Cholesterol-enriched membrane rafts and insulin secretion
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ABSTRACT
The failure of pancreatic β-cells to supply insulin in quantities sufficient to maintain euglycemia is a hallmark of type 2 diabetes. Perturbation of β-cell cholesterol homeostasis, culminating in elevated intracellular cholesterol levels, impairs insulin secretion and has therefore been proposed as a mechanism contributing to β-cell dysfunction. The manner in which this occurs, however, is unclear. Cholesterol is an essential lipid, as well as a major component of membrane rafts, and numerous proteins critical for the regulation of insulin secretion have been reported to associate with these domains. Although this suggests that alterations in membrane rafts could partially account for the reduction in insulin secretion observed when β-cell cholesterol accumulates, this has not yet been demonstrated. In this review, we provide a brief overview of recent work implicating membrane rafts in some of the basic molecular mechanisms of insulin secretion, and discuss the insight it provides into the β-cell dysfunction characteristic of type 2 diabetes. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2012.00200.x, 2012)

KEY WORDS: Cholesterol, Pancreatic β-cell, Type 2 diabetes

INTRODUCTION
The efficient regulation of circulating glucose levels is critical for proper health. This process requires insulin, a peptide hormone that lowers glycemia by triggering the uptake of glucose into cells. As the exclusive source of insulin, pancreatic β-cells play an essential role in glucose homeostasis. They are responsible for the production, storage and release of insulin in a manner that is strictly coupled to demand. The molecular mechanisms and dynamics of β-cell secretion have been widely described1,2. What is clear is that glucose-stimulated insulin secretion (GSIS) is a complex and tightly regulated process, the disruption of which has severe consequences. Evidence of this can be seen in diabetes, a widespread metabolic disease resulting from the failure of β-cells to meet the physiological requirements for insulin. In type 1 diabetes, this is caused by the autoimmune-mediated destruction of β-cells3. In type 2 diabetes, β-cells fail to adequately compensate for the insulin resistance often ensuing in conditions such as obesity4. Early β-cell dysfunction, characterized by impaired GSIS, is considered fundamental in this process5.

The deficits in GSIS observed in type 2 diabetes have been attributed to numerous factors. One receiving significant attention recently is altered β-cell cholesterol homeostasis6. Cholesterol is an essential constituent of mammalian cell membranes, which influences their fluidity, permeability and curvature7. It is also a major component of membrane rafts – dynamic nanoscale assemblies of sterols, sphingolipids and proteins that can be stabilized to coalesce into platforms that facilitate various cellular processes8. These domains are now widely considered to participate in the regulation of GSIS, a conclusion based primarily on two observations. First, numerous proteins involved in either GSIS or the biogenesis of secretory granules (SGs), the intracellular storage organelles from which insulin is secreted, have been shown to partition with detergent-resistant membranes (DRMs) in the light fractions of sucrose density gradients after solubilization at 4°C with Triton X-100 or other non-ionic detergents. Second, perturbation of membrane cholesterol often induced a repartitioning of these proteins that corresponded with alterations in their function, as well as in insulin secretion. These results have prompted speculation that changes in membrane rafts, triggered by altered cellular cholesterol homeostasis, contribute to the impaired GSIS in type 2 diabetes. Here we provide a brief overview of the work linking membrane rafts and GSIS, and the insight it provides into β-cell dysfunction.

CHOLESTEROL HOMEOSTASIS AND β-CELL DYSFUNCTION
Recent studies in mice have emphasized the importance of β-cell cholesterol homeostasis for insulin secretion. Disruption of cholesterol efflux through knockout of the cholesterol transporter adenosine triphosphate (ATP)-binding cassette transporter A1 (ABCA1) selectively in β-cells resulted in the accumulation of islet cholesterol, as well as glucose intolerance and impaired GSIS9. This was in agreement with the previous observation that knockout of liver X receptor β (LXRβ), a
nuclear hormone receptor that upregulates ABCA1 expression in response to cholesterol, caused β-cell dysfunction. Mice lacking the cholesterol acceptor apolipoprotein E (APOE) also showed elevated islet cholesterol levels and reduced insulin secretion. Unlike Abca1−/− mice, however, circulating cholesterol levels in Apoe−/− mice were elevated, suggesting a correlation between hypercholesterolemia and β-cell dysfunction. Knockout of the low density lipoprotein receptor (LDLr), which facilitates cholesterol uptake, also triggered hypercholesterolemia, although its impact on β-cell function was inconsistent. One group observed elevated islet cholesterol levels, impaired glucose tolerance and reduced GSIS in these mice, whereas another reported no significant alterations in islet cholesterol or β-cell function. Despite this discrepancy, the trend emerging from these and other studies is that cholesterol accumulation in β-cells impairs insulin secretion. Work investigating the role of membrane rafts in GSIS has identified numerous stages at which this might occur.

