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Editorial

Functional role of gut microbiota and PCSK9 in the pathogenesis of diabetes mellitus and cardiovascular disease

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In humans, the highest density of bacteria is located in the gut, where microbiota differs throughout the gastrointestinal tract, ranging from 1×10^4 cells/g in the jejunum to 1×10^{14} cells/g in the colon [1,2]. Many factors including diet, drugs, and pathological conditions can induce changes in gut microbial composition, bacterial gene transcription, and metabolism.

1. From gut microbiota dysbiosis to elevated PCSK9 levels: the (missing) metabolic link

Substantial evidence indicates a relation between dysbiosis of the gut microbiota and the pathogenesis of Type 2 Diabetes Mellitus (T2DM). Indeed, cross-sectional studies in humans have demonstrated the compositional and functional difference in the gut bacteria of patients with T2DM or prediabetes compared with normoglycemic individuals [1,3]; furthermore, in a fecal microbiota transplant study, an increase in gut microbial diversity and improved insulin sensitivity was observed as a result of the fecal material transplant from lean donors into patients with metabolic syndrome [4]. Preclinical studies have also shown how fecal transplants from mice with glucose intolerance into healthy germ-free mice can induce glucose intolerance [2]. These data support the hypothesis that dysbiosis of the gut microbiota contributes to insulin resistance.

T2DM, and metabolic dysfunction. Several potential mechanisms have been proposed to explain the role of gut dysbiosis in the pathogenesis of metabolic disorders. Changes in microbial community composition and metabolism along the digestive tract, in particular in case of Small Intestinal Bacterial Overgrowth (SIBO), may play an etiological role in diabetes, non-alcoholic steatohepatitis, and obesity; interestingly, SIBO contributes to the ileum epithelial barrier permeability due to alteration in the structure of epithelial cells tight junction [5]. The dysregulation of the gut mucosal barrier increases the translocation of lipopolysaccharides complex (LPS), also known as endotoxin, through the epithelial barrier, with a perpetuation of the mucosal inflammation and generation of a metabolic endotoxemia condition. Although the mechanisms underlying endotoxemia are not completely understood, mounting evidence suggests an association between the alterations in gut microbiota regulation and the increased activation of inflammatory pathways, leading to an impairment of insulin signaling [1–3]. Pastori, Ettorre, and colleagues in this issue of *Atherosclerosis* elegantly show that patients with atrial fibrillation displaying a concomitant increase of Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) and LPS have a higher risk of cardiovascular risk [6]; consistent with these findings, oxidative stress has been demonstrated to be a key mechanism in the pathogenesis of atrial fibrillation [7,8]. Intriguingly, gut dysbiosis, through means of induction or exacerbation of insulin resistance, may lead to augmented expression of PCSK9, thereby worsening the pre-existing metabolic risk. Importantly, insulin has been shown to trigger PCSK9 expression, using both primary cultures of rat hepatocytes and murine models of insulin resistance, obesity, and diabetes [9]. Furthermore, the “Dallas Heart Study” a cross-sectional and longitudinal study, revealed in an ethnically diverse US population (3138 subjects) a significant positive correlation between PCSK9 and insulin plasma levels in subjects with impaired glucose tolerance or T2DM [10]. These observations were consistent with other epidemiological studies [2,11].

2. PCSK9: structure, biological functions, and mechanistic role in dyslipidemia

PCSK9, a subtilisin-like serine protease, is synthesized and secreted primarily by the liver; to a lesser extent, it is expressed in the small intestine, kidney, adipose tissue, central nervous system, colon epithelia, and vascular smooth muscle cells studies [11]. The liver is not only the key source of PCSK9 biosynthesis but also the main target of its activity studies [11]. *Pcsk9* gene encodes for an inactive glycoprotein (pre-PCSK9) of 692 amino acids comprising a signal peptide, a

