BBA - Molecular Basis of Disease 1865 (2019) 2149–2156

Contents lists available at ScienceDirect

BBA - Molecular Basis of Disease

journal homepage: www.elsevier.com/locate/bbadis

Cholesterol metabolism, pancreatic β-cell function and diabetes

Carla Perego[a,1], Lorenzo Da Dalt[a,1], Angela Pirillo[b], Alessandra Galli[a], Alberico L. Catapano[a,c,*], Giuseppe D. Norata[a,b,**]

[a] Department of Excellence of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy
[b] SISA Centre, Bassini Hospital, Cinisello Balsamo, Milan, Italy
[c] IRCSS Multimedica, Milan, Italy

ARTICLE INFO

Keywords:
Cholesterol
β-cell
Insulin
Hypocholesterolemic drugs
Diabetes

ABSTRACT

Cholesterol plays an essential role in determining cell membrane physico-chemical characteristics and functions. A proper membrane structure is critical in pancreatic β-cells for glucose-mediated insulin secretion, and alterations in cellular cholesterol content may negatively affect this process, leading to β-cell dysfunction. The low density lipoprotein receptor (LDL-R) appears to play a relevant role in ß-cell dysfunction due to cholesterol accumulation. This observation raised the question of whether hypocholesterolemic drugs which increase LDL-R expression might bear diabetogenic properties, thus increasing the risk of new-onset diabetes or worsen glycaemic parameters in diabetic patients.

Being at higher cardiovascular risk, diabetic patients are usually treated with hypolipidemic drugs to correct the atherogenic dyslipidemia characteristic of this pathological condition. Statin therapy has been associated with an increased incidence of new-onset diabetes (NOD), being the diabetogenic effect depending on the type and dose of statin. However, it is worth noting that the benefits on cardiovascular mortality largely exceed the increased risk associated with the development of diabetes. Although genetic variants associated with lower levels of LDL-C are also associated with an increased NOD risk, clinical trials with lipid-lowering drugs other than statins, namely ezetimibe or monoclonal antibodies against PCSK9, did not observe an increase of developing diabetes.

In summary, molecular evidence clearly points to a key role for cholesterol homeostasis in pancreatic β-cell function which, in humans, is negatively affected by statins. Available data exclude that this could be the case for other hypocholesterolemic approaches, but long-term studies are warranted to explore this critical aspect.

1. Introduction

Cholesterol is an essential component of cell membranes and contributes to the control of their physical properties (such as fluidity and curvature), which in turn affect plasma membrane protein functions (i.e. transporters, ion channels and receptors) as well as vesicles formation and fusion. These aspects are also critical for the correct function of pancreatic β-cells and factors influencing cellular cholesterol metabolism are believed to impact β-cell function and the pathogenesis of diabetes. Indeed, in recent years, it became clear that statins, a class of hypolipidemic drugs, largely prescribed in diabetic patients to control their atherogenic dyslipidemia, can increase the risk of developing diabetes.

Aim of this brief review is to discuss the molecular mechanisms controlling insulin production and secretion, the role of cholesterol metabolism in β-cell function and the translational aspects linking hypocholesterolemic treatments with the increased risk of developing diabetes.

2. Molecular mechanisms regulating insulin production and secretion

The endocrine pancreas, through the synthesis and secretion of hormones, is critically involved in the control of glucose homeostasis.

* Correspondence to: A.L. Catapano, Department of Excellence of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy; IRCCS MultiMedica, Milan, Italy.
** Correspondence to: G.D. Norata, Department of Excellence of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy; Center for the Study of Atherosclerosis, E. Bassini Hospital, Cinisello Balsamo, Milan, Italy.
E-mail addresses: alberico.catapano@unimi.it (A.L. Catapano), danilo.norata@unimi.it (G.D. Norata).
[1] Equally contributed.

https://doi.org/10.1016/j.bbadis.2019.04.012
Received 23 February 2018; Received in revised form 20 December 2018; Accepted 6 January 2019
Available online 26 April 2019
0925-4439/

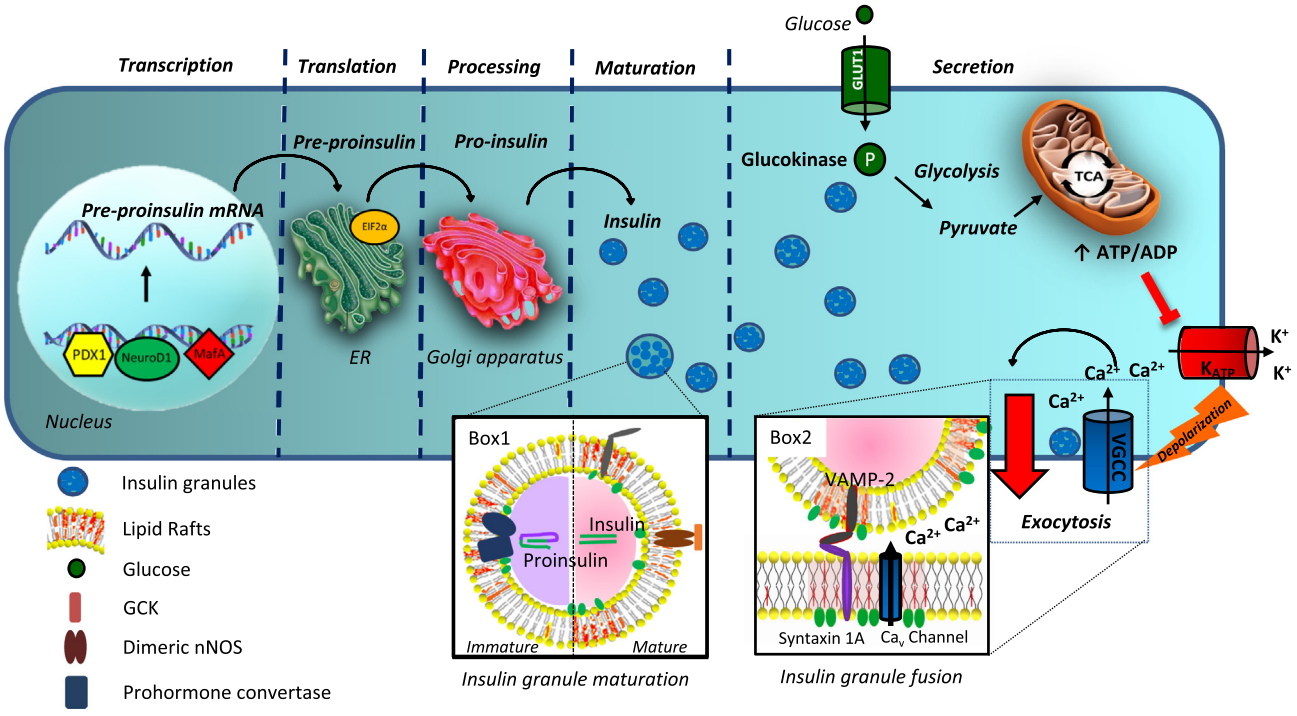

Fig. 1. Insulin production and secretion. Insulin production is controlled at the transcriptional level by several transcription factors including PDX-1 and MafA. The insulin transcript is then translated to pre-proinsulin, a process controlled by EIF2α, and converted to proinsulin in the endoplasmic reticulum (ER). At the trans-Golgi network, proinsulin is sorted to immature secretory granules where it is converted to mature insulin by the action of prohormone convertases, specifically recruited to lipid rafts of granules (box1). Insulin secretion is promoted by glucose -uptake via the GLUT1 transporter and phosphorylation by glucokinase (GCK), the major glucose sensor. Glucose metabolism, via glycolysis and Krebs cycle, results in the elevation of ATP/ADP ratio, closure of K_{ATP} channels, membrane depolarization and activation of voltage-gated calcium channels (VGCC). The resulting increase in intracellular Ca^{2+} represents the triggering signal for insulin granule fusion with the plasma membrane, a process regulated by the SNARE complex (box2).

Among the different endocrine cell populations, β-cells deserve a particular attention given that this is the only cell population able to synthesize and secrete insulin, the major hypoglycemic hormone. In healthy subjects, β-cells sense changes in plasma glucose concentration and respond by releasing insulin. Both insulin production and secretion are tightly regulated to maintain glucose homeostasis (Fig. 1).

Insulin production in β-cells is controlled at the transcriptional level by several transcription factors including the pancreatic and duodenal homeobox-1 (PDX-1), the v-maf musculoaponeurotic fibrosarcoma oncogene homologue (MafA) and the BETA2/Neurogenic differentiation 1 (NeuroD1), which play crucial roles in regulating both the differentiation of β-cells into insulin-producing cells and β-cell function [1]. The intracellular content of glucose regulates the transcription of insulin gene through the modulation of PDX1 and MafA [2] and affects insulin translation to sustain its secretion over time. When extracellular glucose rises above basal level, a specific regulatory element in the untranslated regions of preproinsulin mRNA stabilizes the transcript and favors its translation [3]. Furthermore, glucose controls the phosphorylation state of the eukaryotic initiation factor 2a (eIF2α) [4]. Several kinases and phosphatases control eIF2α phosphorylation and hence insulin translation, but the most interesting protein is the pancreatic endoplasmic reticulum kinase (PERK), which couples protein translation rate with the protein folding capacity of the endoplasmic reticulum (ER) [5,6] (Fig. 1).