**GLUCOSE UPTAKE AND METABOLISM**

Glycemia is normally maintained at concentrations of ~5 mmol/L, and increases above this value trigger glucose uptake in β-cells. In rodents, this is facilitated primarily by glucose transporter (GLUT)2, whereas in humans GLUT1 is the predominant glucose transporter. Although there is little direct evidence for the association of these transporters with membrane rafts in β-cells, GLUT1 partitioned with DRMs in various other cell types. In addition, cholesterol depletion of liver-derived clone 9 cells with methyl-β-cyclodextrin (MβCD), which extracts cholesterol from the plasma membrane, disrupted the raft partitioning of GLUT1 and enhanced glucose transport. Interestingly, Ldlr−/− islets with elevated cholesterol showed reduced glucose uptake, as did cholesterol-loaded primary β-cells. However, the possibility that this represented a membrane raft-dependent effect on glucose transporter activity was not investigated.

After its uptake, glucose is phosphorylated to generate glucose-6-phosphate. This critical rate-limiting step in glucose metabolism is catalyzed by glucokinase (GCK), the primary glucose sensor of β-cell metabolism. This critical rate-limiting step in glucose metabolism inactivate ATP-sensitive K⁺ (KATP) channels, causing membrane depolarization. This triggers Ca²⁺ influx through voltage-gated Ca²⁺ (Caᵥ) channels and subsequent soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated fusion of SGs. Depolarization also activates voltage-gated K⁺ (Kᵥ) channels, which act to re-establish the resting membrane potential and thus suppress insulin secretion.

The inwardly rectifying K⁺ (Kᵃ) channel 6.2 and its associated sulphonylurea receptor (SUR)1 constitute the main Kᵃᵥ channel responsible for glucose-induced depolarization of β-cells. While Kᵃᵥ6.2 associated with membrane rafts in cardiomyocytes, Kᵃᵥ6.2 and SUR1 were excluded from raft fractions in HIT-T15 insulinoma cells. However, Kᵃᵥ2.1 and Caᵥ1.2, and the SNARE proteins syntaxin 1A, synaptosomal-associated protein 25 (SNAP-25) and vesicle-associated membrane protein 2 (VAMP-2) were all partially associated with membrane rafts (Figure 1b). MβCD, which caused their redistribution out of raft fractions, increased GSIS, a result which capacitance measurements indicated was due, in part, to enhanced refilling of the readily releasable SG pool. Kᵃᵥ channel current amplitude was reduced in depleted cells, suggesting that membrane raft disruption promoted insulin secretion by hindering repolarization. In this context, it is noteworthy that SNARE proteins, in addition to their fundamental role in granule fusion, have also been reported to regulate Kᵃᵥ, Kᵥ and Caᵥ channels. Thus, membrane rafts appear to contribute to the modulation of β-cell excitability, either by directly regulating channel activity or by spatially coordinating SNARE-coupled channel gating.

**β-CELL CHANNEL FUNCTION AND SECRETORY GRANULE EXOCYTOSIS**

The enhanced intracellular ATP levels resulting from glucose metabolism inactivate Kᵥ².⁰ (Kᵥ².⁰) channels, causing membrane depolarization. This triggers Ca²⁺ influx through voltage-gated Ca²⁺ (Caᵥ) channels and subsequent soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated fusion of SGs. Depolarization also activates voltage-gated K⁺ (Kᵥ) channels, which act to re-establish the resting membrane potential and thus suppress insulin secretion.