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prosegment, a subtilisin-like catalytic domain, and a cysteine- and histidine-rich C-terminal domain, which is required for trafficking of the PCSK9/LDL-R complex to the lysosome. The signal peptide is cleaved from pre-PCSK9 in the endoplasmic reticulum (ER) to obtain the pro- PCSK9 (the soluble zymogen), subsequently converted to mature secretory PCSK9 through autocatalytic cleavage of the prosegment in the Golgi apparatus [12]. Unlike other serine proteases, PCSK9 prosegment stays noncovalently bound to the active site of the catalytic domain, rendering the protease enzymatically inactive; the prosegment-PCSK9 complex then exits the ER and enters the secretory pathway for the release of mature PCSK9 in the bloodstream. Albeit not enzymatically active, the catalytic domain of secreted PCSK9 associates with LDL receptor (LDL-R) at the hepatocyte surface by interacting with the extracellular Epidermal Growth Factor precursor homology domain-A (EGF-A) of LDL-R [13]. Once the secreted PCSK9 binds the EGF-A domain of LDL-R on the cell membrane, the PCSK9/LDL-R complex enters the endosomal pathway, but contrary to LDL-R/LDL complex, PCSK9 does not dissociate at low pH in the endosome [12,13]. The trafficking of LDL-R back to the cell surface, in fact, depends on the EGF-A domain; the binding of PCSK9 to the EGF-A domain inhibits the LDL-R recycling to the cell surface and enhances the lysosomal degradation of LDL-R [12]. Consequently, when the plasma levels of PCSK9 are elevated there is a significant reduction of LDL-Rs available to clear Low- Density Lipoprotein Cholesterol (LDL-C) from the bloodstream, and this effect leads to elevated plasma levels of LDL-C [14]. In light of PCSK9 role in the direct reduction of LDL-Rs in the liver and other organs, PCSK9 inhibition has recently emerged as one of the most effective LDL-C lowering therapeutical approaches [11].

3. Molecular mechanisms underlying insulin-dependent regulation of PCSK9

Several *in vivo* assays performed in established murine models of diabetes, insulin resistance, and other metabolic conditions, including Liver Insulin Receptor Knockout (LIRKO) mice (hyperglycemic and hyperinsulinemic because their LIRKO hepatocytes lack insulin receptors making them incapable of insulin signaling), streptozotocin treated mice, and mice fed an atherogenic high-fat/high-cholate diet (Paigen diet) [9] have indicated that insulin is able to increase the expression of PCSK9 when initiates the signaling cascade through the insulin receptor, a transmembrane glycoprotein with intrinsic protein tyrosine kinase activity [9,15,16]. Such signaling pathway induces the tyrosine phosphorylation of insulin receptor substrates (IRS)-1 and -2, which bind and activate phosphatidylinositol 3-kinase (PI3K); PI3K then activates protein kinase B (PKB)/Akt; activation of the PI3K/PKB/Akt transduction pathway by insulin increases the expression of sterol response elements binding transcription factor 1c (SREBP-1c) [17], but decreases the expression of Hepatocyte nuclear factor 1α (HNF-1α) through the mammalian target of rapamycin complex 1 (mTORC-1) pathway [16,18]. HNF-1α is a key transcriptional factor of PCSK9, able to induce PCSK9 expression by the binding of highly conserved sequence motif upstream of sterol response elements (SRE) in *Pcsk9* gene, in a coordinated manner with SREBP-1c [17]. Evidence in humans shows that in normal physiological state the inhibitory effect of insulin on PCSK9 gene expression is dominant [11,17], whereas *in vivo* studies in rodents - in which the promoter of PCSK9 gene uses SREBP-1c as principal transcription factor - show that insulin stimulates PCSK9 [9]. However, both in human and rodents, chronically high insulin levels (observed in pre-diabetes or T2DM) lead to inactivation of IRS pathway, with the loss of inhibitory effect of the axis PI3K/PKB/Akt on HNF-1α, that can be expressed to promote the transcription of *Pcsk9* gene [16]. Interestingly, SREBP-1c does not appear to be affected by the inactivation of IRS pathway [17], thus insulin seems to induce SREBP1-c expression through a pathway not completely deciphered. Henceforward, high insulin levels increase PCSK9 expression in both human and rodents mainly through SREBP-1c and HNF-1α transcription factors.

