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Research Article

Increased Steroidogenic Acute Regulatory Protein Contributes to Cholesterol-induced β-Cell Dysfunction

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Abstract

Hypercholesterolemia is often observed in individuals with type 2 diabetes. Cholesterol accumulation in subcellular compartments within islet β-cells can result in insulin secretory dysfunction, which is a key pathological feature of diabetes. Previously, we demonstrated that expression of the mitochondrial cholesterol transport protein, steroidogenic acute regulatory protein (StAR), is induced in islets under conditions of β-cell dysfunction. However, whether it contributes to mitochondrial cholesterol accumulation in β-cells and cholesterol-induced β-cell dysfunction has not been determined. Thus, we sought to examine the role of StAR in isolated mouse islets under conditions of excess exogenous cholesterol. Cholesterol treatment of islets upregulated StAR expression, which was associated with cholesterol accumulation in mitochondria, decreased mitochondrial membrane potential and impaired mitochondrial oxidative phosphorylation. Impaired insulin secretion and reduced islet insulin content were also observed in cholesterol-laden islets. To determine the impact of StAR overexpression in β-cells per se, a lentivirus was used to increase StAR expression in INS-1 cells. Under these conditions, StAR overexpression was sufficient to increase mitochondrial cholesterol content, impair mitochondrial oxidative phosphorylation, and reduce insulin secretion. These findings suggest that elevated cholesterol in diabetes may contribute to β-cell dysfunction via increases in StAR-mediated mitochondrial cholesterol transport and accumulation.

Key Words: diabetes, cholesterol, steroidogenic acute regulatory protein, STARD1, mitochondrial dysfunction, β-cell dysfunction

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; GSIS, glucose-stimulated insulin secretion; HSP90, heat shock protein 90; KRB, Krebs-Ringer bicarbonate; Lt.Control, lentivirus expressing control vector in INS-1 cells; Lt.Star, lentivirus expressing *Star* gene vector in INS-1 cells; OCR, oxygen consumption rate; StAR, steroidogenic acute regulatory proteins.

Hypercholesterolemia is a significant risk factor for the development of cardiovascular disease, a leading cause of morbidity and mortality in people with diabetes (1, 2). Elevated plasma cholesterol has been reported to be associated with increased islet cholesterol content, insulin secretory dysfunction, and hyperglycemia in animal studies (3, 4).

The critical role of mitochondria in β-cell function is well established (5-7). Excess cholesterol accumulation in β-cells has been shown to alter mitochondrial architecture and impair mitochondrial function (8, 9). Mitochondrial cholesterol accumulation is a pathological feature of a number of diseases, including cancer, cardiovascular disease, liver disease, and neurodegenerative disease (10-13); however, whether cholesterol accumulates in β-cell mitochondria resulting in dysfunction has not been examined.

In steroidogenic tissues, mitochondrial cholesterol transport proteins regulate cholesterol trafficking to and metabolism within mitochondria (14). One such protein is steroidogenic acute regulatory (StAR) protein, also known as STARD1 (15, 16). It is predominantly localized to the outer mitochondrial membrane (17) where it facilitates cholesterol transport to the inner mitochondrial membrane (18, 19). In steroidogenic tissues, StAR-mediated mitochondrial cholesterol transport is a critical regulatory step in steroidogenesis (20). In nonsteroidogenic tissues, including hepatocytes and macrophages, StAR regulates bile acid and oxysterol production (21, 22). Upregulation of StAR in hepatocytes increases mitochondrial cholesterol content, which is associated with mitochondrial dysfunction and the pathogenesis of liver diseases including nonalcoholic steatohepatitis and Nieman-Pick disease type C (23, 24).

In mouse islets, we previously showed that StAR is upregulated with islet amyloid formation, a common pathological feature of type 2 diabetes associated with β-cell dysfunction

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(25). StAR is also expressed in human islets and upregulated with islet amyloid formation in cultured human islets (unpublished observation). The role of StAR in regulating mitochondrial cholesterol levels and cholesterol-induced β-cell dysfunction is unknown. Thus, in this study, we investigated whether exposure of islets to excess cholesterol upregulates the expression of StAR and results in mitochondrial cholesterol accumulation, mitochondrial dysfunction, and impaired β-cell function. In addition, we overexpressed StAR specifically in immortalized β-cells to determine whether increasing StAR results in deleterious effects on mitochondrial function and insulin secretion.

Research Design and Methods

Mouse Islet Isolation and Culture

Islets were isolated from 8- to 14-week-old male and female C57BL/6J mice by collagenase digestion as previously described (25). Thereafter, islets were recovered overnight in Roswell Park Memorial Institute (RPMI) 1640 medium containing 11.1 mM glucose, 10% fetal bovine serum, 1% sodium pyruvate, and 1% penicillin/streptomycin (v/v) at 37 °C with 5% CO_2. All mice used in the study were bred at the VA Puget Sound Health Care System, and animal studies were approved by the VA Puget Sound Health Care System Institutional Animal Care and Use Committee.