The insulin gene encodes a 110-amino acid precursor (preproinsulin), containing an N-terminal signal sequence which facilitates its translocation into the lumen of the ER, a process crucial to target insulin to the secretory pathway. The signal peptide is then cleaved to yield proinsulin, which reaches the Golgi apparatus and enters into immature secretory granules, where following the activity of specific endoproteases, it is converted to a mature form [7,8], ready for release on demand. Two different mature secretory granule pools exist: a readily releasable pool (RRP), docked to the plasma membrane and immediately available for the insulin release, and a reserve pool (RP) to sustain the long-term insulin release.

β-cells "sense" elevated plasma glucose (> 100 mg/dL) and rapidly activate the intracellular machinery to secrete insulin. Under hyperglycemic conditions, glucose is internalized by the glucose independent transporters GLUT1 in humans or GLUT2 in rodents [9,10] and then is phosphorylated by glucokinase (GCK); this enzyme controls the rate of entry of glucose into the glycolytic pathway and its subsequent metabolism. Under low intracellular glucose levels, GCK is associated to insulin granules through the interaction with the dimeric neuronal nitric oxide synthase (nNOS) and is not active. As intracellular glucose levels increase, GCK is released from the granules and can phosphorylate glucose, thus controlling its metabolism and, as a consequence, the secretion of insulin [11] (Fig. 1).

Phosphorylated glucose enters the glycolytic pathway, then pyruvate is metabolized by the Krebs cycle to ATP in the mitochondria. The elevation of the cytosolic ATP/ADP ratio consequent to glucose metabolism causes the closure of the K^+ ATP channels, leading to a cellular depolarization and the opening of the L-type voltage-gated Ca^{2+} channels (VGCC). The rapid calcium influx into the cell induces the fusion of the insulin granules with the plasma membrane, a process that requires the presence of specific proteins, known as SNARE proteins [12–15]. In β-cells, specific cell membrane domains called lipid rafts determine the spatial organization of the SNARE proteins as well as the K^+ ATP and voltage-gated Ca^{2+} channels [16], thus maintaining a close association between the calcium influx and the exocytotic machinery and ensuring a fast release of the insulin granules when required [17] (Fig. 1).

The insulin secretion shows a biphasic pattern. The first transient phase involves the release of the RRP granules, reflecting the ability of β-cells to respond quickly to the increase of blood glucose

concentrations [18,19]. The second phase lasts longer and involves the RP granules, which must be mobilized and primed before being released. This phase amplifies the calcium action on exocytosis, through a mechanism independent from the K^+ ATP channels closure.

Many of the processes regulating insulin production, storage and secretion are affected by cellular lipids and cholesterol availability.

3. Cholesterol accumulation and β-cell dysfunction

Lipids, including cholesterol, are transported in the bloodstream via lipoproteins such as low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and high-density lipoprotein (HDL). Lipoproteins are a source of cholesterol and fatty acids for hepatocytes and peripheral cells and are taken up by β-cells through receptors belonging to the LDL receptor (LDL-R) family [20]. All cells, including pancreatic β-cells, are also able to synthesize cholesterol, through the mevalonate pathway. Intracellular synthesis and extracellular cholesterol uptake are under the control of the sterol regulatory element-binding protein 2 (SREBP-2) [21]. Excess cholesterol in β-cells is removed through a process termed reverse cholesterol transport which is mediated by different transporters, including the ATP-binding cassette transporter A1 (ABCA1) which releases cholesterol to lipid-free apolipoprotein A-I (Fig. 2).

In β-cells, cholesterol contributes to the control of the physical properties of the membranes (such as fluidity and curvature), thus affecting the localization and function of membrane proteins (i.e. transporters, ion channels and receptors) as well as vesicles formation and fusion [22]. Together with sphingolipids, cholesterol is an essential component of lipid rafts. Lipid rafts are microdomains of the plasma membrane present in all cells and, in pancreatic β-cells, are specifically enriched in ions channels and receptors as well as in proteins critically involved in coupling glucose-sensing with insulin secretion. Cholesterol is required for the correct formation of secretory granules, as it can influence the interaction of specific granule components with lipid rafts [23].

Several observations suggest that cellular cholesterol accumulation might lead to pancreatic β-cell dysfunction. Patients with type 2 diabetes present a cluster of lipid abnormalities which associate with cholesterol and fatty acid accumulation in pancreatic β-cells and may contribute to the degeneration of pancreatic islets [24]. β-cell SREBP-2 overexpression causes cholesterol accumulation and a severe impairment of cell function [21]. Also, the inhibition of cholesterol biosynthesis, which normally drives the activation of SREBP2, increases expression of the LDL-R and cholesterol uptake with reduced insulin secretion [25]. Similarly, the impairment of membrane transporters involved in the removal of excess cholesterol from cells (such as ABCA1) results in β-cell dysfunction and reduced insulin secretion [26–28].

Mechanistically, cholesterol excess impacts several steps of the metabolic machinery involved in glucose-stimulated insulin release localized at the endoplasmic reticulum, mitochondria and the cell membrane.

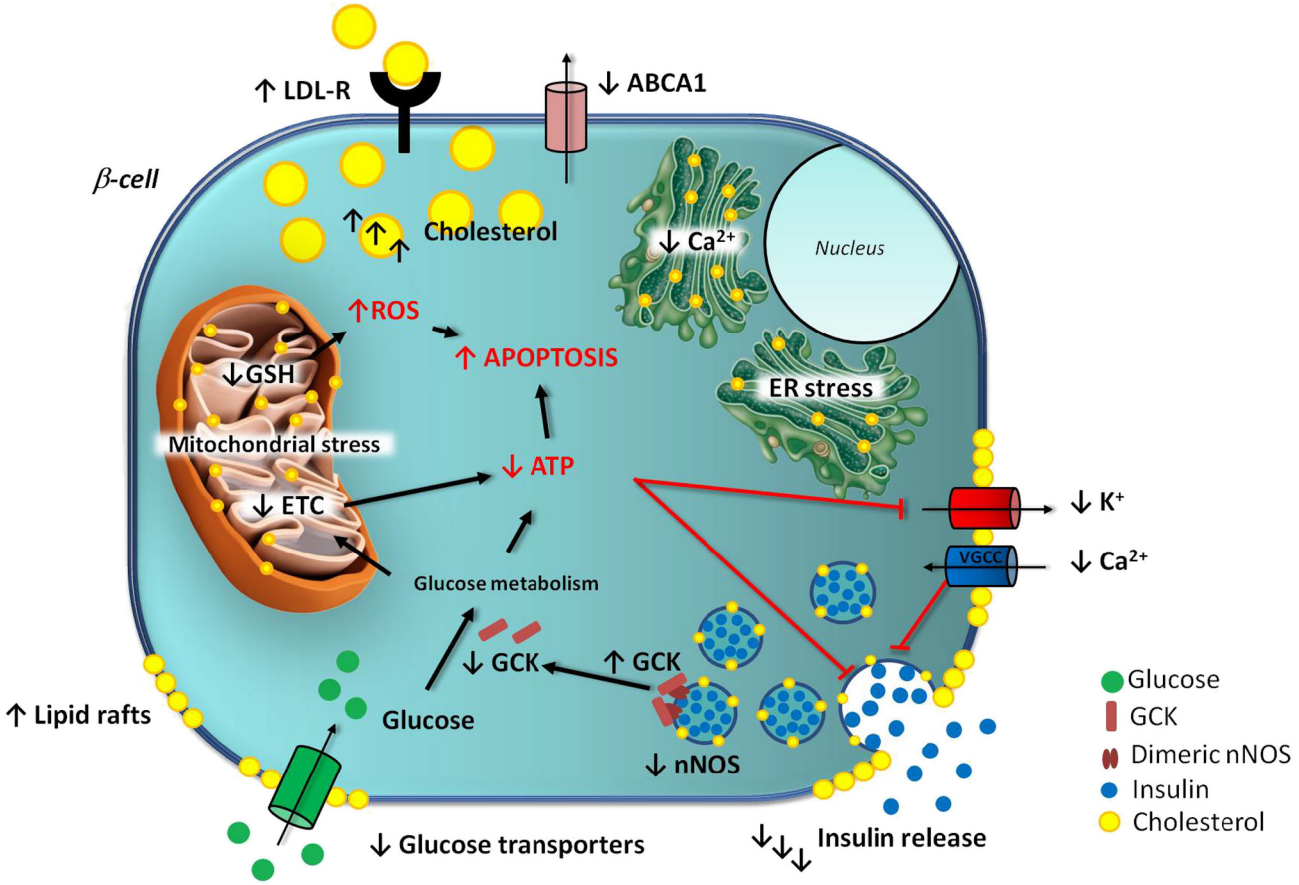

Fig. 2. Cholesterol metabolism and pancreatic β-cell function. Cellular cholesterol levels in β-cells increases following augmented uptake via LDL-R or reduced efflux via ABCA1. Cholesterol accumulation alters lipid raft composition and membrane fluidity; this results in reduced glucose transporter membrane levels, increased glucokinase (GCK) retention in insulin granules and alteration of spatial organization of L-type voltage-gated Ca^{2+} channels (VGCC) and K^+ ATP channels. As a consequence, insulin secretion decreases. Moreover, cholesterol accumulates in mitochondrial membrane, resulting in reduced ability of the electron transport chain to produce energy and increased ROS production; the latter, in turn, favors the depletion of antioxidants (GSH) and leads to mitochondrial stress and cellular apoptosis. Cholesterol accumulation in ER membrane leads to depletion of calcium store, thus increasing ER stress.