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Upregulated GSIS and enhanced depolarization were also observed in MβCD-treated INS-1 cells, as was increased cortical F-actin remodeling. Cholesterol overloading, in contrast, impaired secretion, depolarization and remodeling. These results were attributed to altered membrane phosphatidylinositol 4,5-bisphosphate (PIP2) levels, as MβCD increased PIP₂.
Membrane rafts and insulin secretion

Figure 1 | Insulin secretion – a membrane raft perspective. In pancreatic β-cells, glucose uptake and metabolism triggers membrane depolarization. This initiates Ca\textsuperscript{2+} influx, leading to soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated secretory granule (SG) fusion and insulin secretion. Sphingolipid- and cholesterol-enriched membrane rafts (highlighted in red) have been proposed to regulate various aspects of glucose-stimulated insulin secretion including (a) glucose metabolism, and the secretion-dependent translocation and activation of a SG-associated pool of glucokinase (GCK); (b) K\textsubscript{ATP}, Ca\textsubscript{v}, and K\textsubscript{v} channel activity, SNARE-mediated SG fusion and its spatial coupling to Ca\textsuperscript{2+} entry, fusion pore formation, and the syntaxin-mediated regulation of channel gating; and (c) targeting and retention of prohormones, their processing enzymes, and granins in the cholesterol-enriched membranes of newly forming SG. Ca\textsubscript{v}, voltage-gated Ca\textsuperscript{2+}; CGA, chromogranin A; CPE, carboxypeptidase E; GLUT, glucose transporter; K\textsubscript{ATP}, adenosine triphosphate-sensitive K\textsuperscript{+}; K\textsubscript{i}, inwardly rectifying K\textsuperscript{+}; K\textsubscript{v}, voltage-gated K\textsuperscript{+}; SUR, sulphonylurea receptor; nNOS, neuronal nitric oxide synthase; IA-2, insulinoma-associated protein 2; IA-2B, insulinoma-associated protein 2B; ICAS12, islet cell autoantigen 512; PC1/3, prohormone convertase 1/3; PC2, prohormone convertase 2; PTPRN, protein tyrosine phosphatase, receptor type N; PTPRN2, protein tyrosine phosphatase, receptor type N polypeptide 2; SGII, secretogranin II; SNAP-25, synaptosomal-associated protein 25; VAMP-2, vesicle-associated membrane protein 2.

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Journal of Diabetes Investigation Volume 3 Issue 4 August 2012

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hydrolysis whereas cholesterol loading appeared to enhance membrane PIP\textsubscript{2} levels and disrupt glucose-dependent hydrolysis\textsuperscript{27}. PIP\textsubscript{2} is an important regulator of insulin secretion. Its accumulation impaired insulin secretion by reducing the sensitivity of K\textsubscript{ATP} channels to ATP and stabilizing the cortical F-actin cytoskeleton\textsuperscript{28,29}. This work suggests that the effects of cholesterol accumulation on GSIS are coupled in part through PIP\textsubscript{2}. Do membrane rafts play a role in this process? Possibly,
as Triton X-100 insoluble raft fractions were reportedly enriched in PIP$_2$. However, it is still unclear how this might influence glucose-dependent PIP$_2$ hydrolysis or the regulation of K$_{ATP}$ channels comprised of K$_v$.6.2 and SUR1, both of which appear to be excluded from membrane rafts in β-cells.

In contrast to the observations that cholesterol depletion enhances GSIS, further work by Xia et al. indicated that the inhibition of cholesterol biosynthesis in MIN6 insulinoma cells, which also disrupted the raft partitioning of K$_v$.1.2, K$_v$.2.1 and SNARE proteins, impaired GSIS. In this case, Ca$_v$, K$_v$ and K$_{ATP}$ channel activity were all reduced. Similar results were reported in β-cells after cholesterol overloading, suggesting that these channels are sensitive to any deviations from optimal cholesterol levels, regardless of direction. As reducing K$_v$ and K$_{ATP}$ channel activity promotes secretion, the component of impaired GSIS resulting from altered channel function was attributed in these studies to the observed deficit in Ca$_v$ channel activity.