Establishment of Stable Cell Lines Expressing Star or Control Vector

INS-1 cells, an immortalized rat β-cell line, were cultured in RPMI 1640 medium containing 11.1 mM glucose, 10% fetal bovine serum, 1% sodium pyruvate, and 1% penicillin/streptomycin (v/v) at 37 °C with 5% CO_2. At 50% confluency, cells were transfected for 24 hours with lentivirus (multiplicity of infection = 1) expressing mouse *Star* or a control vector under a cytomegalovirus promoter with a C-Myc-DDK tag (#PS199964V and #MR203861L1V; OriGene Technologies, Rockville, MD). *Star* gene and protein expression were confirmed by quantitative PCR and Western blot, respectively. Experiments were performed on passages 60 to 66, with *Star* gene transcription and protein levels quantified for each experimental set. These transfected cells are hereafter referred to as lentivirus expressing *Star* gene vector in INS-1 cells (Lt.Star) or lentivirus expressing control vector in INS-1 cells (Lt.Control). Cells were plated in triplicate at 10 to 15 K per well and cultured to confluence, unless specified otherwise.

Cholesterol Treatment of Islets

Isolated islets were recovered overnight, then treated for 24 hours with 0.25 or 0.5 mM water-soluble cholesterol (cholesterol-cyclodextrin complex; Sigma-Aldrich, # C4951) in RPMI 1640 media containing 11.1 mM glucose, 10% fetal bovine serum, 1% sodium pyruvate, and 1% penicillin/streptomycin (v/v). Control islets were handled similarly except the media did not contain cholesterol. Mixtures of islets from male and female mice were used.

Mitochondrial Fractionation

One hundred fifty to 200 islets were collected for quantification of mitochondrial cholesterol content. Briefly, using a modified version of the protocol described by Wieckowski et al (26), islet cells in ice-cold sodium-chloride-tris-EDA buffer were homogenized using a Dounce homogenizer. Lysed islets were subjected to multiple centrifugation steps until the crude mitochondrial fraction was obtained. Briefly, after homogenization, the cell lysate was centrifuged at 1000 g for 10 minutes at 4 °C, after which the resultant supernatant was centrifuged at 10 400 g for 10 minutes at 4 °C. The pellet was resuspended in the ice-cold sodium-chloride-tris-EDA buffer and centrifuged at 16 000 g for 2 minutes at 4 °C to obtain the crude mitochondrial fraction. The crude isolated mitochondrial fraction was lysed in PBS buffer containing 0.1% Triton-X. Similarly, 1×10^8 INS-1 cells were lysed and the resulting supernatant was subjected to multiple centrifugation steps until the final pellet of crude mitochondria was obtained, which was then resuspended in 250 mM mannitol containing 5 mM HEPES and 0.5 mM EGTA (pH 7.4).

Cholesterol Quantification

Forty to 50 islets were harvested in lysis buffer containing PBS with 0.1% Triton-X. Islet cholesterol concentration was measured in the supernatant using the Amplex red cholesterol assay kit (Thermo Fisher Scientific, #A12216). The cholesterol content of the islets was quantified by normalizing the islet cholesterol concentration to the protein concentration, the latter measured via bicinchoninic acid protein assay. Mitochondrial cholesterol content in islets or INS-1 cells was measured as described earlier and normalized to mitochondrial protein concentration.

Real-time Quantitative PCR

RNA extraction and reverse transcription were done as previously described (25). mRNA of each gene was measured in triplicate using TaqMan real-time quantitative PCR gene expression assays (Thermo Fisher Scientific/Life Technologies). Peptidyl-prolyl cis-trans isomerase B (*Ppib*, cyclophilin) was used as a housekeeping gene (25). The TaqMan primer-probe sets used are *Star* (mouse Mm00441558, rat Rn00580695) and *Ppib* (mouse Mm00478295, rat Rn03302274). mRNA expression was calculated using the ΔΔCt method.

Protein Quantification

Western blot analysis was done as previously described (27). Briefly, islet or INS-1 cell proteins (20-40 μg) were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Blots were probed with primary antibodies for StAR (rabbit, 1:1000, Cell Signaling Technology, #8449, RRID:AB_10889737) and heat shock protein 90 (HSP90) (rabbit, 1:1000, Cell Signaling Technology, #4874, RRID: AB_2121214), and anti-rabbit horseradish peroxidase secondary antibody (Goat, 1:3000, Abcam, #ab6721, RRID: AB_955447). They were then visualized via enhanced chemiluminescence. Protein quantification was performed using ImageJ software (National Institutes of Health, Bethesda, MD). StAR protein expression was normalized to HSP90.

Mitochondrial Function Tests

Islets were plated in duplicate in a Seahorse XF96 spheroid microplate (Agilent # 102978-100) at 20 islets/well in RPMI growth medium containing 2.8 mM glucose. Islets were maintained at 37 °C in a non-CO_2 incubator for 60 minutes. Basal mitochondrial oxygen consumption rate (OCR), 22.5 mM glucose-stimulated OCR, 5 μM oligomycin inhibited OCR,

2 μM carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP)-induced maximal OCR, and 3 μM rotenone- and antimycin-induced OCR were quantified sequentially using a Seahorse XF96 Analyzer.

INS-1 cells were seeded in triplicate in a Seahorse XF24 culture microplate (Agilent #100777-004) at 3.5×10^4 cells/well in RPMI growth medium containing 11.1 mM glucose. After a 48-hour culture, cells were incubated with assay medium supplemented with 11.1 mM glucose for 60 minutes at 37 °C. Thereafter, basal mitochondrial OCR and maximal OCR were quantified using a Seahorse Bioscience XF24 Analyzer.