Cholesterol accumulation on endoplasmic reticulum can favor the depletion of calcium stores necessary for insulin release [29,30] and induces ER-stress by activating the PERK-eIF2α pathway [31]; a prolonged activation of this pathway can, in turn, lead to β-cell injury and reduced insulin production [6].

Cholesterol is also enriched in the trans-Golgi network, where it plays a key role in the biogenesis and trafficking of secretory granules, essential steps for the proper storage, processing, and secretion of insulin in pancreatic β-cells [32]. Both cholesterol depletion and accumulation can alter insulin release by altering granule trafficking [32]. Cholesterol depletion, in fact, can directly affect the granule formation, while excess cholesterol, which accumulates in insulin granules, triggers granule enlargement, alteration of granule membrane protein distribution, and reduction of the docking and fusion of granules with the plasma membrane [32]. This effect may be consequent also on the alteration of β-cell lipid rafts. Indeed, the sorting of granins (constituent of the secretory granules) and endoproteases (necessary for the prohormone processing and insulin maturation) to secretory granules is mediated by their interaction with lipid rafts [23]. Furthermore, lipid rafts determine the spatial organization of the SNARE proteins syntaxin and SNAP25, as well as of K^+ ATP and voltage-gated Ca^{2+} channels [16]. A close association between the calcium influx and the exocytotic machinery ensures a fast release of the insulin granules when a stimulus is applied [17]; thus, cholesterol depletion redistributes SNARE proteins and channel proteins out of the cholesterol-rich lipid rafts, thus resulting in an impairment of insulin secretion [25].

Cholesterol accumulation plays also a direct role in dampening glucose stimulated insulin secretion. First, at least in mice, cholesterol impacts β-cell glucose uptake by reducing glucose transporter activity [33]. Second, cholesterol stabilizes the dimeric form of nNOS, thus preventing the translocation of GCK from insulin granules to the cytoplasm, which results in a reduced glycolytic flow, decreased production of ATP and reduced exocytosis of insulin granules (Fig. 2). Third, elevated cellular cholesterol levels affect mitochondrial membrane fluidity, thus resulting in the alteration of the electron transport chain (ETC) and a reduced proton efflux [34]; this impacts the ability of mitochondria in β-cells to produce ATP following the activation of glycolysis and TCA cycle with the net effect of reducing insulin secretion. Finally, elevated cholesterol levels also affect the density of voltage-gated Ca^{2+} channels, leading to the reduction in intracellular flux of Ca^{2+}, again affecting insulin granules exocytosis (Fig. 2) [33].

It is important to notice that the loss of mitochondrial potential as a consequence of cholesterol accumulation in mitochondrial membrane also favors the opening of the transition pores with the release of cytochrome C and the induction of apoptosis [35]. Also, the antioxidant capacity of the mitochondria is affected by cholesterol driven alteration in membrane fluidity as a consequence of reduced GSH transport in the mitochondria (Fig. 2) which decreases the ability to resist to the oxidative damage induced by an increased presence of ROS and the consequent lipid peroxidation [36]. Similarly, cholesterol accumulation in plasma membrane was shown to induce β-cell apoptosis, mediated by an increased ROS production and the subsequent activation of stress-activated protein kinases (SAPKs), such as p38 MAPK and JNK [30].

Experimental data point therefore to a critical role for cholesterol metabolism in affecting β-cell function.

4. Translational relevance of cholesterol metabolism in β-cells and diabetes

Diabetes is associated with higher risk of cardiovascular events [37] mainly as a consequence of atherosclerotic cardiovascular disease. Patients with type 2 diabetes typically present a cluster of lipid anomalies, referred to as diabetic dyslipidemia, which includes low HDL-C, high TG, increased levels of small dense LDL (despite the presence of LDL-C levels commonly in the normal range) and remnant lipoproteins [38]. Moreover, they are characterized by an increased postprandial hyperlipidemia, resulting from an imbalanced metabolism of TG-rich lipoproteins (hepatic overproduction and delayed clearance) [38]. Non-fasting TG-rich lipoproteins indeed are associated with increased risk of CVD [39], and play a key role in the atherogenic process [40,41].

Although targeting TG-rich lipoproteins offers new opportunities for the management of diabetic dyslipidemia [42], LDL-C lowering still represents the major target for the treatment of atherogenic dyslipidemia in diabetes and is commonly managed with statin therapy as first approach [43]. Statins lower LDL-C by inhibiting the HMG-CoA reductase (HMGCR) and upregulating the expression of LDL-R in the liver, thus favoring cholesterol uptake in the hepatocyte and reducing plasma cholesterol levels [44]. Indeed diabetic patients treated with statins present a reduction in all-cause mortality per mmol/L LDL-C reduction similar to that observed in patients without diabetes [45].

However, meta-analyses of randomized controlled trials as well as observational studies have reported that statin therapy is dose-dependently associated with a 12% increased risk of new-onset diabetes [46–50] which is mainly dependent on the dose and the intensity of the statin. Whether the increased risk of NOD in patients treated with statins is a class effect is still controversial, but overall it appears that atorvastatin, rosuvastatin and simvastatin have a higher diabetogenic effect compared with pravastatin or pitavastatin [51,52]. The diabetogenic effect of atorvastatin is related to the dose [53] and to the presence of baseline risk factors for diabetes (which include higher fasting blood glucose, TG levels, body mass index, systolic and diastolic blood pressure and lower HDL-C levels), with patients presenting two to four diabetes risk factors being more likely to develop new onset diabetes (NOD) [54]. A similar observation was reported in the JUPITER study, in which rosuvastatin increased the incidence of NOD in patients presenting risk factors for diabetes [55].

Are these only associations or is there a causal link between statin treatment and the new onset diabetes? In Table 1 we reported the prevalence of type 2 diabetes in subjects carrying genetic variants in genes associated with LDL-C level control (either reducing or increasing LDL-C levels), and in individuals treated with major cholesterol-lowering drugs (statins and PCSK9 mAbs). Mendelian randomization studies reported that genetic variants in the *HMGCR* gene (encoding for the target of statin activity), which are associated with lower LDL-C levels (thus mimicking the activity of statins), are also causally associated with increased bodyweight (which is physiologically linked to insulin resistance) and increased risk of diabetes [50] (Table 1). The latter observation points to the possibility that reduced cholesterol biosynthesis (genetically driven by reduced HMG-CoA reductase

Table 1
Association between LDL-C-lowering genetic variants or drugs and risk of new-onset diabetes.

Ref.	Target	Effect on LDL-C	Type 2 diabetes risk	
	Genetic variant		OR per allele	OR per 1 mmol/L LDL-C reduction
[50,78]	*HMGCR*	LDL-C↓	1.02–1.06	1.39
[78,87]	*Npc1l1*	LDL-C↓	1.027–1.051	2.42
	PCSK9	LDL-C↓	1.089	1.19–1.293
	LDLR	LDL-C↓	1.028	1.13
[58]	*LDLR*	LDL-C↑		0.45

Ref.	Target	Effect on LDL-C	Type 2 diabetes risk
	LDL-C-lowering drug		OR/RR
[47–49,93–96]	Statins (HMGCR inhibitors)	LDL-C↓	1.09–1.13 (RCTs) 1.44 (observational studies)
[81,82,88,89]	anti PCSK9 mAbs	LDL-C↓	0.95–1.06

activity) might impact not only the liver, which would result in increased LDL-R expression and cholesterol uptake, but might affect other tissues as well, including pancreatic β-cells.