4. LPS absorption leads to insulin resistance and triggers PCSK9 expression

Metabolic endotoxemia consists of a low-grade inflammation triggered by LPS complex, a component presents in the outer membrane of Gram-negative bacteria; LPS can also be internalized by intestinal epithelial cells and transported to the Golgi compartment of the enterocyte, where newly assembled chylomicrons are located before their basolateral secretion; another possibility, supported by the observation that intestinal tight-junction integrity is impaired in obese mice, is that dietary fat leads to paracellular leakage of LPS across the intestinal epithelium [19]. Additionally, some dysbiosis conditions, including SIBO [5], can affect the tight-junction integrity in the gut, increasing LPS permeability through the intestinal epithelial barrier. In clinical cross-sectional observational studies, increased levels of systemic LPS or LPS binding protein were associated with low-grade chronic inflammation in obesity, metabolic syndrome, insulin resistance, and T2DM [19].

LPS stimulates an inflammatory response via a synergic activation of Toll-Like Receptor-2 and 4 (TLR-2, TLR- 4), leading to the expression of several cytokines and interfering with insulin signaling; when TLR-4 is activated, through possible cooperation with TLR-2, it upregulates specific pathways including c-Jun NH2-terminal kinase (JNK) and IκB kinase complex (IKKβ)/inhibitor of nuclear factor-κB (IκBα)/nuclear factor-κB (NF-κB) [20]. Chronic hyperinsulinemia leads to inactivation of IRS pathway, with the consequent loss of inhibitory effect on HNF-1α transcription factor, that can induce PCSK9 expression. The binding between circulating LPS absorbed by the gut epithelium and TLR-2 and -4 activates JNK and IKKβ/IκBα/NF-κB pathways, eventually leading to inactivation of IRS1-2 [2]. Therefore, LPS can induce similar effects of chronic hyperinsulinemia and alter the insulin pathway in hepatocytes and/or adipocytes, with increased expression of PCSK9: loss-of-function mutations of TLR-4 prevent insulin resistance induced by obesity or free fatty acids, suggesting a crucial role for TLR-4 in the interface of innate immune system and energetic metabolism; moreover, activation of TLR-4 by LPS in pre-adipocytes increases the expression of several cytokines, including TNF-α and IL-6, and impairs the insulin signaling in adipocytes [16].

5. Conclusions

Although the precise mechanisms of PCSK9 regulation by insulin are still not fully determined, the available epidemiological observations and data from *in vivo* and *in vitro* studies suggest a trend towards a positive association between the incidence of pre-diabetes or T2DM and plasma PCSK9 levels. Insulin appears to regulate PCSK9 through a dual effect: induction via up-regulation of SREBP-1c transcription factor and inhibition via repression of HNF-1α expression.

Gut microbial dysbiosis can be a major determinant of LPS translocation through the intestinal epithelial barrier and consequent mucosal inflammation and endotoxemia. LPS binding to TLRs can interfere with insulin signaling, leading to insulin resistance and inducing PCSK9 transcription. PCSK9 protein, in turn, promotes the reduction of LDL-R in hepatocytes and adipocytes, thereby limiting the clearance of LDL-C and LPS from the bloodstream. Hence, it is possible to infer that endotoxemia and insulin resistance can contribute synergically to increase plasma levels of PCSK9, therefore representing two important risk factors for cardiovascular disease.