Mitochondrial Membrane Potential Quantification

Islets were plated in duplicate at 25 islets/well and INS-1 cells were plated in triplicate at 10 000 to 15 000 per well in 96-well plates. Mitochondrial membrane potential in islets and INS-1 cells was measured using the JC-10 Assay (Abcam, #ab112134), where a fluorescence ratio of 590/525 nm was determined and expressed as a percentage relative to the control condition.

Glucose-stimulated Insulin Secretion

Glucose-stimulated insulin secretion (GSIS) by islets and INS-1 cells was measured as described previously (28). Islets or INS-1 cells (plated at 10 000-15 000 per well in a 96-well plate) were cultured in Krebs-Ringer bicarbonate (KRB) buffer containing 2.8 mM glucose for 90 minutes. Following incubation in 2.8 mM glucose, triplicate sets of 5 islets from each experimental condition were transferred to KRB buffer containing either 2.8 or 20 mM glucose and incubated for 60 minutes at 37 °C with 5% CO_2. Thereafter, KRB buffer samples were collected to determine insulin secretion. Like in the islet experiments, INS-1 cells (in triplicate) were then cultured in KRB buffer containing either 2.8 or 20 mM glucose and incubated for 60 minutes at 37 °C with 5% CO_2, after which media was collected to quantify insulin secretion. Islets cultured in 2.8 mM glucose were extracted using 0.2 M HCl-ethanol to measure insulin content. INS-1 cells in 20 mM glucose were extracted using 0.2 M HCl-ethanol to quantify insulin content. Insulin in the media and islet/cell extracts was quantified using the insulin (mouse) ultrasensitive ELISA assay (ALPCO Diagnostics, #80-INSMSU-E01, RRID:AB_2792981). Total insulin content was determined as the sum of the insulin release and the insulin content at the end of the experiment in a given glucose concentration. Fractional insulin secretion was computed as the ratio of insulin secretion to total islet insulin content.

Cell Viability

Islets were plated in duplicate at 20 to 25 islets/well and INS-1 cells were in triplicate at 10 000-15 000 per well in 96-well plates. Islet viability was measured by incubating islets for an hour with Cell Titer Fluor reagent (Promega, #G6080). INS-1 cell viability was quantified by incubating cells for 6 hours with XTT reagent (Sigma-Aldrich, #1146501500) (29). Data are expressed as a percentage relative to the control condition.

Statistical Analysis

Data are presented as mean ± SEM with each endpoint measure comprised of 3 to 6 independent experiments. A paired Student's *t*-test was used to compare the means between 2 groups. One-way ANOVA followed by Dunnett's multiple comparisons test was used to compare 3-group data. Two-way ANOVA followed by Sidak's test was used to compare GSIS data. Differences were considered statistically significant with $P < .05$. Prism version 10.4.1 (GraphPad) was used for all statistical analyses.

Results

Islet Cholesterol Accumulation Results in Upregulation of StAR

We first sought to determine whether cholesterol accumulation results in increased expression of StAR within islets. Following 24-hour treatment with 0.25 and 0.5 mM cholesterol, islet cholesterol content was significantly increased by 2.0- and 2.4-fold, respectively, compared to islets cultured without cholesterol (Fig. 1A). Increased cholesterol content was associated with elevated StAR mRNA levels by 3.2- and 6.8-fold (Fig. 1B) and protein levels by 2.8- and 7.4-fold (Fig. 1C) at 0.25 and 0.5 mM cholesterol, respectively, compared to control islets. Cholesterol treatment did not change HSP90 protein expression (0 mM cholesterol: 100 ± 20.4%; 0.25 mM cholesterol: 111.5 ± 16.9%; 0.5 mM cholesterol: 97.9 ± 15.8%; $P = 0.33$) and islet protein concentrations (0 mM cholesterol: 0.28 ± 0.05 mg/mL; 0.25 mM cholesterol: 0.28 ± 0.06 mg/mL; 0.5 mM cholesterol: 0.27 ± 0.07 mg/mL; $P = .95$).

Islet Cholesterol Accumulation is Associated With Increased Mitochondrial Cholesterol Content and Mitochondrial Dysfunction

In addition to islet cholesterol content being increased with cholesterol loading, mitochondrial cholesterol content was elevated 2.2-fold in 0.5 mM cholesterol-treated vs control islets (Fig. 2A). Mitochondrial membrane potential, a critical indicator of mitochondrial function and health, was significantly decreased by 13% with cholesterol accumulation in islets (Fig. 2B). Further, we measured mitochondrial respiration in islets in real time (Fig. 2C). There were no changes in non-mitochondrial respiration (Fig. 2D), and proton leak (Fig. 2E) at 22.5 mM glucose, whereas mitochondrial respiration at 22.5 mM glucose was significantly decreased by 47% (Fig. 2F) in cholesterol-treated islets. Significant decreases were also observed in adenosine triphosphate (ATP)-linked respiration (37%; Fig. 2G) and coupling efficiency (49%; Fig. 2H) in cholesterol-treated islets. Further, islet cholesterol treatment resulted in a 30% decrease in maximal respiration (with FCCP) (Fig. 2I) and a 46% decrease in mitochondrial spare capacity (Fig. 2J).

