadel M, Varret M, Rabès JP, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia[J]. *Nat Genet*, 2003,34(2):154-156.

[5] Albom WE, Cao G, Careskey HE, et al. Serum proprotein convertase subtilisin kexin 9 is correlated directly with serum LDL cholesterol[J]. *Clin Chem*, 2007,53(10):1814-1819.

[6] Dubuc G, Tremblay M, Paré G, et al. A new method for measurement of total plasma PCSK9: clinical applications[J]. *J Lipid Res*, 2010,51(1):140-149.

[7] Lambert G, Ancellin N, Charlton F, et al. Plasma PCSK9 concentrations correlate with LDL and total cholesterol in diabetic patients and are decreased by fenofibrate treatment[J]. *Clin Chem*, 2008,54(6):1038-1045.

[8] Mayne J, Dewpura T, Raymon A, et al. Plasma PCSK9 levels are significantly modified by statins and fibrates in humans[J]. *Lipids Health Dis*, 2008,7(1):22.

[9] Lagace TA, Curtis DE, Garuti R, et al. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice[J]. *J Clin Invest*, 2006, 116(11):2905-3005.

[10] Zhang DW, Lagace TA, Garuti R, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat A of low density lipoprotein receptor decreases receptor recycling and increases degradation[J]. *J Biol Chem*, 2007,282(25):1802-1812.

[11] Nassoury N, Blasiole DA, Tebon Oler A, et al. The cellular trafficking of the secretory proprotein convertase PCSK9 and its dependence on the LDLR[J]. *Traffic*, 2007,8(6):718-732.

[12] Seidah NG, Awan Z, Chrétien M, et al. PCSK9: a key modulator of cardiovascular health[J]. *Circ Res*, 2014, 114(6):1022-1036.

[13] Li J, Tumanut C, Gavigan JA, et al. Secreted PCSK9 promotes LDL receptor degradation independently of proteolytic activity[J]. *Biochem J*, 2007,406(2):203-207.

[14] Benjannet S, Rhainds D, Essalmani R, et al. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol[J]. *J Biol Chem*, 2004,279(47):48865-48875.

[15] Han B, Eacho PI, Knierman MD, et al. Isolation and Characterization of the Circulating Truncated Form of PCSK9[J]. *J Lipid Res*, 2014,55(7):1505-1514.

[16] Arsenault BJ, Pelletier-Beaumont E, Alméras N, et al. PCSK9 levels in abdominally obese men: association with cardiometabolic risk profile and effects of a one-year lifestyle modification program[J]. *Atherosclerosis*, 2014, 236(2):321-326.

[17] Baass A, Dubuc G, Tremblay M, et al. Plasma PCSK9 is associated with age, sex, and multiple metabolic markers in a population-based sample of children and adolescents[J]. *Clin Chem*, 2009,55(9):1637-1645.

[18] Brouwers MCGJ, van Greevenbroek MMJ, Konrad RJ, et al. Circulating PCSK9 is a strong determinant of plasma triacylglycerols and total cholesterol in homozygous carriers of apolipoprotein e2[J]. *Clin Sci (Lond)*, 2014,126(9):679-684.

[19] Cariou B, Langhi C, Le Bras M, et al. Plasma PCSK9 concentrations during an oral fat load and after short-term high-fat, high-fat-high-protein and high-fructose diets[J]. *Natr Metab (Lond)*, 2013,10(1):4.

[20] Chan DC, Wong ATY, Pang J, et al. Inter-relationships between proprotein convertase subtilisin/kexin type 9, apolipoprotein C-III and plasma apolipoprotein B-48 transport in obese subjects: a stable isotope study in the postprandial state[J]. *Clin Sci (Lond)* 2015,128(6):379-385.

[21] Chernogubova E, Strawbridge R, Mahdessian H, et al. Common and low-frequency genetic variants in the PCSK9 locus influence circulating PCSK9 levels[J]. *Arterioscler Thromb Vasc Biol*, 2012,32(6):1526-1534.
PCSK9 and triglyceride-rich lipoprotein metabolism

[22] Kwakernaak AJ, Lambert G, Dullaart RPF. Plasma proprotein convertase subtilisin-kexin type 9 is predominantly related to intermediate density lipoproteins[J]. Clin Biochem, 2014, 47(7-8):679-682.

[23] Lakoski SG, Lagace TA, Cohen JC, et al. Genetic and metabolic determinants of plasma PCSK9 levels[J]. J Clin Endocrinol Metab, 2009, 94(7):2537-2543.