If this was the case, then patients with genetically impaired LDL-R activity should be less prone to develop diabetes. Familial hypercholesterolaemia (FH) is a genetic disease mainly caused by a mutation in the LDL-R which results in a dramatic increase in plasma cholesterol levels and premature risk of cardiovascular disease and mortality [56]. Of note FH patients, in spite of the increased CVD risk, have a prevalence of type 2 diabetes mellitus significantly lower compared to that of unaffected subjects [57,58], being the severity of LDL-R mutation inversely related with diabetes prevalence [57,58]. In agreement with this observation, in mice lacking the LDL-R, β-cells are protected from accumulation of cholesterol and cholesterol-induced dysfunction, while mice expressing more LDL-R show pancreatic islet abnormalities [26,59]. These findings clearly point to the LDL-R as a potential player linking statins and cholesterol metabolism to pancreatic β-cell function.

As statins increase LDL-R also in extrahepatic tissues, could this effect have deleterious consequence on pancreatic β-cell function [60]?

When the diabetogenic effect of statins in patients with FH (bearing a mutation in the LDL-R) was compared to that in patients with familial combined hypercholesterolemia (FCH), which is the most common type of genetic dyslipidemia, characterized by several metabolic disturbances including hypertension, obesity, insulin resistance, and metabolic syndrome, the latter group clearly outweighed FH patients for the prevalence of diabetes (13% vs 2%) [61]. In this study, during follow-up (~10 years) 14% of FCH and only 1% of FH patients developed diabetes [61], a finding further confirmed by other studies [62,63], suggesting that long-term statin treatment do not seem to be associated with increased risk of developing diabetes in FH patients (Table 1). This is likely due to the reduced functionality of LDL-R in FH patients, which cannot sustain cholesterol accumulation in β-cells in spite of an increased expression as a consequence of statin treatment. These findings support the possibility that the upregulation of LDL-R may be involved in the diabetogenic effect of statins, likely by inducing cholesterol accumulation and dysfunction in pancreatic β-cells [64]. In agreement with this hypothesis, and since the diabetogenic properties of statins vary based on the type and dose of statin, patients presenting multiple risk factors for diabetes may benefit more from the use of less diabetogenic statins (such as pravastatin or pitavastatin) [65]. Indeed, more lipophilic statins, such as simvastatin, atorvastatin and pitavastatin, have a higher extrahepatic tissue penetration and can freely diffuse into cells; this may result in the worsening of insulin sensitivity or the inhibition of insulin secretion from β-cells [66]. Pitavastatin, however, represents an exception, probably due to its ability to also increase plasma levels of adiponectin [67]. Less lipophilic statins, such as pravastatin and rosuvastatin, have a higher hepatoselectivity and require a carrier-mediated uptake. This would suggest a less pronounced effect on diabetes; however, rosuvastatin, is transported with an elevated affinity into cells, thus resulting in a more potent inhibition of cholesterol biosynthesis, but also an increased diabetogenic effect compared to pravastatin. Statin therapy was also shown to increase HbA1c levels and worsen glycaemic control in diabetic patients, again with differences according to the statin [68–72].

Is the combination of statins with other lipid lowering drugs with a different mechanism of action able to limit their diabetogenic effect? Data are available for ezetimibe which inhibits the activity of the cholesterol transporter NPC1L1 [73] (involved in dietary cholesterol absorption in the intestine) and for the inhibitors of the proprotein convertase subtilisin kexin 9 (PCSK9) which modulate LDL-R catabolism. Studies in experimental models have suggested that ezetimibe improves hepatic insulin resistance and glycaemic control [74,75]. In humans, a recent meta-analysis including 16 RCTs showed that ezetimibe alone or ezetimibe plus statin had a neutral effect on HbA1c and on fasting blood glucose in patients with or without diabetes compared with placebo or the same statin [76]. In combination with a low-dose statin, it presents a better profile compared with that observed in patients treated with a high-dose statin alone [76]. These data may suggest the possibility that ezetimibe combined to a low statin dose could mitigate the negative effects of high dose statins on glycaemic control in spite of similar LDL-C-lowering capacity. In patients with diabetes at baseline, the combination treatment produced a greater relative and absolute benefit compared with patients without diabetes [77]. However, it is worth noting that genetic variants in *NPC1L1* gene associated with low LDL-C levels were also associated with a higher risk of type 2 diabetes [78] (Table 1). Whether this is the direct result of NPC1L1 deficiency or depends on the indirect effect in increasing LDL-R expression remains to be addressed.

Also PCSK9 inhibitors were shown to be extremely effective in reducing LDL-C levels, approximately by 50–60% [79,80], thus resulting in a beneficial effect also on cardiovascular outcomes [81,82]. Nevertheless PCSK9 was shown to control LDL-R expression not only in the liver but also in pancreatic β-cells [83]. In mice pancreatic islet, PCSK9 is mainly expressed by δ-cells, while its expression is not detectable in β- and α-cells [84]. Interestingly, when PCSK9 is knocked out in all tissues, LDL-R expression is significantly increased also in β-cells and the consequent cholesterol accumulation leads to morphological abnormalities in pancreatic islets [85]. In line with this, mice lacking PCSK9 have lower insulin plasma levels that is retained and less released from pancreatic β-cells [85]. Moreover, mice lacking only hepatic PCSK9 production do not present alterations in plasma and pancreatic insulin levels [85], suggesting that circulating PCSK9 (most of hepatic origin) does not affect significantly LDL-R expression in pancreas. This observation is critical, as mAbs to PCSK9 target only circulating PCSK9 and may not have a significant impact on LDL-R in pancreatic β-cells.

Of note, similarly to what previously shown for genetic variants associated with reduced HMGCR function, also genetic variants associated with reduced PCSK9 mass or activity are associated with increased risk of diabetes [78,86,87] (Table 1). Two different meta-analysis of randomized controlled trials with PCSK9 inhibitors reported different findings; in 26,123 patients without diabetes at baseline no significant diabetogenic effect with anti-PCSK9 antibodies was observed [88]. Another meta-analysis, however, reported a small but significant increase in fasting blood glucose and HbA1c in trials with median follow-up of 78 weeks, without an increased risk of NOD [89]. This study also reported a progressively greater imbalance in glucose homeostasis with longer treatment period [89], an observation suggesting that a longer exposure to these drugs might result in an increased risk of incident diabetes. A prespecified analysis of the FOURIER trial with a median follow-up of 2.2 years showed that, among patients with atherosclerotic cardiovascular disease, evolocumab did not worsen glycaemia nor increase the risk of NOD [90]. This observation seems to be supported by another analysis of the FOURIER trial, showing a lack of increased risk of NOD even in subjects achieving very low levels of LDL-C [91]. To date, the ODYSSEY OUTCOMES trial, with a median duration follow-up of 2.8 years, could not detect any increased incidence of new-onset diabetes among patients treated with alirocumab compared to those in the placebo group (9.6% and 10.1%, respectively) [82]. Longer follow-up will be critical to exclude a diabetogenic effect for this class of drugs.

5. Conclusions

Cholesterol metabolism is tightly linked to β-cell function. Cholesterol accumulates in β-cells as a consequence of increased LDL-R expression/activity (driving increased cholesterol uptake) or reduced ABCA1 expression (limiting cholesterol efflux). Elevated cellular cholesterol levels affect membrane fluidity, glucokinase shuttling in the cytoplasm, mitochondrial activity and insulin release.

In humans, genetic variants which reduce LDL-C by different molecular mechanisms associate with an increased risk of developing

diabetes. This does not automatically translate into increased risk of developing diabetes in patients treated with lipid-lowering therapies in spite most of the drugs act indirectly by increasing hepatic LDL-R expression. Statins present a diabetogenic effect that relates to their potency and perhaps also physico-chemical characteristics. Ezetimibe presents a neutral effect even in combination with statins and also data from anti-PCSK9 inhibitors, which induce an extensive LDL-C reduction, do not suggest an increased risk of developing diabetes at least with the follow-up data available up to now.

In summary, although statins present a diabetogenic potential, it is important to point out that the benefits on cardiovascular mortality largely exceed the increased risk associated with the development of diabetes. Moreover, available data indicate that the diabetogenic effect of statin therapy seems to increase the risk of new onset diabetes mainly in subjects with a “pre-diabetic” status. Whether the subtle metabolic changes underlying the pre-diabetic situation contribute the effect of statins on glycaemia is unclear and deserves further investigation. Further, this paves the road for monitoring the impact of the combination therapy statin-metformin, to potentially reduce the risk of statin-induced T2DM [92], although specific trials are warranted to verify this possibility.

Authors' contributions

All authors have made equal intellectual contributions to the writing of this manuscript. All authors read and approved the final manuscript.

Funding

The authors are supported by Fondazione Cariplo 2015-0524 and 2015-0564 (ALC), and 2016-0852 (GDN); H2020 REPROGRAM PHC-03-2015/667837-2 (ALC); Ministero della Salute GR-2011-02346974 (GDN); PCSK9 Competitive Grant (GDN and CP), European Foundation for the Study of Diabetes, EFSD 2018 (GDN).

