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Apolipoprotein A-I improves pancreatic β-cell function independent of the ATP-binding cassette transporters ABCA1 and ABCG1

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ABSTRACT: Apolipoprotein A-I (apoA-I), the main protein constituent of HDLs, increases insulin synthesis and insulin secretion in pancreatic β cells. ApoA-I also accepts cholesterol that effluxes from cells expressing ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G_1 (ABCG1). Mice with conditional deletion of ABCA1 and ABCG1 in β cells [β-double knockout (DKO) mice] have increased islet cholesterol levels and reduced glucose-stimulated insulin secretion (GSIS). The project asks whether metabolic pathways are dysregulated in β-DKO mouse islets and whether this can be corrected, and GSIS improved, by treatment with apoA-I. β-DKO mice were treated with apoA-I or PBS, and islets were isolated for determination of GSIS. Total RNA was extracted from β-DKO and control mouse islets for microarray analysis. Metabolic pathways were interrogated by functional enrichment analysis. ApoA-I treatment improved GSIS in β-DKO but not control mouse islets. Plasma lipid and lipoprotein levels and islet cholesterol levels were also unaffected by treatment with apoA-I. Cholesterol metabolism, glucose metabolism, and inflammation pathways were dysregulated in β-DKO mouse islets. This was not corrected by treatment with apoA-I. In summary, apoA-I treatment improves GSIS by a cholesterol-independent mechanism, but it does not correct metabolic dysregulation in β-DKO mouse islets.—Hou, L., Tang, S., Wu, B. J., Ong, K.-L., Westerterp, M., Barter, P. J., Cochran, B. J., Tabet, F., Rye, K.-A. Apolipoprotein A-I improves pancreatic β-cell function independent of the ATP-binding cassette transporters ABCA1 and ABCG1. FASEB J. 33, 8479–8489 (2019). www.fasebj.org

KEY WORDS: apoA-I · β cells · cholesterol metabolism · glucose metabolism · inflammation

Diabetes and related health issues are a major public health priority that is expected to exceed 15% of the total global health expenditure by 2040. Type 1 diabetes (T1D), an autoimmune disorder that selectively destroys insulin producing β cells in the pancreas, accounts for 5–10% of all diabetes (1–3). Type 2 diabetes (T2D), the most common form of the disease, is characterized by hyperglycemia, insulin resistance, and dyslipidemia (4). T2D initially manifests as insulin resistance, but disease progression is driven by the failure of β cells to secrete insulin in amounts sufficient to meet metabolic demands (5). The β-cell dysfunction in T1D and T2D is associated with impaired mitochondrial function, oxidative stress, endoplasmic reticulum stress, and glucolipotoxicity (6–9). High cholesterol levels are an additional cause of β-cell dysfunction (10). Preclinical and *in vitro* studies have established that insulin secretion is impaired in β cells with elevated cholesterol levels (11, 12).

Elevated plasma HDL cholesterol levels are associated with a reduced risk of developing cardiovascular disease (13). We and others have reported that HDLs and apolipoprotein A-I (apoA-I), the main HDL apolipoprotein, also have antidiabetic properties (14–16). In addition to increasing insulin synthesis and improving insulin secretion in β cells,

ABBREVIATIONS: ABCA1, ATP-binding cassette transporter A1; ABCG1, ATP-binding cassette transporter G_1; *Akt1*, thymoma viral proto-oncogene 1; apoA-I, apolipoprotein A-I; DKO, double knockout; *Fasn*, fatty acid synthase; Fox, forkhead box; *Gbp11*, guanylate binding protein 11; Gck, glucokinase; *Glut2*, solute carrier family 2, member 2; GSIS, glucose-stimulated insulin secretion; *Hmgcr*, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; *Insig1*, insulin-induced gene 1; *Irs1*, insulin receptor substrate 1; KRBB, Krebs-Ringer bicarbonate buffer; *Ldlr*, low-density lipoprotein receptor; *Pik3r1*, phosphoinositide-3-kinase regulatory subunit 1; *Prkg1*, cGMP-dependent protein kinase type I; RQV, relative quantitative value; *Sftpd*, surfactant-associated protein D; *Srebf2*, sterol regulatory element binding factor 2; T1D, type 1 diabetes; T2D, type 2 diabetes

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HDLs and apoA-I alleviate insulin resistance by increasing glucose uptake into skeletal muscle (14, 16, 17). These findings are consistent with results from a clinical trial in which a single infusion of reconstituted HDLs consisting of apoA-I complexed with phospholipid improved glycemic control and β-cell function in patients with T2D (18). Improved glycemic control in people with T2D and a reduction in the incidence of new onset diabetes have also been reported in randomized clinical trials of cholesteryl ester transfer protein inhibitors that increase HDL and apoA-I levels (19, 20).

The ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G_1 (ABCG1) maintain cellular cholesterol homeostasis by effluxing excess cholesterol to apoA-I and HDLs. They are also essential for maintaining normal β-cell function (21–24). Deletion of either ABCA1 or ABCG1 in β cells increases intracellular cholesterol levels and impairs insulin secretion (23, 24). We recently reported that conditional deletion of ABCA1 as well as ABCG1 in β cells in mice [β-double knockout (DKO) mice] increases islet cholesterol levels, resulting in glucose intolerance, impaired insulin secretion, and preferential disposal of glucose into adipose tissue instead of skeletal muscle (25).

Whether the β-cell dysfunction in β-DKO mice is reversible and whether ABCA1 and ABCG1 have metabolic effects that extend beyond decreasing insulin secretion in β-DKO mouse islets is unknown. These issues are addressed in the present study, which seeks to identify metabolic pathways that are regulated by ABCA1 and ABCG1 in β-DKO mouse islets and determine whether treatment with apoA-I improves β-cell function in these animals.

MATERIALS AND METHODS

Animals

β-DKO mice were generated by crossing hemizygous B6.Cg-Tg (Ins-2-Cre)25Mgn/J (Cre) mice (The Jackson Laboratory, Bar Harbor, ME, USA) with homozygous *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice as previously described (25). All experiments were approved by the University of New South Wales Animal Care and Ethics Committee (approval 13/135B) and conducted in accordance with their guidelines. Animals were housed under 12-h light/dark cycles and maintained on a standard laboratory chow diet. Mouse genotypes were confirmed by PCR amplification of DNA as previously described (25). Only male mice were used in this study. Female mice were not used to ensure that the results would not be confounded by hormonal cycles that may affect body fat distribution and insulin sensitivity.

ApoA-I treatment

ApoA-I was isolated from pooled samples of human plasma and provided to us by Commonwealth Serum Laboratories, Parkville, VIC, Australia. Prior to use, the apoA-I was chromatographed on a Q-Sepharose Fast-Flow Column attached to an AKTA fast protein liquid chromatography (FPLC) system (26). The preparations appeared as a single band when electrophoresed on a 20% SDS-polyacrylamide PhastGel (GE Healthcare, Waukesha, WI, USA) and stained with Coomassie Blue. Twelve-week-old mice were randomly allocated to be injected intraperitoneally with either apoA-I (8 mg/kg) or an equivalent volume of PBS twice weekly for 4 wk and euthanized 24 h after the final injection. Islets were immediately isolated for assessment of glucose-stimulated insulin secretion (GSIS) or quantification of islet cholesterol levels or total RNA isolation. Plasma levels of human apoA-I were determined by ELISA as previously described (25).

Biochemical analyses

Blood was collected from the euthanized animals and immediately placed on ice. Plasma was isolated by centrifugation and total cholesterol, unesterified cholesterol, and triglyceride concentrations were determined enzymatically using a Beckman AU480 autoanalyzer (Beckman Coulter, Brea, CA, USA). Plasma HDL cholesterol levels were quantified after precipitating apoB-containing lipoproteins with polyethylene glycol PEG-6000 (27). Lipoprotein profiles were determined by loading plasma samples (200 μl) onto 2 Superdex 200 columns connected in series. Lipoproteins were resolved at a flow rate of 0.25 ml/min (AKTA FPLC system; GE Healthcare) and fractions were collected at 1-min intervals.

Islet isolation

Islets were isolated by cannulating the common bile duct and clamping the duodenal end of the pancreas. A perfusion solution [2.5 ml of 0.25 mg/ml Liberase and 7.5 μg/ml Thermolysin (Roche, Basel, Switzerland) in Krebs-Ringer bicarbonate buffer (KRBB)] was injected into the common bile duct. The pancreas was excised, cleared of fat and lymph nodes, and incubated at 37°C for 15 min. After addition of a quenching reagent [15 ml, 10% (v/v) new born calf serum in KRBB], undigested tissue was removed by filtration through a nylon mesh (500 μm; Corning, Corning, NY, USA). The digested tissue was washed with quenching reagent and centrifuged (3 × 1 min, 235 *g*, 10°C). The samples were then resuspended in Ficoll-Paque (30 ml; GE Healthcare), overlayed with quenching reagent (10 ml), and centrifuged (22 min, 1360 *g*, 10°C). Islets were collected, passed through a 70-μm netwell filter, washed with KRBB, and hand-picked under a low-magnification (×400) microscope (Olympus, Shinjuku, Japan).