Alterations of the membrane lipid environment also disrupted insulin secretion by dispersing Ca$_{2+}$ influx independent of changes in channel activity or SG release competence. This spatial uncoupling of Ca$_{2+}$ influx and SGs has been proposed to hinder fusion pore formation, thus reducing the frequency of full fusion events and the efficiency of insulin release. A similar effect could partly explain the decreased GSIS resulting from cholesterol depletion. However, a direct disruption of exocytosis was also demonstrated in cholesterol-depleted islet cells. In addition, reduced SG exocytosis, detected in the absence of altered channel function, was a primary deficit in the β-cells of Abca1$^{-/-}$ islets. This indicated that altered membrane cholesterol levels likely impaired SNARE function.

The core SNARE fusion complex is comprised of the target (t)-SNAREs syntaxin 1A, and SNAP-25 on the plasma membrane and the vesicle (v)-SNARE VAMP-2 on vesicles. As previously described in neurons, cholesterol-sensitive syntaxin 1 and SNAP-25 define clusters where SGs preferentially dock and fuse. In islet cells, MBlCD dispersed syntaxin 1 and induced a redistribution of SNAP-25 to the cytosol, both of which were accompanied by reductions in the number of docked SGs and GSIS. Interestingly, diffusion of syntaxin 1A from granule docking sites and reduced GSIS were also observed in INS-1 cells cultured in high glucose, a condition that down-regulated cholesterol biosynthesis and disrupted syntaxin 1A-containing membrane rafts.

These results suggest that membrane rafts promote SNARE clustering and fusion. However, studies demonstrating cholesterol-dependent t-SNARE clustering did not always detect SNARE proteins in membrane raft fractions. In addition, syntaxin 1A clustering was detected in proteoliposomes that did not support the formation of raft-like liquid ordered (L$_n$) domains. When β-cell SNARE proteins did partition with DRMs, a substantial pool was also detected in soluble fractions. In neuroendocrine cells, cross-linked syntaxin 1A and SNAP-25 complexes partitioned in both raft and non-raft fractions. More recently, these proteins have been shown to form two conformationally distinct and spatially segregated t-SNARE dimer intermediates, only one of which supports fusion. After cholesterol depletion, only the fusion competent form was detected. In addition, cross-linked complexes containing syntaxin 1A and the Sec1/Munc18-like (SM) protein mammalian uncoordinated 18 (Munc-18), an accessory protein essential for secretion that participates in multiple stages of SNARE assembly and fusion, were restricted to non-raft fractions. Although these observations suggest that SNARE assembly and fusion might proceed in non-raft membrane regions, more work is required to understand the role of membrane rafts in the multiple, and often spatially and temporally distinct, stages of SNARE assembly and function.

**GRANULE BIOGENESIS: CHOLESTEROL AND SECRETORY GRANULE MEMBRANE INTEGRITY**

Proper SG biogenesis is an obvious prerequisite for secretion, and cholesterol, which is highly enriched in the granule membrane, is critical for their formation and integrity. In neuroendocrine cells, disruption of cholesterol biosynthesis with lovastatin, a 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitor, blocked the formation of granules from the trans-Golgi network (TGN) and caused swelling of the TGN cisterna. In MIN6 cells, lovastatin decreased SG numbers while increasing their average size, and this corresponded with reduced insulin content and impaired regulated secretory response.

SG malformation was also observed in two mouse models with deficient cholesterol production. Disruption of either lanosterol-5-desaturase (SCS5) or 7-dehydrocholesterol reductase (DHCR7), the enzymes responsible for the last two steps in cholesterol synthesis, decreased pancreatic exocrine and endocrine SG numbers, and increased granule size. Studies in model membranes demonstrated that lanosterol enhanced membrane flexibility while reducing intrinsic membrane curvature relative to cholesterol, suggesting that the altered SG biogenesis in Sc5d$^{-/-}$ mice resulted from the accumulation of lanosterol in granule membranes. Cholesterol accumulation in Abca1$^{-/-}$ islets was also suggested to disrupt SG biogenesis, a conclusion based on observations that β-cell Golgi ultrastructure was altered and circulating proinsulin levels were enhanced. However, although the insulin SGs of these mice were described to be ‘heterogeneous with respect to mean diameter’ compared with controls, no significant changes in SG numbers, size or distribution were observed.