[24] Mayne J, Ooi TC, Raymond A, et al. Differential effects of PCSK9 loss of function variants on serum lipid and PCSK9 levels in Caucasian and African Canadian populations[J]. Lipids Health Dis, 2013, 12(70).

[25] Sullivan S, Fabbrini E, Horton JD, et al. Lack of relationship between plasma PCSK9 concentrations and hepatic lipoprotein kinetics in obese people[J]. Trans Res, 2012, 158(5):302-306.

[26] Kwakernaak AJ, Lambert G, Slagman MCJ, et al. Proprotein convertase subtilisin-kexin type 9 is elevated in proteinuric subjects: relationship with lipoprotein response to antiproteinuric treatment[J]. Atherosclerosis, 2013, 226(2):459-465.

[27] Abujrad H, Mayne J, Ruzicka M, et al. Chronic kidney disease on hemodialysis is associated with decreased serum PCSK9 levels[J]. Atherosclerosis, 2014, 233(1):123-129.

[28] Naoumovna RP, Tosi I, Patel D, et al. Severe hypercholesterolemia in four British families with the D374Y mutation in the PCSK9 gene: long-term follow-up and treatment response[J]. Arterioscler Thromb Vasc Biol, 2005, 25(12):2654-2660.

[29] Raal F, Panz V, Immelman A, et al. Elevated PCSK9 levels in untreated patients with heterozygous or homozygous familial hypercholesterolemia and the response to high-dose statin therapy[J]. J Am Heart Assoc, 2013, 2(2):1-7.

[30] Humphries SE, Neely RDG, Whittall RA, et al. Healthy individuals carrying the PCSK9 p.R46L variant and familial hypercholesterolemia patients carrying PCSK9 p.D374Y exhibit lower plasma concentrations of PCSK9[J]. Clin Chem, 2009, 55(12):2153-2161.

[31] Costet P, Cariou B, Labert G, et al. Plasma PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory-element protein 1c[J]. J Biol Chem, 2006, 281(10):6211-6218.

[32] Kapelle PJWH, Lambert G, Dullaart RPF. Plasma proprotein convertase subtilisin-kexin type 9 does not change during 24h insulin infusion in healthy subjects and type 2 diabetic patients[J]. Atherosclerosis, 2011, 214(2):432-435.

[33] Awan Z, Dubuc G, Faraj M, et al. The effect of insulin on circulating PCSK9 in postmenopausal obese women[J]. Clin Biochem, 2014, 47(12):1033-1039.

[34] Brouwers MCGJ, Troutt JS, van Greevenbroek MMJ, et al. Plasma proprotein convertase subtilisin kexin type 9 is not altered in subjects with impaired glucose metabolism and type 2 diabetes mellitus, but its relationship with non-HDL cholesterol and apolipoprotein B may be modified by type 2 diabetes mellitus[J]. Atherosclerosis, 2011, 217(1):263-267.

[35] Mbikay M, Sirois F, Mayne J, et al. PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities[J]. FEBs Lett, 2010, 584(4):701-706.

[36] Le May C, Kourimate S, Langhi C, et al. Proprotein convertase subtilisin kexin type 9 null mice are protected from postprandial triglycerideremia[J]. Arterioscler Thromb Vasc Biol, 2009, 29(5):684-690.

[37] Malloy MJ, Kane JP. Fibric Acid Derivatives (Fibrates) - Mechanism of Action[J]. In: Katzung BG, Editor. Basic and Clinical Pharmacology. 10th edition. New York: McGraw Hill Lange, 2007:569.

[38] Chan DC, Hamilton SJ, Rye KA, et al. Fenofibrate concomitantly decreases serum proprotein convertase subtilisin/kexin type 9 and very-low-density lipoprotein particle concentrations in statin-treated type 2 diabetic patients[J]. Diabetes Obes Metab, 2010, 12(9):752-756.

[39] Costet P, Hoffmann MM, Cariou B, et al. Plasma PCSK9 is increased by fenofibrate and atorvastatin in a non-additive fashion in diabetic patients[J]. Atherosclerosis, 2010, 212(1):246-251.

[40] Noguchi T, Kobayashi J, Yagi K, et al. Comparison of effects of bezafibrate and fenofibrate on circulating proprotein convertase subtilisin/kexin type 9 and adipocytokine levels in dyslipidemic subjects with impaired glucose tolerance or type 2 diabetes mellitus: results from a crossover study[J]. Atherosclerosis, 2011, 217(1):165-170.