Transparency document

The Transparency document associated this article can be found, in online version.

References

[1] Y. Zhu, Q. Liu, Z. Zhou, Y. Ikeda, PDX1, Neurogenin-3, and MAFA: critical transcription regulators for beta cell development and regeneration, Stem Cell Res Ther 8 (2017) 240.
[2] L.M. de Vargas, J. Sobolewski, R. Siegel, L.G. Moss, Individual beta cells within the intact islet differentially respond to glucose, J. Biol. Chem. 272 (1997) 26573–26577.
[3] B. Wicksteed, T.P. Herbert, C. Alarcon, M.K. Lingohr, L.G. Moss, C.J. Rhodes, Cooperativity between the preproinsulin mRNA untranslated regions is necessary for glucose-stimulated translation, J. Biol. Chem. 276 (2001) 22553–22558.
[4] D. Vander Mierde, D. Scheuner, R. Quintens, R. Patel, B. Song, K. Tsukamoto, M. Beullens, R.J. Kaufman, M. Bollen, F.C. Schuit, Glucose activates a protein phosphatase-1-mediated signaling pathway to enhance overall translation in pancreatic beta-cells, Endocrinology 148 (2007) 609–617.
[5] H.P. Harding, Y. Zhang, D. Ron, Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase, Nature 397 (1999) 271–274.
[6] F.J. Kong, J.H. Wu, S.Y. Sun, J.Q. Zhou, The endoplasmic reticulum stress/autophagy pathway is involved in cholesterol-induced pancreatic beta-cell injury, Sci. Rep. 7 (2017) 44746.
[7] X.F. Huang, P. Arvan, Intracellular transport of proinsulin in pancreatic beta-cells. Structural maturation probed by disulfide accessibility, J. Biol. Chem. 270 (1995) 20417–20423.
[8] J.D. Dikeakos, T.L. Reudelhuber, Sending proteins to dense core secretory granules: still a lot to sort out, J. Cell Biol. 177 (2007) 191–196.
[9] A. De Vos, H. Heimberg, E. Quartier, P. Huypens, L. Bouwens, D. Pipeleers, F. Schuit, Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression, J. Clin. Invest. 96 (1995) 2489–2495.
[10] L.J. McCulloch, M. van de Bunt, M. Braun, K.N. Frayn, A. Clark, A.L. Gloyn, GLUT2 (SLC2A2) is not the principal glucose transporter in human pancreatic beta cells: implications for understanding genetic association signals at this locus, Mol. Genet. Metab. 104 (2011) 648–653.
[11] M. Hao, W.S. Head, S.C. Gunawardana, A.H. Hasty, D.W. Piston, Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction, Diabetes 56 (2007) 2328–2338.
[12] J.C. Henquin, Triggering and amplifying pathways of regulation of insulin secretion by glucose, Diabetes 49 (2000) 1751–1760.
[13] P. Rorsman, M. Braun, Regulation of insulin secretion in human pancreatic islets, Annu. Rev. Physiol. 75 (2013) 155–179.
[14] D. Bruns, R. Jahn, Molecular determinants of exocytosis, Pflugers Archiv 443 (2002) 333–338.
[15] A.T. Brunger, Structural insights into the molecular mechanism of calcium-dependent vesicle-membrane fusion, Curr. Opin. Struct. Biol. 11 (2001) 163–173.
[16] F. Xia, X. Gao, E. Kwan, P.P. Lam, L. Chan, K. Sy, L. Sheu, M.B. Wheeler, H.Y. Gaisano, R.G. Tsushima, Disruption of pancreatic beta-cell lipid rafts modifies Kv2.1 channel gating and insulin exocytosis, J. Biol. Chem. 279 (2004) 24685–24691.
[17] M. Fryirs, P.J. Barter, K.A. Rye, Cholesterol metabolism and pancreatic beta-cell function, Curr. Opin. Lipidol. 20 (2009) 159–164.
[18] A.D. Cherrington, D. Sindelar, D. Edgerton, K. Steiner, O.P. McGuinness, Physiological consequences of phasic insulin release in the normal animal, Diabetes 51 (Suppl. 1) (2002) S103–S108.
[19] J.C. Henquin, M. Nenquin, P. Stiernet, B. Ahren, In vivo and in vitro glucose-induced biphasic insulin secretion in the mouse: pattern and role of cytoplasmic Ca2+ and amplification signals in beta-cells, Diabetes 55 (2006) 441–451.
[20] A.Y. Grupping, M. Cnop, C.F. Van Schravendijk, J.C. Hannaert, T.J. Van Berkel, D.G. Pipeleers, Low density lipoprotein binding and uptake by human and rat islet beta cells, Endocrinology 138 (1997) 4064–4068.
[21] M. Ishikawa, Y. Iwasaki, S. Yatoh, T. Kato, S. Kumadaki, N. Inoue, T. Yamamoto, T. Matsuzaka, Y. Nakagawa, N. Yahagi, K. Kobayashi, A. Takahashi, N. Yamada, H. Shimano, Cholesterol accumulation and diabetes in pancreatic beta-cell-specific SREBP-2 transgenic mice: a new model for lipotoxicity, J. Lipid Res. 49 (2008) 2524–2534.
[22] M.A. Churchward, J.R. Coorssen, Cholesterol, regulated exocytosis and the physiological fusion machine, The Biochemical journal 423 (2009) 1–14.
[23] M.C. Gondre-Lewis, H.I. Petrache, C.A. Wassif, D. Harries, A. Parsegian, F.D. Porter, Y.P. Loh, Abnormal sterols in cholesterol-deficiency diseases cause secretory granule malformation and decreased membrane curvature, J. Cell Sci. 119 (2006) 1876–1885.
[24] M. Cnop, Fatty acids and glucolipotoxicity in the pathogenesis of type 2 diabetes, Biochem. Soc. Trans. 36 (2008) 348–352.
[25] F. Xia, L. Xie, A. Mihic, X. Gao, Y. Chen, H.Y. Gaisano, R.G. Tsushima, Inhibition of cholesterol biosynthesis impairs insulin secretion and voltage-gated calcium channel function in pancreatic beta-cells, Endocrinology 149 (2008) 5136–5145.
[26] J.K. Kruit, P.H. Kremer, L. Dai, R. Tang, P. Ruddle, W. de Haan, L.R. Brunham, C.B. Verchere, M.R. Hayden, Cholesterol efflux via ATP-binding cassette transporter A1 (ABCA1) and cholesterol uptake via the LDL receptor influences cholesterol-induced impairment of beta cell function in mice, Diabetologia 53 (2010) 1110–1119.
[27] L.R. Brunham, J.K. Kruit, M.R. Hayden, C.B. Verchere, Cholesterol in beta-cell dysfunction: the emerging connection between HDL cholesterol and type 2 diabetes, Current diabetes reports 10 (2010) 55–60.
[28] L.R. Brunham, J.K. Kruit, T.D. Pape, J.M. Timmins, A.Q. Reuwer, Z. Vasanji, B.J. Marsh, B. Rodrigues, J.D. Johnson, J.S. Parks, C.B. Verchere, M.R. Hayden, Beta-cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment, Nat. Med. 13 (2007) 340–347.
[29] M. Cnop, J.C. Hannaert, A.Y. Grupping, D.G. Pipeleers, Low density lipoprotein can cause death of islet beta-cells by its cellular uptake and oxidative modification, Endocrinology 143 (2002) 3449–3453.
[30] X. Lu, J. Liu, F. Hou, Z. Liu, X. Cao, H. Seo, B. Gao, Cholesterol induces pancreatic beta cell apoptosis through oxidative stress pathway, Cell Stress Chaperones 16 (2011) 539–548.
[31] Y. Kouroku, E. Fujita, I. Tanida, T. Ueno, A. Isoai, H. Kumagai, S. Ogawa, R.J. Kaufman, E. Kominami, T. Momoi, ER stress (PERK/eIF2alpha phosphorylation) mediates the polyglutamine-induced LC3 conversion, an essential step for autophagy formation, Cell Death Differ. 14 (2007) 230–239.
[32] J.S. Bogan, Y. Xu, M. Hao, Cholesterol accumulation increases insulin granule size and impairs membrane trafficking, Traffic 13 (2012) 1466–1480.
[33] A.K. Lee, V. Yeung-Yam-Wah, F.W. Tse, A. Tse, Cholesterol elevation impairs glucose-stimulated Ca(2+) signaling in mouse pancreatic beta-cells, Endocrinology 152 (2011) 3351–3361.
[34] M.D. Brand, D.G. Nicholls, Assessing mitochondrial dysfunction in cells, The Biochemical journal 435 (2011) 297–312.
[35] C. Carrasco-Pozo, K.N. Tan, M. Reyes-Farias, N. De La Jara, S.T. Ngo, D.F. Garcia-Diaz, P. Llanos, M.J. Cires, K. Borges, The deleterious effect of cholesterol and protection by quercetin on mitochondrial bioenergetics of pancreatic beta-cells, glycemic control and inflammation: in vitro and in vivo studies, Redox Biol. 9 (2016) 229–243.
[36] Y.F. Zhao, L. Wang, S. Lee, Q. Sun, Y. Tuo, Y. Wang, J. Pei, C. Chen, Cholesterol induces mitochondrial dysfunction and apoptosis in mouse pancreatic beta-cell line MIN6 cells, Endocrine 37 (2010) 76–82.
[37] E.W. Gregg, Q. Gu, Y.J. Cheng, K.M. Narayan, C.C. Cowie, Mortality trends in men and women with diabetes, 1971 to 2000, Ann. Intern. Med. 147 (2007) 149–155.
[38] M.R. Taskinen, J. Boren, New insights into the pathophysiology of dyslipidemia in type 2 diabetes, Atherosclerosis 239 (2015) 483–495.
[39] B.G. Nordestgaard, A. Varbo, Triglycerides and cardiovascular disease, Lancet 384 (2014) 626–635.