Enlarged SGs and impaired GSIS downstream of depolarization were also observed in islets and insulinoma cells lacking the cholesterol transporter ATP-binding cassette transporter G1 (ABCG1). In β-cells, ABCG1 was reportedly expressed on SGs, and its loss led to reductions in SG cholesterol levels without altering total cellular or circulating cholesterol levels. Therefore, ABCG1 has been proposed to maintain SG integrity by facilitating the retention of cholesterol within the granule inner...
membrane leaflet, thus counteracting the carrier-mediated diffusion of cholesterol from the SG outer membrane leaflet.\textsuperscript{45} Granule membranes account for a large portion of total β-cell cholesterol. In addition to reducing SG membrane integrity, the dispersal of this pool could alter raft-dependent processes elsewhere – a possibility supported by the observation that MjCD extracted more cholesterol from cells lacking ABCG1.\textsuperscript{46} This apparent redistribution of cholesterol to the plasma membrane might explain the late-stage deficit in secretion (i.e. downstream of Ca\textsuperscript{2+} influx) detected in these islets, although it is tempting to speculate that a corresponding reduction of SG membrane cholesterol might have also perturbed raft-associated SG proteins, such as VAMP-2. Whatever the case, this work suggests a unique mechanism of β-cell dysfunction that could be of particular interest given the observation that ABCG1 expression is reduced in diabetic mice.\textsuperscript{45}

### GRANULE BIOGENESIS: PROTEIN SORTING TO SECRETORY GRANULES

Biogenesis of SGs requires proper sorting of cargo proteins to immature secretory granules. Although the mechanisms governing this process are still debated,\textsuperscript{46} membrane rafts appear to play a role (Figure 1c). Prohormone convertase 1/3 (PC1/3) and 2 (PC2), two cargo endoproteases responsible for the conversion of proinsulin to insulin during SG maturation, both partitioned with DRMs in neuroendocrine cells.\textsuperscript{47,48} In addition, PC1/3 associated with the luminal leaflet of SGs in a cholesterol-sensitive manner,\textsuperscript{49} and deletion of its membrane-binding domain led to mis-sorting of PC1/3 and its constitutive release.\textsuperscript{49} The membrane raft association of PC2, in contrast, appeared to be sphingolipid-dependent. Inhibition of sphingolipid synthesis with fumonisin blocked its targeting to SGs,\textsuperscript{49} as did truncation of its C-terminal raft-binding domain.\textsuperscript{51}

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#### Table: Cholesterol Redistribution

**Reduced Cholesterol (MjCD)**

- Raft Association of Ca\textsubscript{1.2}, K\textsubscript{v} 2.1, Syntaxin 1A, SNAP-25, VAMP-2 (↓)\textsuperscript{25}
- K\textsubscript{v} Channel Activity (↓)\textsuperscript{25}
- Exocytosis (↓)\textsuperscript{25}
- Depolarization (↓)\textsuperscript{27}
- Cortical Actin Reorganization (↓)\textsuperscript{27}
- PIP\textsubscript{2} Hydrolysis (↓)\textsuperscript{27}
- Ga\textsubscript{4} Channel Activity (↓)\textsuperscript{34}
- Membrane Association of SNAP-25 (↓)\textsuperscript{34}
- Exocytosis (↓)\textsuperscript{34}
- Syntaxin 1 Clustering (↓)\textsuperscript{37}
- Docked SGs (↓)\textsuperscript{34,37}

**Elevated Cholesterol**

- Glucose Uptake & Oxidation (↑)\textsuperscript{13}
- Exocytosis (↑)\textsuperscript{15}