Conflict of interest

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

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References

[1] G.G. Schiattarella, B. Trimarco, Microbial metabolites as predictive biomarkers: a paradigm shift for cardiovascular risk stratification, Eur. Heart J. (2019).
[2] R.D. Hills Jr., B.A. Pontefract, H.R. Mishcon, et al., Gut Microbiome: Profound Implications for Diet and Disease, Nutrients, (2019), p. 11.
[3] J. Qin, Y. Li, Z. Cai, et al., A metagenome-wide association study of gut microbiota in type 2 diabetes, Nature 490 (2012) 55–60.
[4] A. Vrieze, E. Van Nood, F. Holleman, et al., Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome, Gastroenterology 143 (2012) 913–916 e917.
[5] A. Adike, J.K. DiBaise, Small intestinal bacterial Overgrowth: nutritional implications, diagnosis, and management, Gastroenterol. Clin. N. Am. 47 (2018) 193–208.
[6] D. Pastori, E. Ettorre, R. Carnevale, et al., Interaction between Serum Endotoxemia and Proprotein Convertase Subtilisin/kexin 9 (PCSK9) in Patients with Atrial Fibrillation: A Post-hoc Analysis from the ATHERO- AF Cohort, Atherosclerosis, 2019, pp. 195–200.
[7] W. Xie, G. Santulli, S.R. Reiken, et al., Mitochondrial oxidative stress promotes atrial fibrillation, Sci. Rep. 5 (2015) 11427.
[8] J. Gambardella, D. Sorriento, M. Ciccarelli, et al., Functional role of mitochondria in arrhythmogenesis, Adv. Exp. Med. Biol. 982 (2017) 191–202.
[9] J. Miao, P.V. Manthena, M.E. Haas, et al., Role of insulin in the regulation of Proprotein Convertase subtilisin/kexin type 9, Arterioscler. Thromb. Vasc. Biol. 35 (2015) 1589–1596.
[10] S.G. Lakoski, T.A. Lagace, J.C. Cohen, et al., Genetic and metabolic determinants of plasma PCSK9 levels, J. Clin. Endocrinol. Metab. 94 (2009) 2537–2543.
[11] M.S. Sabatine, PCSK9 inhibitors: clinical evidence and implementation, Nat. Rev. Cardiol. 16 (2019) 155–165.
[12] H.J. Kwon, T.A. Lagace, M.C. McNutt, et al., Molecular basis for LDL receptor recognition by PCSK9, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 1820–1825.
[13] S. Poirier, G. Mayer, V. Poupon, et al., Dissection of the endogenous cellular pathways of PCSK9- induced low density lipoprotein receptor degradation: evidence for an intracellular route, J. Biol. Chem. 284 (2009) 28856–28864.
[14] F.J. Raal, R.P. Giugliano, M.S. Sabatine, et al., PCSK9 inhibition-mediated reduction in Lp(a) with evolocumab: an analysis of 10 clinical trials and the LDL receptor's role, J. Lipid Res. 57 (2016) 1086–1096.
[15] D. Sorriento, J. Gambardella, A. Fiordelisi, et al., Mechanistic role of kinases in the regulation of mitochondrial fitness, Adv. Exp. Med. Biol. 982 (2017) 521–528.
[16] D. Ai, C. Chen, S. Han, et al., Regulation of hepatic LDL receptors by mTORC1 and PCSK9 in mice, J. Clin. Investig. 122 (2012) 1262–1270.
[17] P. Costet, B. Cariou, G. Lambert, et al., Hepatic PCSK9 expression is regulated by nutritional status via insulin and sterol regulatory element-binding protein 1c, J. Biol. Chem. 281 (2006) 6211–6218.
[18] G. Santulli, H. Totary-Jain, Tailoring mTOR-based therapy: molecular evidence and clinical challenges, Pharmacogenomics 14 (2013) 1517–1526.
[19] T. Sakura, T. Morioka, A. Shioi, et al., Lipopolysaccharide-binding protein is associated with arterial stiffness in patients with type 2 diabetes: a cross-sectional study, Cardiovasc. Diabetol. 16 (2017) 62.
[20] D.M. Tsukumo, M.A. Carvalho-Filho, J.B. Carvalheira, et al., Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance, Diabetes 56 (2007) 1986–1998.

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