Islet Cholesterol Accumulation Leads to Reduced β-Cell Function

Exposure of islets to 20 mM glucose resulted in a 26-fold increase in insulin secretion compared to exposure to 2.8 mM glucose, whereas with cholesterol treatment the insulin response to 20 mM glucose only increased 2.5-fold (Fig. 3A). While insulin secretion was numerically greater in the presence of 0.5 mM cholesterol at 2.8 mM glucose, this difference was not significant ($P = .12$). Insulin content was decreased by 27% in islets treated with cholesterol (Fig. 3B). To determine whether the reduced glucose-mediated insulin secretion in cholesterol-treated islets was independent of the reduction in insulin content, we calculated fractional insulin secretion, which

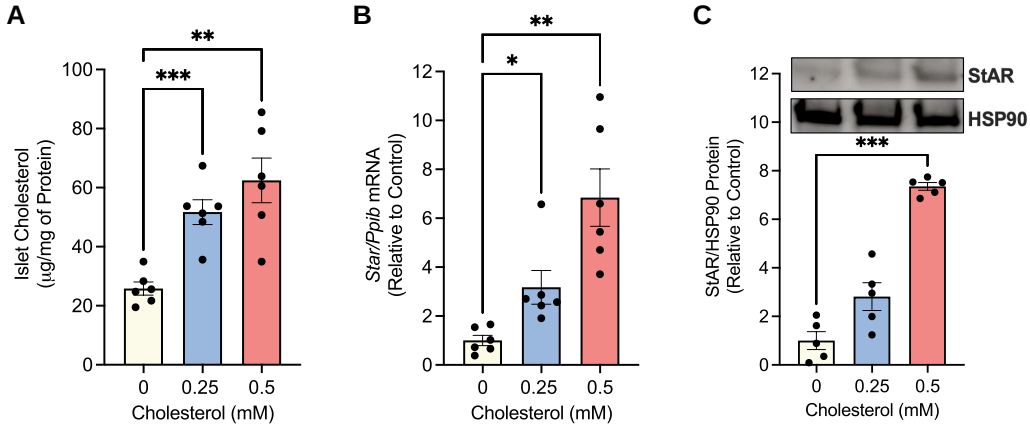

Figure 1. Islet cholesterol accumulation results in the upregulation of StAR. Islets were treated with 0 (control), 0.25, or 0.5 mM water-soluble cholesterol for 24 hours. (A) Islet cholesterol content relative to protein content; n = 6. (B) *Star* mRNA expression relative to the housekeeping gene *Ppib*. Data are relative to control islets; n = 6. (C) StAR protein expression relative to HSP90. A representative blot is shown. Data are relative to control islets; n = 5. $*P < .05$, $**P < .01$, $***P < .01$.

Abbreviations: HSP90, heat shock protein 90; StAR, steroidogenic acute regulatory protein.