Determination of islet cholesterol levels

Isolated islets were lysed with water (100 μl) and homogenized by passage through a 1-ml syringe connected to a 29-gauge needle. Protein concentrations were determined using the bicinchoninic acid assay. Samples (60 μg protein) were extracted with cold methanol (2.5 ml) and hexane (10 ml), vortexed (30 s), then centrifuged (4 min, 675 *g*, 4°C). The hexane layer was collected, evaporated (SpeedVac; Thermo Fisher Scientific, Waltham, MA, USA), and redissolved in acetonitrile/isopropanol [130 μl, 30/70 (v/v)]. The samples (100 μl) were injected onto an Ascentis C18 HPLC Column (MilliporeSigma, Burlington, MA, USA), eluted with acetonitrile/isopropanol [30/70 (v/v)], and the absorbance at 204 nm was measured (28). The results were analyzed with Chemstation software (Agilent Technologies, Santa Clara, CA, USA).

Glucose tolerance test

Mice had food withheld for 5 h prior to administration of an intraperitoneal injection of D-glucose (2 g/kg). Blood glucose levels were monitored at 15, 30, 45, 60, 90, and 120 min using an Accu-Chek blood glucose monitor (Roche).

TABLE 1. *List of primers used for quantitative RT-PCR analysis*

Gene	Primer sequence, 5′–3′	
	Forward	Reverse
Abca1	GAACGGGTTACTATCTGACC	GAGAAACACTGTCCTCCTTT
Abcg1	GGATGAATCAGCGAATGTTG	CACACTTGGGTATTTTCTGC
Akt1	CCTGATGTTTTGTTTCTCGG	GATAGTTTTCCTCCTGACCT
ApoB	CCACACCTTCTTGATTCTGA	CTTCCAGTTCCATCTTCCTC
Fasn	GTCAGTGTGAAGAAGTGTCT	ACCCATAAGTATCAGAGCCT
Foxa2	TAACTGTAACGGGGAGGG	TGTTGCTCACGGAAGAGTA
GAPDH	GTATGTCGTGGAGTCTACTG	TTGCTGACAATCTTGAGTGA
Gbp11	CAGTGATTTCTTTGTGGACAG	CAGTCTCTCATTTGCTCCTA
Glut1	TAGTCTTCACCTTGATTGGC	TCGGTATTAGTGTGTCCTTG
Glut2	ATCATTGGCACATCCTACTT	TTTGGTGACATCCTCAGTTC
Glut4	CCCCAGATACCTCTACATCA	ACTTCCGTTTCTCATCCTTC
Hmgcr	CGATAGAGATAGGAACCGTG	ATCACAGTGCCACATACAAT
IL1β	CACCTTTTGACAGTGATGAGA	CACAGCCACAATGAGTGATA
Insig1	GTGTCACAGTGGGAAACATA	GACCAGTGTCTCTACATCCT
Irs1	GATCAGGCTATCTTCCTTGG	GTGTTGAAAAACTGGGTGAG
Ldlr	AAACGAAGCCATTTTCAGTG	TTGTCTCACACCAGTTCAC
Pik3r1	GCAGTAAAATCAGACGACAG	GTCCTTCTCAGCAACTTGT
Ppargc1a	CAGTTCACTCTCAGTAAGGG	CAGCACACTCTATGTCACTC
Prkg1	TAAACTGTGGAATCGTCCTC	TGTGGTCCTATCCTGAAAGA
Scap	TCTTGGACAGGAGGATTGTA	CAGATGAGGAAGGAGAACTG
Scarb1	CAGGTGTGCTCTTCTAAATG	GGGAACTAAGGCTTTCAGAC
Sftpd	CGTGGACTAAGTGGACCTC	GCCTTTTGCCCCTGTAGAT
Srebf2	TGATTGTCTTGAGCGTCTTT	GGATAAGCAGGTTTGTAGGT

GSIS

Isolated islets were cultured overnight at 37°C in Roswell Park Memorial Institute (RPMI) 1640 medium containing 10% (v/v) fetal bovine serum and 1% (v/v) penicillin/streptomycin, placed in 96-well plates (5 islets/well, 8 replicates/sample), and preincubated (30 min, 37°C) with KRBB containing 2.8 mM glucose and 0.25% (w/v) BSA. The islets were then incubated at 37°C for 1 h with KRBB containing either 2.8 or 25 mM glucose. The insulin concentration in the culture medium was quantified using a Rat Insulin Radioimmunoassay Kit (MilliporeSigma) with 100% cross-reactivity to mouse insulin. Insulin secreted into the cell culture medium was expressed as % islet total insulin content and normalized to basal levels as previously described (22). For determination of GSIS *in vivo*, β-DKO mice were treated with apoA-I (8 mg/kg) or an equivalent volume of PBS twice weekly for 4 wk and had food withheld for 5 h before an intraperitoneal injection of D-glucose (3 g/kg). Blood was sampled from the lateral tail vein at 0, 5, 10, and 15 min. Plasma insulin concentrations were determined by ELISA (MilliporeSigma).