[40] A. Pirillo, G.D. Norata, A.L. Catapano, Postprandial lipemia as a cardiometabolic risk factor, Curr. Med. Res. Opin. 30 (2014) 1489–1503.

[41] G.D. Norata, L. Grigore, S. Raselli, P.M. Seccomandi, A. Hamsten, F.M. Maggi, P. Eriksson, A.L. Catapano, Triglyceride-rich lipoproteins from hypertriglyceridemic subjects induce a pro-inflammatory response in the endothelium: molecular mechanisms and gene expression studies, J. Mol. Cell. Cardiol. 40 (2006) 484–494.

[42] C. Xiao, S. Dash, C. Morgantini, R.A. Hegele, G.F. Lewis, Pharmacological targeting of the atherogenic dyslipidemia complex: the next frontier in CVD prevention beyond lowering LDL cholesterol, Diabetes 65 (2016) 1767–1778.

[43] A. American Diabetes, 9. Cardiovascular disease and risk management: standards of medical care in diabetes-2018, Diabetes Care 41 (2018) S86–S104.

[44] G.D. Norata, C.M. Ballantyne, A.L. Catapano, New therapeutic principles in dyslipidaemia: focus on LDL and Lp(a) lowering drugs, Eur. Heart J. 34 (2013) 1783–1789.

[45] P.M. Kearney, L. Blackwell, R. Collins, A. Keech, J. Simes, R. Peto, J. Armitage, C. Baigent, Efficacy of cholesterol-lowering therapy in 18,686 people with diabetes in 14 randomised trials of statins: a meta-analysis, Lancet 371 (2008) 117–125.

[46] P. Kohli, D.D. Waters, R. Nemr, B.J. Arsenault, M. Messig, D.A. DeMicco, R. Laskey, J.J.P. Kastelein, Risk of new-onset diabetes and cardiovascular risk reduction from high-dose statin therapy in pre-diabetics and non-pre-diabetics: an analysis from TNT and IDEAL, J. Am. Coll. Cardiol. 65 (2015) 402–404.

[47] D. Thakker, S. Nair, A. Pagada, V. Jamdade, A. Malik, Statin use and the risk of developing diabetes: a network meta-analysis, Pharmacoepidemiol. Drug Saf. 25 (2016) 1131–1149.

[48] M. Casula, F. Mozzanica, L. Scotti, E. Tragni, A. Pirillo, G. Corrao, A.L. Catapano, Statin use and risk of new-onset diabetes: a meta-analysis of observational studies, Nutrition, metabolism, and cardiovascular diseases 27 (2017) 396–406.

[49] S. Wang, R. Cai, Y. Yuan, Z. Varghese, J. Moorhead, X.Z. Ruan, Association between reductions in low-density lipoprotein cholesterol with statin therapy and the risk of new-onset diabetes: a meta-analysis, Sci. Rep. 7 (2017) 39982.

[50] D.I. Swerdlow, D. Preiss, K.B. Kuchenbaecker, M.V. Holmes, J.E. Engmann, T. Shah, R. Sofat, S. Stender, P.C. Johnson, R.A. Scott, M. Leusink, N. Verweij, S.J. Sharp, Y. Guo, C. Giambartolomei, C. Chung, A. Peasey, A. Amuzu, K. Li, J. Palmen, P. Howard, J.A. Cooper, F. Drenos, Y.R. Li, G. Lowe, J. Gallacher, M.C. Stewart, I. Tzoulaki, S.G. Buxbaum, A.D. van der, N.G. Forouhi, N.C. Onland-Moret, Y.T. van der Schouw, R.B. Schnabel, J.A. Hubacek, R. Kubinova, M. Baceviciene, A. Tamosiunas, A. Pajak, R. Topor-Madry, U. Stepaniak, S. Malyutina, D. Baldassarre, B. Sennblad, E. Tremoli, U. de Faire, F. Veglia, I. Ford, J.W. Jukema, R.G. Westendorp, G.J. de Borst, P.A. de Jong, A. Algra, W. Spiering, A.H. Maitland-van der Zee, O.H. Klungel, A. de Boer, P.A. Doevendans, C.B. Eaton, J.G. Robinson, D. Duggan, J. Kjekshus, J.R. Downs, A.M. Gotto, A.C. Keech, R. Marchioli, G. Tognoni, P.S. Sever, N.R. Poulter, D.D. Waters, T.R. Pedersen, P. Amarenco, H. Nakamura, J. J. McMurray, J.D. Lewsey, D.I. Chasman, P.M. Ridker, A.P. Maggioni, L. Tavazzi, K. K. Ray, S.R. Seshasai, J.E. Manson, J.F. Price, P.H. Whincup, R.W. Morris, D.A. Lawlor, G.D. Smith, Y. Ben-Shlomo, P.J. Schreiner, M. Fornage, D.S. Siscovick, M. Cushman, M. Kumari, N.J. Wareham, W.M. Verschuren, S. Redline, S.R. Patel, J.C. Whittaker, A. Hamsten, J.A. Delaney, C. Dale, T.R. Gaunt, A. Wong, D. Kuh, R. Hardy, S. Kathiresan, B.A. Castillo, P. van der Harst, E.J. Brunner, A. Tybjaerg-Hansen, M.G. Marmot, R.M. Krauss, M. Tsai, J. Coresh, R.C. Hoogeveen, B.M. Psaty, L.A. Lange, H. Hakonarson, F. Dudbridge, S.E. Humphries, P.J. Talmud, M. Kivimaki, N.J. Timpson, C. Langenberg, F.W. Asselbergs, M. Voevoda, M. Bobak, H. Pikhart, J.G. Wilson, A.P. Reiner, B.J. Keating, A.D. Hingorani, N. Sattar, HMG-coenzyme A reductase inhibition, type 2 diabetes, and bodyweight: evidence from genetic analysis and randomised trials, Lancet, 385 (2015) 351–361.

[51] A.J. Vallejo-Vaz, S.R. Kondapally Seshasai, K. Kurogi, I. Michishita, T. Nozue, S. Sugiyama, S. Tsimikas, H. Yoshida, K.K. Ray, Effect of pitavastatin on glucose, HbA1c and incident diabetes: a meta-analysis of randomized controlled clinical trials in individuals without diabetes, Atherosclerosis 241 (2015) 409–418.

[52] E.P. Navarese, A. Buffon, F. Andreotti, M. Kozinski, N. Welton, T. Fabiszak, S. Caputo, G. Grzesk, A. Kubica, I. Swiatkiewicz, A. Sukiennik, M. Kelm, S. De Servi, J. Kubica, Meta-analysis of impact of different types and doses of statins on new-onset diabetes mellitus, Am. J. Cardiol. 111 (2013) 1123–1130.

[53] A.M. Angelidi, E. Stamboliu, K.I. Adamopoulou, A.A. Kousoulis, Is Atorvastatin Associated with New Onset Diabetes or Deterioration of Glycemic Control? Systematic Review Using Data from 1.9 Million Patients, International journal of endocrinology, 2018 (2018) 8380192.

[54] D.D. Waters, J.E. Ho, S.M. Boekholdt, D.A. DeMicco, J.J. Kastelein, M. Messig, A. Breazna, T.R. Pedersen, Cardiovascular event reduction versus new-onset diabetes during atorvastatin therapy: effect of baseline risk factors for diabetes, J. Am. Coll. Cardiol. 61 (2013) 148–152.

[55] P.M. Ridker, A. Pradhan, J.G. MacFadyen, P. Libby, R.J. Glynn, Cardiovascular benefits and diabetes risks of statin therapy in primary prevention: an analysis from the JUPITER trial, Lancet 380 (2012) 565–571.