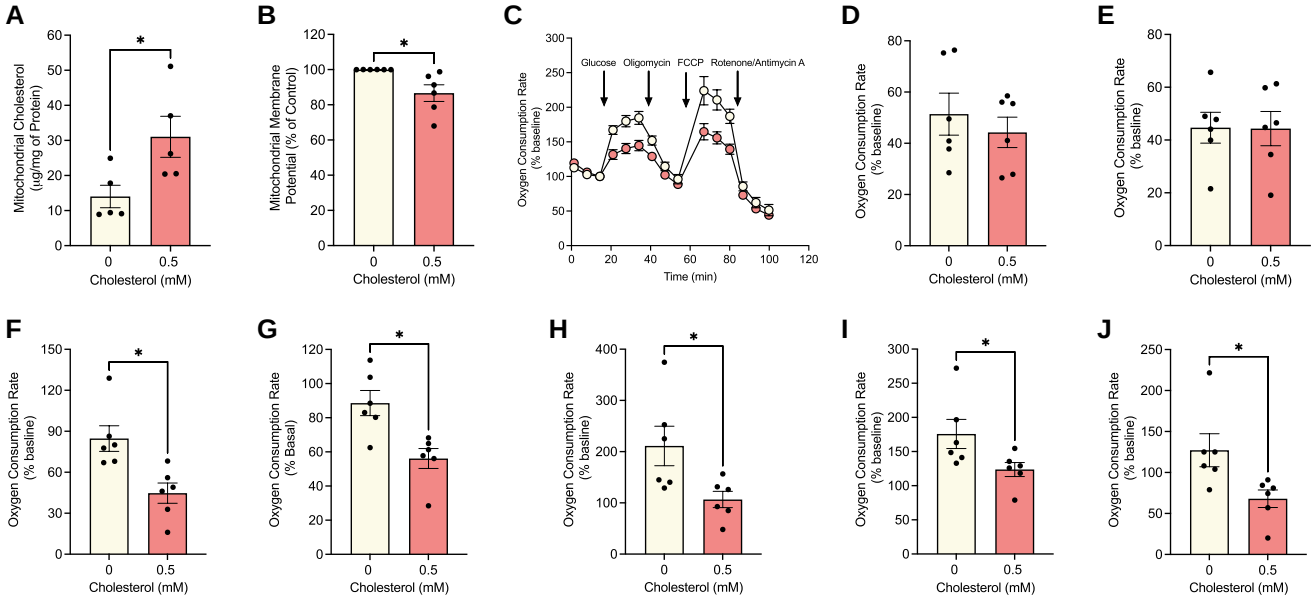

Figure 2. Islet cholesterol accumulation increases mitochondrial cholesterol content and induces mitochondrial dysfunction. Islets were treated with 0 (control) or 0.5 mM water-soluble cholesterol for 24 hours. (A) Mitochondrial cholesterol content relative to mitochondrial protein content; n = 5. (B) Mitochondrial membrane potential. Data represents emission wavelength ratio, 590/525 nm as percentage of control; n = 6. (C) Mitochondrial respiration profile of control islets and 0.5 mM cholesterol-treated islets as percentage of baseline respiration. (D) Nonmitochondrial respiration, (E) proton leak-associated mitochondrial respiration, and (F) glucose-stimulated mitochondrial respiration. (G) ATP-linked respiration and (H) coupling efficiency after the addition of 22.5 mM glucose. (I) Maximal mitochondrial respiration after the addition of chemical uncoupler FCCP and (J) spare respiratory capacity; n = 6 for studies in C-J. $*P < .05$.

Abbreviations: ATP, adenosine triphosphate; FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone.

also demonstrated impaired β-cell secretory function with cholesterol treatment (Fig. 3C). Cell viability was not different between 0.5 mM cholesterol-treated and control islets (Fig. 3D).

β-cell StAR Overexpression Increases Mitochondrial Cholesterol in the Absence of Exogenous Cholesterol

We next used stably transduced Lt.Star and Lt.Control INS-1 cells to investigate if β-cell overexpression of StAR could recapitulate the deleterious effects on mitochondrial function in the absence of exogenous cholesterol loading. Only cells with Lt.Star exhibited mRNA expression of mouse *Star* (Fig. 4A), with total StAR protein levels being increased 4.5-fold compared to Lt.Control cells (Fig. 4B). Lentiviral mouse *Star* overexpression did not affect the expression of the endogenous rat *Star* as determined over 5 passages (Fig. 4C). Consistent with StAR's role in transporting cholesterol into mitochondria, β-cell StAR overexpression was associated with a 1.15-fold increase in mitochondrial cholesterol content (Fig. 4D). However, β-cell StAR overexpression did not affect mitochondrial membrane potential (Fig. 4E).

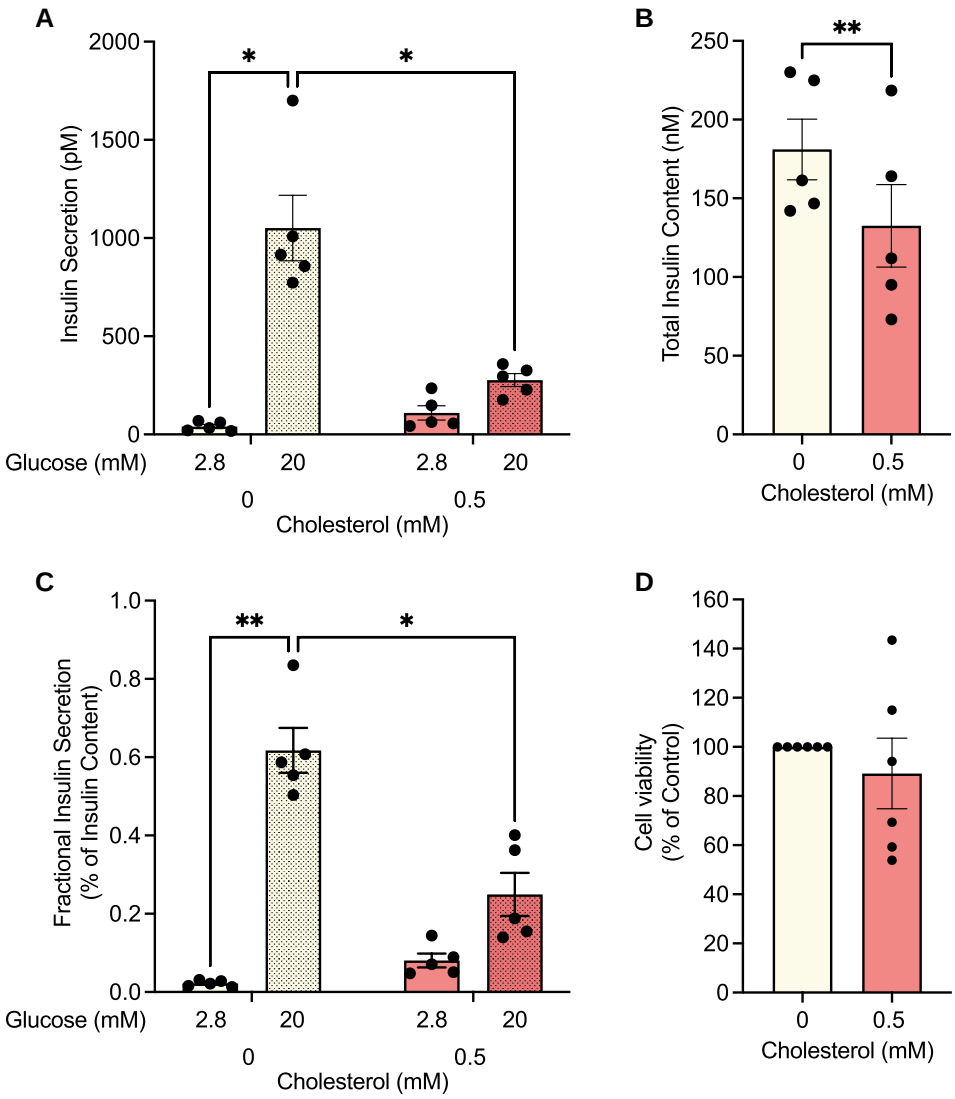

Figure 3. Islet cholesterol accumulation leads to reduced β-cell secretory function. Islets were treated with 0 (control) or 0.5 mM water-soluble cholesterol for 24 hours. (A) Insulin secretion in response to 2.8 or 20 mM glucose; n = 5. (B) Total islet insulin content; n = 5. (C) Fractional insulin secretion in response to 2.8 or 20 mM glucose; n = 5. (D) Cell viability; n = 6. $^{*}P < .05$, $^{**}P < .01$.