[41] Troutt JS, Albom WE, Cao G, et al. Fenofibrate treatment increases human serum proprotein convertase subtilisin kexin type 9 levels[J]. J Lipid Res, 2010, 51(2):345-351.

[42] Sahebkar A. Circulating levels of proprotein convertase subtilisin kexin type 9 are elevated by fibrate therapy: a systematic review and meta-analysis of clinical trials[J]. Cardiol Rev, 2014, 22(6):306-312.

[43] Kourimate S, Le May C, Langhi C, et al. Dual mechanisms for the fibrate-mediated repression of proprotein convertase subtilisin/kexin type 9[J]. J Biol Chem, 2008, 283(15):9666-9673.

[44] Roubtsova A, Munkonda MN, Awan Z, et al. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue[J]. Arterioscler Thromb Vasc Biol, 2011, 31(4):785-791.

[45] Mbikay M, Sirois F, Gyama-Acheampong C, et al. Variable effects of gender and Western diet on lipid and glucose homeostasis in aged PCSK9-deficient C57BL/6 mice CSK9PC57BL/6[J]. J Diabetes, 2015, 7(1):74-84.

[46] Sun XM, Eden ER, Tosi I, et al. Evidence for effect of mutant PCSK9 on apolipoprotein B secretion as the cause of unusually severe dominant hypercholesterolaemia[J]. Hum Mol Genet, 2005, 14(9):1161-1169.

[47] Sun H, Samarghandi A, Zhang N, et al. Proprotein convertase subtilisin kexin type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor[J]. Arterioscler Thromb Vasc Biol, 2012, 32(7):1585-1595.

[48] Rashid S, Curtis DE, Garuti R, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9[J]. Proc Natl Acad Sci USA, 2005, 102(15):5374-5379.

[49] Ouguerram K, Chetiveaux M, Zair Y, et al. Apolipoprotein B100 metabolism in autosomal-dominant hypercholesterolemia related to mutations in PCSK9[J]. Arterioscler Thromb Vasc Biol, 2004, 24(8):1448-1453.

[50] Lambert G, Jarnoux AL, Pineau T, et al. Fasting induces hyperlipidemia in mice overexpressing proprotein convertase subtilisin kexin type 9: lack of modulation of
very-low-density lipoprotein hepatic output by the low-density lipoprotein receptor[J]. *Endocrinology*, 2006, 14(10):4965-4995.

[51] Tavori H, Fan D, Blakemore JL, et al. Serum proprotein convertase subtilisin/kexin type 9 and cell surface low-density lipoprotein receptor: evidence for a reciprocal regulation[J]. *Circulation*, 2013,127(24):2403-2413.

[52] Blasiole DA, Oler AT, Attie AD. Regulation of ApoB secretion by the low density lipoprotein receptor requires exit from the endoplasmic reticulum and interaction with ApoE or ApoBJU[J]. *J Biol Chem*, 2008,283(17):11374-11381.

[53] Levy E, Ben Djoudi Ouadda A, Spahis S, et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells[J]. *Atherosclerosis*, 2013,227(2):297-306.

[54] Rashid S, Tavori H, Brown PE, et al. Proprotein convertase subtilisin/kexin type 9 promotes intestinal overproduction of triglyceride-rich apolipoprotein B lipoproteins through both low-density lipoprotein receptor-dependent and -independent mechanisms[J]. *Circulation*, 2014,130(5):431-441.

[55] Poirier S, Mayer G, Benjannet S, et al. The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2[J]. *J Biol Chem*, 2000,275(4):2363-2372.

[56] Canuel M, Sun X, Asselin MC, et al. Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1)[J]. *PLoS One*, 2013,8(5):e64115.

[57] Frykman PK, Brown MS, Yamamoto T, et al. Normal plasma lipoproteins and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very low density lipoprotein receptor[J]. *Proc Natl Acad Sci USA*, 1995,92(18):8453-8457.

[58] Demers A, Lauzier B, Des Rosiers C, et al. Proprotein Convertase Subtilisin/kexin Type 9 (PCSK9) Targets the CD36 Receptor for Degradation[J]. *Arterioscler Thromb Vasc Biol*, 2013,33(5):A77.

[59] Masuda D, Hirano K, Oku H, et al. Chylomicron remnant clearance is associated with plasma lipoprotein and fertility in gene-targeted mice homozygous for a disruption in the gene encoding very-low-density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2[J]. *J Am Coll Cardiol*, 2014,63(23):2531-2540.