[56] B.G. Nordestgaard, M.J. Chapman, S.E. Humphries, H.N. Ginsberg, L. Masana, O.S. Descamps, O. Wiklund, R.A. Hegele, F.J. Raal, J.C. Defesche, A. Wiegman, R.D. Santos, G.F. Watts, K.G. Parhofer, G.K. Hovingh, P.T. Kovanen, C. Boileau, M. Averna, J. Boren, E. Bruckert, A.L. Catapano, J.A. Kuivenhoven, P. Pajukanta, K. Ray, A.F. Stalenhoef, E. Stroes, M.R. Taskinen, A. Tybjaerg-Hansen, Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society, Eur Heart J, 34 (2013) 3478-3490a.

[57] M.C. Vohl, D. Gaudet, S. Moorjani, G. Tremblay, P. Perron, C. Gagne, D. Lesiege, J. Bergeron, P.J. Lupien, J.P. Despres, Comparison of the effect of two low-density lipoprotein receptor class mutations on coronary heart disease among French-Canadian patients heterozygous for familial hypercholesterolaemia, Eur. J. Clin. Investig. 27 (1997) 366–373.

[58] J. Besseling, J.J. Kastelein, J.C. Defesche, B.A. Hutten, G.K. Hovingh, Association between familial hypercholesterolemia and prevalence of type 2 diabetes mellitus, Jama 313 (2015) 1029–1036.

[59] M. Mbikay, F. Sirois, J. Mayne, G.S. Wang, A. Chen, T. Dewpura, A. Prat, N.G. Seidah, M. Chretien, F.W. Scott, PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities, FEBS Lett. 584 (2010) 701–706.

[60] M. Paseban, A.E. Butler, A. Sahebkar, Mechanisms of statin-induced new-onset diabetes, J. Cell. Physiol. 234 (8) (2019) 12551–12561.

[61] I. Skoumas, C. Masoura, C. Pitsavos, D. Tousoulis, L. Papadimitriou, K. Aznaouridis, C. Chrysohoou, N. Giotsas, M. Toutouza, C. Tentolouris, C. Antoniades, C. Stefanadis, Evidence that non-lipid cardiovascular risk factors are associated with high prevalence of coronary artery disease in patients with heterozygous familial hypercholesterolemia or familial combined hyperlipidemia, Int. J. Cardiol. 121 (2007) 178–183.

[62] J. Skoumas, C. Liontou, C. Chrysohoou, C. Masoura, K. Aznaouridis, C. Pitsavos, C. Stefanadis, Statin therapy and risk of diabetes in patients with heterozygous familial hypercholesterolemia or familial combined hyperlipidemia, Atherosclerosis 237 (2014) 140–145.

[63] F. Fuentes, J.F. Alcala-Diaz, G.F. Watts, R. Alonso, O. Muniz, J.L. Diaz-Diaz, N. Mata, J.F. Sanchez Munoz-Torrero, A. Brea, J. Galiana, R. Figueras, R. Aguado, M. Piedecausa, J.M. Cepeda, J.I. Vidal, F. Rodriguez-Cantalejo, J. Lopez-Miranda, P. Mata, S. Investigators, Statins do not increase the risk of developing type 2 diabetes in familial hypercholesterolemia: the SAFEHEART study, Int. J. Cardiol. 201 (2015) 79–84.

[64] Q. Yu, Y. Chen, C.B. Xu, Statins and new-onset diabetes mellitus: LDL receptor may provide a key link, Front. Pharmacol. 8 (2017) 372.

[65] P. Chan, L. Shao, B. Tomlinson, Y. Zhang, Z.M. Liu, An evaluation of pitavastatin for the treatment of hypercholesterolemia, Expert. Opin. Pharmacother. (2018) 1–11.

[66] M. Brault, J. Ray, Y.H. Gomez, C.S. Mantzoros, S.S. Daskalopoulou, Statin treatment and new-onset diabetes: a review of proposed mechanisms, Metabolism 63 (2014) 735–745.

[67] L. Arnaboldi, A. Corsini, Could changes in adiponectin drive the effect of statins on the risk of new-onset diabetes? The case of pitavastatin, Atheroscler Suppl 16 (2015) 1–27.

[68] S. Erqou, C.C. Lee, A.I. Adler, Statins and glycaemic control in individuals with diabetes: a systematic review and meta-analysis, Diabetologia 57 (2014) 2444–2452.

[69] J.Y. Cui, R.R. Zhou, S. Han, T.S. Wang, L.Q. Wang, X.H. Xie, Statin therapy on glycemic control in type 2 diabetic patients: a network meta-analysis, J. Clin. Pharm. Ther. 43 (2018) 556–570.

[70] S. Simsek, C.G. Schalkwijk, B.H. Wolffenbuttel, Effects of rosuvastatin and atorvastatin on glycaemic control in type 2 diabetes—the CORALL study, Diabetic medicine 29 (2012) 628–631.

[71] T. Takano, T. Yamakawa, M. Takahashi, M. Kimura, A. Okamura, Influences of statins on glucose tolerance in patients with type 2 diabetes mellitus, J. Atheroscler. Thromb. 13 (2006) 95–100.

[72] S.M. Liew, P.Y. Lee, N.S. Hanafi, C.J. Ng, S.S. Wong, Y.C. Chia, P.S. Lai, N.F. Zaidi, E.M. Khoo, Statins use is associated with poorer glycaemic control in a cohort of hypertensive patients with diabetes and without diabetes, Diabetology & metabolic syndrome 6 (2014) 53.

[73] A. Pirillo, A.L. Catapano, G.D. Norata, Niemann-pick C1-like 1 (NPC1L1) inhibition and cardiovascular diseases, Curr. Med. Chem. 23 (2016) 983–999.

[74] S.J. Yang, J.M. Choi, L. Kim, B.J. Kim, J.H. Sohn, W.J. Kim, S.E. Park, E.J. Rhee, W.Y. Lee, K.W. Oh, S.W. Park, S.W. Kim, C.Y. Park, Chronic administration of ezetimibe increases active glucagon-like peptide-1 and improves glycemic control and pancreatic beta cell mass in a rat model of type 2 diabetes, Biochem. Biophys. Res. Commun. 407 (2011) 153–157.

[75] Y. Zhong, J. Wang, P. Gu, J. Shao, B. Lu, S. Jiang, Effect of ezetimibe on insulin secretion in db/db diabetic mice, Exp. Diabetes Res. 2012 (2012) 420854.

[76] H. Wu, H. Shang, J. Wu, Effect of ezetimibe on glycemic control: a systematic review and meta-analysis of randomized controlled trials, Endocrine 60 (2018) 229–239.

[77] R.P. Giugliano, C.P. Cannon, M.A. Blazing, J.C. Nicolau, R. Corbalan, J. Spinar, J.G. Park, J.A. White, E.A. Bohula, E. Braunwald, I.-I. Investigators, Benefit of adding ezetimibe to statin therapy on cardiovascular outcomes and safety in patients with versus without diabetes mellitus: results from IMPROVE-IT (improved reduction of outcomes: vytorin efficacy international trial), Circulation 137 (2018) 1571–1582.

[78] L.A. Lotta, S.J. Sharp, S. Burgess, J.R.B. Perry, I.D. Stewart, S.M. Willems, J. Luan, E. Ardanaz, L. Arriola, B. Balkau, H. Boeing, P. Deloukas, N.G. Forouhi, P.W. Franks, S. Grioni, R. Kaaks, T.J. Key, C. Navarro, P.M. Nilsson, K. Overvad, D. Palli, S. Panico, J.R. Quiros, E. Riboli, O. Rolandsson, C. Sacerdote, E.C. Salamanca, N. Slimani, A.M. Spijkerman, A. Tjonneland, R. Tumino, A.D. van der, Y.T. van der Schouw, M.I. McCarthy, I. Barroso, S. O'Rahilly, D.B. Savage, N. Sattar, C. Langenberg, R.A. Scott, N.J. Wareham, Association Between Low-Density Lipoprotein Cholesterol-Lowering Genetic Variants and Risk of Type 2 Diabetes: A Meta-analysis, Jama, 316 (2016) 1383–1391.

[79] N.G. Seidah, A. Prat, A. Pirillo, A.L. Catapano, G.D. Norata, Novel strategies to target PCSK9: beyond monoclonal antibodies, Cardiovasc. Res. 115 (3) (2019) 510–518.

[80] G.D. Norata, G. Tibolla, A.L. Catapano, Targeting PCSK9 for hypercholesterolemia, Annu. Rev. Pharmacol. Toxicol. 54 (2014) 273–293.

[81] M.S. Sabatine, R.P. Giugliano, A.C. Keech, N. Honarpour, S.D. Wiviott, S.A. Murphy, J.F. Kuder, H. Wang, T. Liu, S.M. Wasserman, P.S. Sever, T.R. Pedersen, F.S. Committee, Investigators, Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease, N Engl J Med 376 (2017) 1713–1722.