Assessment of mitochondrial function (Fig. 4F) showed a significant 18% decrease in basal mitochondrial respiration (Fig. 4G) and 20% decrease in maximal respiration with FCCP (Fig. 4H) in Lt.Star vs Lt.Control cells.

β-cell StAR Overexpression Results in β-cell Dysfunction and Reduced Cell Viability

β-cell StAR overexpression in Lt.Star cells was associated with a 46% decrease in GSIS, with no difference in basal insulin secretion (Fig. 5A) or insulin content (Fig. 5B), when compared to Lt.Control cells. Similarly, fractional insulin secretion data showed a significant decrease in GSIS, with no change in basal insulin secretion (Fig. 5C). Further, StAR overexpression reduced cell viability by 15% (Fig. 5D).

Discussion

We report for the first time that cholesterol accumulation in islets upregulates mRNA and protein expression of the mitochondrial cholesterol transport protein, StAR. This is associated with mitochondrial cholesterol accumulation, as well as mitochondrial dysfunction and impaired insulin secretion. Further, we show that β-cell upregulation of StAR per se (in the absence of cholesterol loading) is sufficient to increase mitochondrial cholesterol levels and can recapitulate the mitochondrial dysfunction and impaired insulin secretion seen in islets treated with exogenous cholesterol.

Exogenous cholesterol treatment of islets increased mitochondrial cholesterol content, which was associated with decreased insulin release and insulin content. By expressing insulin secretion as a function of content, we established that the β-cell secretory dysfunction in islets was independent of the deficit in insulin content. In β-cells not exposed to cholesterol but with lentivirus-mediated StAR overexpression, we also observed increased mitochondrial cholesterol content as well as reduced insulin secretion, although insulin content was unchanged. Together, these data would suggest that increased cholesterol transport into mitochondria by StAR is sufficient to produce mitochondrial and secretory dysfunction