[60] Mckenney JM, Harm PD, Koren MJ, et al. Safety and Efficacy of a Monoclonal Antibody to Proprotein Convertase Subtilisin/Kexin Type 9 Serine Protease, SAR236553/REGN727, in Patients With Primary Hypercholesterolemia Receiving Ongoing Stable Atorvastatin Therapy[J]. *J Am Coll Cardiol*, 2012,59(25):2344-2353.

[61] Robinson RG, Nedergaard BS, Rogers WJ, et al. Effect of Evolocumab or Ezetimibe Added to Moderate- or High-Intensity Statin Therapy on LDL-C Lowering in Patients With Hypercholesterolemia The LAPLACE-2 Randomized Clinical Trial[J]. *JAMA*, 2014,311(18):1870-1882.

[62] Roth EM, Taskinen M, Ginsberg HN, et al. Monotherapy with the PCSK9 inhibitor alirocumab versus ezetimibe in patients with hypercholesterolemia: Results of a 24 week double-blind, randomized Phase 3 trial[J]. *Int J Cardiol*, 2014,176(1):55-61.

[63] Sullivan D, Olsson AG, Scott R, et al. Effect of a Monoclonal Antibody to PCSK9 on Low-Density Lipoprotein Cholesterol[J]. *JAMA*, 2014,308(23):2497-2506.

[64] Stein EA, Gipe D, Bergeron J, et al. Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial[J]. *Lancet*, 2012,380(9836):29-36.

[65] Stein EA, Honarpour N, Wasserman SM, et al. Effect of the Proprotein Convertase Subtilisin/Kexin 9 Monoclonal Antibody, AMG 145, in Homozygous Familial Hypercholesterolemia[J]. *Circulation*, 2013,127(2):2408-2417.

[66] Stein EA, Honarpour N, Wasserman SM, et al. Effect of the Proprotein Convertase Subtilisin/Kexin 9 Monoclonal Antibody, AMG 145, in Homozygous Familial Hypercholesterolemia[J]. *Circulation*, 2013,127(24):2408-2417.

[67] Stein EA, Giugliano RP, Koren MJ, et al. Anti-PCSK9 Monotherapy for Hypercholesterolemia Clinical Trial of Evolocumab[J]. *J Am Coll Cardiol*, 2014,63(23):2531-2540.

[68] Stein EA, Gipe D, Bergeron J, et al. Effect of a monoclonal antibody to PCSK9, REGN727/SAR236553, to reduce low-density lipoprotein cholesterol in patients with heterozygous familial hypercholesterolemia on stable statin dose with or without ezetimibe therapy: a phase 2 randomised controlled trial[J]. *Lancet*, 2012,380(9836):29-36.

[69] Stein EA, Honarpour N, Wasserman SM, et al. Effect of the Proprotein Convertase Subtilisin/Kexin 9 Monoclonal Antibody, AMG 145, in Homozygous Familial Hypercholesterolemia[J]. *Circulation*, 2013,128(19):2113-2120.

[70] Ral F, Scott R, Somaratne R, et al. Low-Density Lipoprotein Cholesterol - Lowering Effects of AMG 145, a Monoclonal Antibody to Proprotein Convertase Subtilisin/Kexin Type 9 Serine Protease in Patients With Heterozygous Familial Hypercholesterolemia[J]. *Circulation*, 2012,126(20):2408-2417.

[71] Stein EA, Honarpour N, Wasserman SM, et al. Effect of the Proprotein Convertase Subtilisin/Kexin 9 Monoclonal Antibody, AMG 145, in Homozygous Familial Hypercholesterolemia[J]. *Circulation*, 2013,128(19):2113-2120.

[72] Raal FJ, Honarpour N, Blom DJ, et al. Inhibition of PCSK9 with evolocumab in homozygous familial hypercholesterolemia (TESLA Part B): a randomised, double-blind, placebo-controlled trial[J]. *Lancet*, 2015,385(9965):341-350.

[73] Raal FJ, Stein EA, et al. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial[J]. *Lancet*, 2015,385(9965):331-340.

[74] Stein EA, Giugliano RP, Koren MJ, et al. Efficacy and safety of evolocumab (AMG 145), a fully human monoclonal antibody to PCSK9, in hyperlipidaemic patients on various background lipid therapies?: pooled analysis of 1359 patients in four phase 2 trials[J]. *Eur Heart J*, 2014,35(33):2249-2259.