[82] G.G. Schwartz, P.G. Steg, M. Szarek, D.L. Bhatt, V.A. Bittner, R. Diaz, J.M. Edelberg, S.G. Goodman, C. Hanotin, R.A. Harrington, J.W. Jukema, G. Lecorps, K.W. Mahaffey, A. Moryusef, R. Pordy, K. Quintero, M.T. Roe, W.J. Sasiela, J.F. Tamby, P. Tricoci, H.D. White, A.M. Zeiher, O.O. Committees, Investigators, Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome, N Engl J Med 379 (2018) 2097–2107.

[83] G.D. Norata, H. Tavori, A. Pirillo, S. Fazio, A.L. Catapano, Biology of proprotein convertase subtilisin kexin 9: beyond low-density lipoprotein cholesterol lowering, Cardiovasc. Res. 112 (2016) 429–442.

[84] C. Langhi, C. Le May, V. Gmyr, B. Vandewalle, J. Kerr-Conte, M. Krempf, F. Pattou, P. Costet, B. Cariou, PCSK9 is expressed in pancreatic delta-cells and does not alter insulin secretion, Biochem. Biophys. Res. Commun. 390 (2009) 1288–1293.

[85] L. Da Dalt, M. Ruscica, F. Bonacina, G. Balzarotti, A. Dhyani, E. Di Cairano, A. Baragetti, L. Arnaboldi, S. De Metrio, F. Pellegatta, L. Grigore, M. Botta, C. Macchi, P. Uboldi, C. Perego, A.L. Catapano, G.D. Norata, PCSK9 deficiency reduces insulin secretion and promotes glucose intolerance: the role of the low-density lipoprotein receptor, Eur. Heart J. 40 (2019) 357–368.

[86] B.A. Ference, J.G. Robinson, R.D. Brook, A.L. Catapano, M.J. Chapman, D.R. Neff, S. Voros, R.P. Giugliano, G. Davey Smith, S. Fazio, M.S. Sabatine, Variation in PCSK9 and HMGCR and risk of cardiovascular disease and Diabetes, N. Engl. J. Med. 375 (2016) 2144–2153.

[87] A.F. Schmidt, D.I. Swerdlow, M.V. Holmes, R.S. Patel, Z. Fairhurst-Hunter, D.M. Lyall, F.P. Hartwig, B.L. Horta, E. Hypponen, C. Power, M. Moldovan, E. van Iperen, G.K. Hovingh, I. Demuth, K. Norman, E. Steinhagen-Thiessen, J. Demuth, L. Bertram, T. Liu, S. Coassin, J. Willeit, S. Kiechl, K. Willeit, D. Mason, J. Wright, R. Morris, G. Wanamethee, P. Whincup, Y. Ben-Shlomo, S. McLachlan, J.F. Price, M. Kivimaki, C. Welch, A. Sanchez-Galvez, P. Marques-Vidal, A. Nicolaides, A.G. Panayiotou, N.C. Onland-Moret, Y.T. van der Schouw, G. Matullo, G. Fiorito, S. Guarrera, C. Sacerdote, N.J. Wareham, C. Langenberg, R. Scott, J. Luan, M. Bobak, S. Malyutina, A. Pajak, R. Kubinova, A. Tamosiunas, H. Pikhart, L.L. Husemoen, N. Grarup, O. Pedersen, T. Hansen, A. Linneberg, K.S. Simonsen, J. Cooper, S.E. Humphries, M. Brilliant, T. Kitchner, H. Hakonarson, D.S. Carrell, C.A. McCarty, H. L. Kirchner, E.B. Larson, D.R. Crosslin, M. de Andrade, D.M. Roden, J.C. Denny, C. Carty, S. Hancock, J. Attia, E. Holliday, M. O'Donnell, S. Yusuf, M. Chong, G. Pare, P. van der Harst, M.A. Said, R.N. Eppinga, N. Verweij, H. Snieder, T. Christen, D.O. Mook-Kanamori, S. Gustafsson, L. Lind, E. Ingelsson, R. Pazoki, O. Franco, A. Hofman, A. Uitterlinden, A. Dehghan, A. Teumer, S. Baumeister, M. Dorr, M.M. Lerch, U. Volker, H. Volzke, J. Ward, J.P. Pell, D.J. Smith, T. Meade, A.H. Maitland-van der Zee, E.V. Baranova, R. Young, I. Ford, A. Campbell, S. Padmanabhan, M.L. Bots, D.E. Grobbee, P. Froguel, D. Thuillier, B. Balkau, A. Bonnefond, B. Cariou, M. Smart, Y. Bao, M. Kumari, A. Mahajan, P.M. Ridker, D.I. Chasman, A.P. Reiner, L.A. Lange, M.D. Ritchie, F.W. Asselbergs, J.P. Casas, B.J. Keating, D. Preiss, A.D. Hingorani, N. Sattar, PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study, Lancet Diabetes Endocrinol, 5 (2017) 97–105.

[88] Y.X. Cao, H.H. Liu, Q.T. Dong, S. Li, J.J. Li, Effect of proprotein convertase subtilisin/kexin type 9 (PCSK9) monoclonal antibodies on new-onset diabetes mellitus and glucose metabolism: a systematic review and meta-analysis, Diabetes Obes. Metab. 20 (2018) 1391–1398.

[89] L.S.F. de Carvalho, A.M. Campos, A.C. Sposito, Proprotein convertase Subtilisin/Kexin type 9 (PCSK9) inhibitors and incident type 2 Diabetes: a systematic review and meta-analysis with over 96,000 patient-years, Diabetes Care 41 (2018) 364–367.

[90] M.S. Sabatine, L.A. Leiter, S.D. Wiviott, R.P. Giugliano, P. Deedwania, G.M. De Ferrari, S.A. Murphy, J.F. Kuder, I. Gouni-Berthold, B.S. Lewis, Y. Handelsman, A.L. Pineda, N. Honarpour, A.C. Keech, P.S. Sever, T.R. Pedersen, Cardiovascular safety and efficacy of the PCSK9 inhibitor evolocumab in patients with and without diabetes and the effect of evolocumab on glycaemia and risk of new-onset diabetes: a prespecified analysis of the FOURIER randomised controlled trial, Lancet Diabetes Endocrinol, 5 (2017) 941–950.

[91] R.P. Giugliano, T.R. Pedersen, J.G. Park, G.M. De Ferrari, Z.A. Gaciong, R. Ceska, K. Toth, I. Gouni-Berthold, J. Lopez-Miranda, F. Schiele, F. Mach, B.R. Ott, E. Kanevsky, A.L. Pineda, R. Somaratne, S.M. Wasserman, A.C. Keech, P.S. Sever, M.S. Sabatine, F. Investigators, Clinical efficacy and safety of achieving very low LDL-cholesterol concentrations with the PCSK9 inhibitor evolocumab: a pre-specified secondary analysis of the FOURIER trial, Lancet 390 (2017) 1962–1971.

[92] M.F. van Stee, A.A. de Graaf, A.K. Groen, Actions of metformin and statins on lipid and glucose metabolism and possible benefit of combination therapy, Cardiovasc. Diabetol. 17 (2018) 94.

[93] S.N. Rajpathak, D.J. Kumbhani, J. Crandall, N. Barzilai, M. Alderman, P.M. Ridker, Statin therapy and risk of developing type 2 diabetes: a meta-analysis, Diabetes Care 32 (2009) 1924–1929.

[94] N. Sattar, D. Preiss, H.M. Murray, P. Welsh, B.M. Buckley, A.J. de Craen, S.R. Seshasai, J.J. McMurray, D.J. Freeman, J.W. Jukema, P.W. Macfarlane, C.J. Packard, D.J. Stott, R.G. Westendorp, J. Shepherd, B.R. Davis, S.L. Pressel, R. Marchioli, R.M. Marfisi, A.P. Maggioni, L. Tavazzi, G. Tognoni, J. Kjekshus, T.R. Pedersen, T.J. Cook, A.M. Gotto, M.B. Clearfield, J.R. Downs, H. Nakamura, Y. Ohashi, K. Mizuno, K.K. Ray, I. Ford, Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials, Lancet, 375 (2010) 735–742.

[95] E.J. Mills, P. Wu, G. Chong, I. Ghement, S. Singh, E.A. Akl, O. Eyawo, G. Guyatt, O. Berwanger, M. Briel, Efficacy and safety of statin treatment for cardiovascular disease: a network meta-analysis of 170,255 patients from 76 randomized trials, QJM 104 (2011) 109–124.

[96] D. Preiss, S.R. Seshasai, P. Welsh, S.A. Murphy, J.E. Ho, D.D. Waters, D.A. DeMicco, P. Barter, C.P. Cannon, M.S. Sabatine, E. Braunwald, J.J. Kastelein, J.A. de Lemos, M.A. Blazing, T.R. Pedersen, M.J. Tikkanen, N. Sattar, K.K. Ray, Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy: a meta-analysis, Jama 305 (2011) 2556–2564.