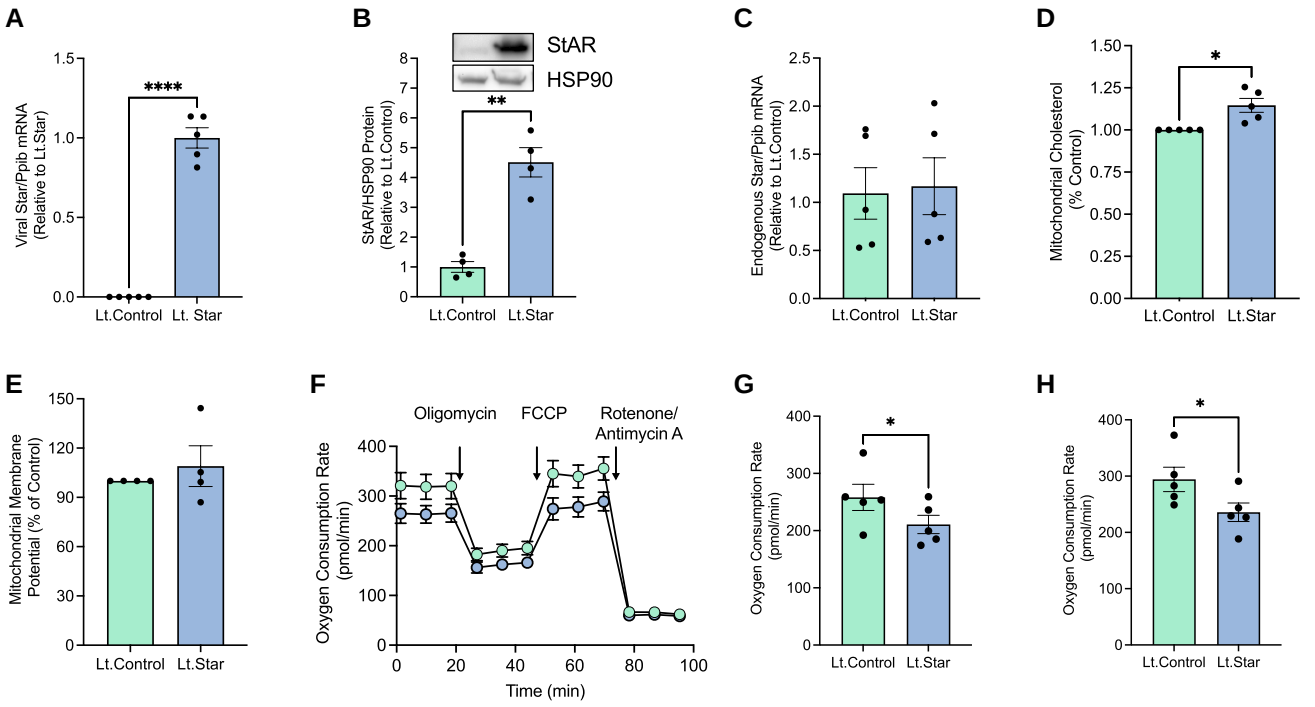

Figure 4. β-cell StAR overexpression in INS-1 cells in the absence of exogenous cholesterol increases mitochondrial cholesterol and reduces mitochondrial function. In Lt.Control cells and Lt.Star cells, the following were quantified: (A) Virally expressed (mouse) *Star* mRNA; n = 5. (B) Total StAR protein expression relative to HSP90 protein with a representative blot shown above; n = 4. (C) Endogenous *Star* mRNA (rat); n = 5. (D) Mitochondrial cholesterol content. Data are expressed relative to Lt.Control cells; n = 6. (E) Mitochondrial membrane potential. Data represents emission wavelength ratio, 590/525 nm as percentage of control; n = 4. (F) Mitochondrial respiration profile; Lt.Control and Lt.Star cells; n = 5. (G) Basal mitochondrial respiration; n = 5. (H) Maximal respiration after the addition of the chemical uncoupler FCCP; n = 5. $^{*}P < .05$, $^{**}P < .01$, $^{****}P < .0001$.

Abbreviations: FCCP, carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone; HSP90, heat shock protein 90; Lt.Control, lentivirus expressing control vector in INS-1 cells; Lt.Star, lentivirus expressing *Star* gene vector in INS-1 cells; StAR, steroidogenic acute regulatory protein.

in the absence of excess exogenous cholesterol and that these defects are likely independent of changes in insulin content. However, it is possible that the quantity of cholesterol transported may also be a determinant of the severity of change given we observed that the fold increase in mitochondrial cholesterol with StAR upregulation was greater in islets than INS-1 cells.

Previous studies have established that excess cholesterol adversely affects the mitochondria in β-cells. For example, cholesterol accumulation in β-cells was shown to decrease mitochondrial antioxidant capacity, induce reactive oxygen species production, reduce mitochondrial membrane potential and ATP production, and impair oxidative phosphorylation (8, 9, 30, 31). The impairment of oxidative phosphorylation alters the intracellular ATP/adenosine diphosphate (ADP) ratio and reduces insulin secretion (6, 7). With islet cholesterol loading, we observed a reduction in mitochondrial membrane potential, as well as impaired mitochondrial respiration, which would be consistent with our finding of decreased insulin secretion. Although increased mitochondrial cholesterol in INS-1 cells with StAR overexpression was associated with impaired mitochondrial respiration and insulin secretion, mitochondrial membrane potential remained unchanged. These observations suggest that the magnitude of the adverse effects may depend in part on the quantity of cholesterol transported into the mitochondria. Given the critical role of mitochondria in the insulin secretory process, it is not surprising that mitochondrial defects associated with StAR-mediated increases in cholesterol transport would be linked to reduced β-cell function.

The cholesterol-induced mitochondrial defects may be explained, in part, by increased mitochondrial cholesterol accumulation. Our data support that the increased transport of cholesterol into the mitochondria may be a major driver of mitochondrial cholesterol accumulation in islets. That is, we observed a significant increase in mRNA and protein expression of StAR upon treatment of islets with exogenous cholesterol. Moreover, StAR overexpression in β-cells without cholesterol loading resulted in increased mitochondrial cholesterol content and impaired insulin secretion. In other cell types, excess cholesterol has been shown to induce the expression of StAR. For example, in hepatocytes and neuroblastoma cells, upregulation of endogenous cholesterol increases mitochondrial cholesterol transport protein levels and mitochondrial cholesterol content (23, 32-34). In a nonalcoholic fatty liver disease mouse model, overexpression of StAR in hepatocytes improved glucose metabolism and attenuated insulin resistance via farnesoid X receptor activation by bile acids (35). Further, increased StAR expression in hepatocytes promotes cholesterol metabolism and bile acid synthesis (36). Our findings that StAR overexpression is deleterious in β-cells may be related in part to the β-cell lacking many of the cytochrome P450 family enzymes required for cholesterol metabolism as observed in liver (unpublished data). Further work, including in vivo studies, in which β-cell StAR expression is modified should be informative.