**Expansion of Cholesterol Homeostasis and Glucose-Stimulated Insulin Secretion (GSIS)**

| **Reduced Cholesterol (MjCD)** | **Elevated Cholesterol** |
|--------------------------------|-------------------------|
| Cholesterol Depletion (MjCD)  | Cholesterol Overloading (Incubation with soluble cholesterol) |
| - Raft Association of: Ca\textsubscript{1.2}, K\textsubscript{v} 2.1, Syntaxin 1A, SNAP-25, VAMP-2 (↓)\textsuperscript{25} | - Glucose Uptake (↑)\textsuperscript{16} |
| - K\textsubscript{v} Channel Activity (↓)\textsuperscript{25} | - Mitochondrial Metabolism (↑)\textsuperscript{16} |
| - Exocytosis (↓)\textsuperscript{25} | - Ca\textsubscript{1.2}, K\textsubscript{ATP}, K\textsubscript{v} Channel Activity (↑)\textsuperscript{16} |
| - Depolarization (↓)\textsuperscript{27} | - Depolarization (↓)\textsuperscript{27} |
| - Cortical Actin Reorganization (↓)\textsuperscript{27} | - Cortical Actin Reorganization (↓)\textsuperscript{27} |
| - PIP\textsubscript{2} Hydrolysis (↓)\textsuperscript{27} | - PIP\textsubscript{2} Hydrolysis (↑)\textsuperscript{27} |
| - Ga\textsubscript{4} Channel Activity (↓)\textsuperscript{34} | - GCK Translocation & Activation (↑)\textsuperscript{11} |
| - Membrane Association of SNAP-25 (↓)\textsuperscript{34} | |
| - Exocytosis (↓)\textsuperscript{34} | |
| - Syntaxin 1 Clustering (↓)\textsuperscript{37} | |
| - Docked SGs (↓)\textsuperscript{34,37} | |

**Disruption of Cholesterol Biosynthesis**

(NB598: squalene epoxidase inhibitor)

- DRM association of Ca\textsubscript{1.2}, K\textsubscript{v} 2.1, Syntaxin 1A, SNAP-25, VAMP-2 (↓)\textsuperscript{31}
- Ca\textsubscript{1.2}, K\textsubscript{ATP}, K\textsubscript{v} Channel Activity (↓)\textsuperscript{31}
- Exocytosis (↓)\textsuperscript{31}

| Hyperglycemia |
|---------------|
| - Syntaxin 1A Clustering (↑)\textsuperscript{38} |
| - DRM Association of Syntaxin 1A (↑)\textsuperscript{38} |

**Cholesterol RedISTRIBUTION (Abca1–/– mice)**

- Enlarged SG Morphology\textsuperscript{43,44}
- SG Number (↑)\textsuperscript{43,44}
- (Incubation with soluble cholesterol)

**Cholesterol Accumulation**

(Sc5d–/– mice)

- Ldlr–/– mice
- Altered Golgi Morphology\textsuperscript{35}
- Exocytosis (↑)\textsuperscript{15}
- Glucose Uptake & Oxidation (↑)\textsuperscript{13}

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\textsuperscript{43}The deficit in regulated secretion associated with the cassette transporter G1; DRM, detergent resistant membrane; GCK, glucokinase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; K\textsubscript{v}, adenosine triphosphate-binding cassette transporter G1; DRM, detergent resistant membrane; GCK, glucokinase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; K\textsubscript{v}, adenosine triphosphate-sensitive K\textsuperscript{+} channel; K\textsubscript{ATP}, voltage-gated K\textsuperscript{+} channel; Ldlr, low density lipoprotein receptor; MjCD, methyl-β-cyclodextrin; PIP\textsubscript{2}, phosphatidylinositol 4,5-bisphosphate; Sc5d, lanthosterol-5-desaturase; SNAP-25, synaptosomal-associated protein 25; VAMP-2, vesicle-associated membrane protein 2.

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Figure 2 | Cholesterol homeostasis and glucose-stimulated insulin secretion (GSIS). A summary of the reported effects of cholesterol perturbation in pancreatic islets, β-cells or insulinoma cells. Arrows indicate increase (↑) or decrease (↓) in the described effect. Those effects associated with enhanced or reduced GSIS are listed in green or red, respectively. Instances of cholesterol-sensitive repartitioning of raft-associated proteins are highlighted in yellow. Superscripted numbers: references. Except as noted below, exocytosis is listed only when observed directly (i.e. changes in Ca\textsuperscript{2+}-dependent exocytotic response monitored by capacitance measurements taken independent of voltage-gated Ca\textsuperscript{2+} channel activity). *The deficit in regulated secretion associated with the cassette transporter G1; DRM, detergent resistant membrane; GCK, glucokinase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; K\textsubscript{v}, adenosine triphosphate-binding cassette transporter G1; DRM, detergent resistant membrane; GCK, glucokinase; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A; K\textsubscript{v}, adenosine triphosphate-sensitive K\textsuperscript{+} channel; K\textsubscript{ATP}, voltage-gated K\textsuperscript{+} channel; Ldlr, low density lipoprotein receptor; MjCD, methyl-β-cyclodextrin; PIP\textsubscript{2}, phosphatidylinositol 4,5-bisphosphate; Sc5d, lanthosterol-5-desaturase; SNAP-25, synaptosomal-associated protein 25; VAMP-2, vesicle-associated membrane protein 2.
The insulin processing enzyme carboxypeptidase E (CPE) was also reported to associate with membrane rafts in SG membranes purified from pituitary cells. Cholesterol depletion dissociated CPE from SG membranes, and its sorting to SGs was disrupted in lovastatin-treated AtT-20 pituitary cells. A similar missorting was observed in neuroendocrine cells when the C-terminus of CPE, which mediated its partitioning with DRMs, was removed. It should be noted, however, that CPE did not partition with raft fractions in MIN6 cells.