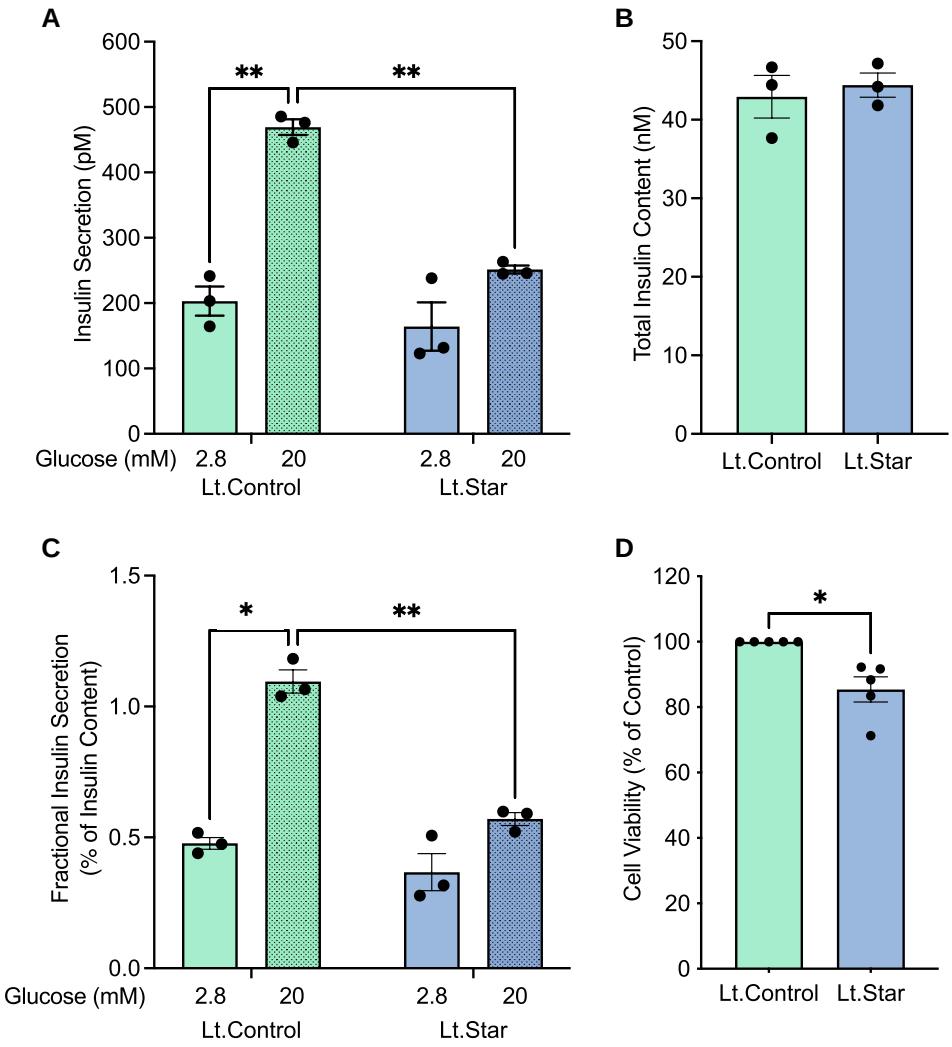

Figure 5. β-cell StAR overexpression in INS-1 cells in the absence of exogenous cholesterol results in insulin secretory dysfunction. (A) Insulin secretion from Lt.Control and Lt.Star cells in response to 2.8 or 20 mM glucose; n = 3. (B) Total insulin content of Lt.Control and Lt.Star cells; n = 3. (C) Fractional insulin secretion from Lt.Control and Lt.Star cells in response to 2.8 or 20 mM glucose; n = 3. (D) Cell viability of Lt.Control and Lt.Star cells; n = 5. $*P < .05$, $**P < .01$.

Abbreviations: Lt.Control, lentivirus expressing control vector in INS-1 cells; Lt.Star, lentivirus expressing *Star* gene vector in INS-1 cells; StAR, steroidogenic acute regulatory protein.

Cholesterol accumulation is known to lead to β-cell dysfunction and apoptosis (9). While we observed comparable levels of StAR expression in islets treated with cholesterol and INS-1 cells with viral overexpression, only in the latter did we observe a significant reduction in cell viability. The reasons for this are unclear, but 1 possibility is that in INS-1 cells, StAR overexpression occurred for a more prolonged period than the relatively short upregulation of StAR in the presence of cholesterol in islets. We did observe a numerical decrease in cell viability in cholesterol-treated islets, which may have been more pronounced and significant if exposure to cholesterol had been more prolonged.

StAR expression is known to be regulated by the cAMP/protein kinase A pathway (37, 38). In β-cells, cholesterol accumulation has been reported to increase cAMP generation (8). Thus, our data showing a dose-dependent increment of StAR expression with increased islet cholesterol accumulation suggests that induction of StAR may be downstream of cholesterol-induced upregulation of cAMP—this possibility could be explored in future studies.

Our experimental design using islets involved 24-hour exposure to exogenous cholesterol, which differs from what would be occurring in humans where exposure could be occurring over months to years. Experimentally increasing cholesterol concentrations in healthy humans for a prolonged period is not feasible, but studies in animal models of hypercholesterolemia could shed more light on the possibility that StAR plays a critical role in the deterioration of β-cell function under conditions of chronic cholesterol exposure.

Gene mutations in StAR are associated with lipoid congenital adrenal hyperplasia in humans (39, 40). This disorder is associated with diminished cortisol production; increased levels of androgens; and an increased risk of diabetes, obesity, and other metabolic disorders (41). Although the β-cell is not a steroidogenic cell, our observation of StAR expression and its modulation by cholesterol suggests that the protein may have physiological and pathological roles in β-cells. Further studies are required to provide additional insight.

In conclusion, we have shown that cholesterol accumulation leads to increased StAR expression and mitochondrial

cholesterol accumulation, which is associated with mitochondrial dysfunction and impaired insulin secretion. Collectively, these data provide new insights that may be relevant to cholesterol-induced β-cell dysfunction in diabetes and suggest that targeting StAR could be a novel therapeutic approach to prevent the deleterious β-cell effects of excess cholesterol.

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Disclosures

The authors have no relevant conflicts of interest to declare.

Data Availability

Original data generated and analyzed during this study are included in this published article.

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