Cholesterol appears essential for targeting the granin protein, secretogranin III (SGIII), to immature granules, as it bound to SG-like liposomes and isolated SG membranes in a cholesterol-dependent manner, and deletion of its N-terminal membrane-binding domain caused its constitutive secretion. In INS-1 cells, SGIII binds CPE, and its interaction with chromogranin A (CGA), another granin that promotes prohormone aggregation in the acidic luminal milieu of SGs, targeted CGA to insulin SG membranes. Therefore, SGIII has been described as a multi-functional adaptor that facilitates the retention of prohormone aggregates in the cholesterol-enriched membranes of nascent SGs, thus promoting their processing and maturation by raft-associated convertases. Does SGIII associate with membrane rafts? This does not appear to be the case, as it was soluble in Triton X-100 and several other detergents typically used to designate raft association. It has instead been proposed to reside in distinct cholesterol-enriched membrane domains. The transmembrane SG protein phogrin, additionally referred to as insulinoma-associated protein 2 (IP2) or IP2β), is also expressed in rat insulinoma cells. Phogrin and its paralog, ICA512, like SGIII were also primarily soluble in Triton X-100. However, ICA512 partitioned to a greater extent with DRMs after solubilization in Lubrol (Dirkx R and Solimena M, unpublished data, 2007), a finding similar to that reported previously for VAMP-2. Phogrin and its paralog, ICA512, like SGIII were also primarily soluble in Triton X-100. However, ICA512 partitioned to a greater extent with DRMs after solubilization in Lubrol (Dirkx R and Solimena M, unpublished data, 2007), a finding similar to that reported previously for VAMP-2. These results suggest that SG membranes, although highly enriched with cholesterol and sphingolipids, still appear to be organized in heterogeneous functional membrane raft domains with distinct protein profiles.

CONCLUSIONS

Cholesterol is essential for proper β-cell function, and despite discrepancies regarding the effect of cholesterol depletion on some aspects of GSIS, there now appears to be a clear consensus that accumulation of cellular cholesterol impairs insulin secretion (Figure 2). In the end, however, the question remains – do membrane rafts regulate GSIS? Conceivably yes, as compelling evidence indicates that numerous proteins essential for granule biogenesis and insulin secretion associate with these domains in a cholesterol-depletion sensitive fashion. However, at this time there is little evidence that physiological stimuli that trigger or augment insulin secretion (e.g. glucose, glucagon-like peptide 1 etc.) do so by altering the partitioning of these proteins in or out of membrane rafts. In addition, although a recent study suggested that hyperglycemia-induced reductions in cholesterol might impair GSIS in part by disrupting membrane rafts, it has not been clearly demonstrated that physiopathological conditions that induce cholesterol accumulation also alter membrane rafts. For these reasons, it is difficult to assess at this time if membrane raft perturbation is a key factor contributing to β-cell dysfunction in type 2 diabetes. Thus, while it remains an appealing possibility, the hypothesis that impaired cellular cholesterol homeostasis disrupts insulin secretion by altering β-cell membrane rafts remains to be proven.

ACKNOWLEDGEMENTS

We are grateful to Andreas Müller for the EM micrograph of a high pressure frozen rat β-cell, Erdinc Sezgin and Petra Schwille for discussion, and Kai Simons for critical reading of this manuscript. This work has been supported by funds from the Deutsche Forschungsgemeinschaft (grant SO 818/1-1) and the German Ministry for Education and Research (BMBF) to the German Centre for Diabetes Research (DZD; http://www.dzd-ev.de). We declare no conflicts of interest related to this manuscript.

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