Whole-genome microarrays and real-time PCR

Islet total RNA was isolated using Qiazol miRNeasy Kits (Qiagen, Valencia, CA, USA) and quantified by spectrophotometry (NanoDrop; Thermo Fisher Scientific). Gene expression profiling was determined using Affymetrix GeneChip microarrays according to the manufacturer's protocol (Thermo Fisher Scientific). Total RNA (1 μg) was reverse transcribed (T-7 dT primers), amplified, and biotinylated (Affymetrix IVT Labeling Kit; Thermo Fisher Scientific). The samples were hybridized to GeneChip Mouse Gene 2.0 ST whole-genome arrays (35,240 genes) (Thermo Fisher Scientific), washed, stained (Affymetrix Fluidics Station), and scanned (Affymetrix GeneChip Scanner). The data were analyzed with Partek Microarray Data Analysis (Partek, Singapore, Singapore) and pathway analyses were performed using Ingenuity Pathway Analysis software (Qiagen, Venlo, The Netherlands). Differentially expressed mRNAs were identified using prespecified threshold values of >1.2 or <-1.2-fold change and a false discovery rate of $P \leq 0.05$. Differential expression of selected genes was validated by real-time PCR using the iQ SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) as previously described (14). Relative quantitative values (RQVs) in mRNA expression were determined as $2^{[Ct(\text{gene of interest}) - Ct(\text{GAPDH})]}$. Primer sequences are shown in **Table 1**.

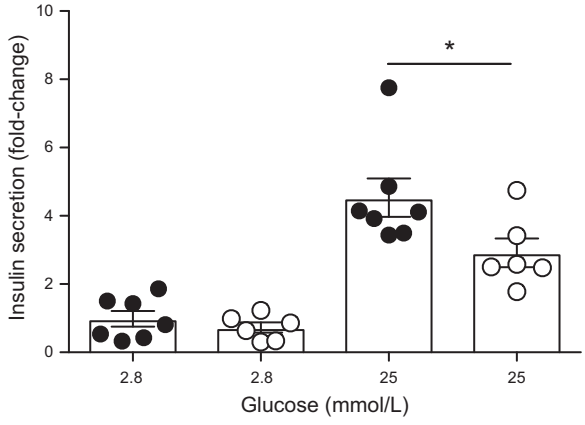

Figure 1. GSIS is impaired in β-DKO mouse islets. Islets from 16-wk-old *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ (black circles) and β-DKO mice (open circles) were isolated and incubated for 1 h with 2.8 or 25 mM glucose. GSIS was quantified by radioimmunoassay. Values are expressed as the percentage insulin secreted relative to total islet insulin content and normalized to 1 for mice maintained under basal glucose (2.8 mM) conditions. Fold change in insulin secretion in response to 25 mM glucose is shown; n = 6–7/group. Data were compared by 2-way ANOVA with Bonferroni's posttest. *$P < 0.05$.

Statistical analysis

All statistical analyses were performed using Prism v.6 (GraphPad Software, La Jolla, CA, USA). Nonlinear fit analysis was performed on all time-course data. Comparisons were made by 2-way ANOVA with Bonferroni's posttest or with a Mann-Whitney test, where appropriate. A 2-tailed value of $P < 0.05$ was considered significant. All results are presented as means ± SEM unless otherwise specified.

RESULTS

GSIS is impaired in β-DKO mouse islets

Insulin secretion was comparable when isolated islets from *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice (**Fig. 1**, closed symbols) and β-DKO mice (Fig. 1, open circles) were incubated in the presence of 2.8 mM glucose. When isolated islets from *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice were incubated under high glucose (25 mM) conditions, insulin secretion increased by 3.6-fold, from 1.0 ± 0.2 to 4.5 ± 0.6% of the total islet insulin content (Fig. 1, black circles). Insulin secretion also increased when islets from β-DKO mice were incubated with 25 mM glucose (Fig. 1). However, insulin secretion from the isolated β-DKO mouse islets (3.0 ± 0.4% of the total islet insulin content) was impaired relative to what was observed for the isolated *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets (4.5 ± 0.6% of the total islet insulin content) (Fig. 1, $P < 0.05$). This is consistent with what has been previously reported for mice with conditional deletion of ABCA1 in β cells (24).

ApoA-I treatment improves glucose tolerance in β-DKO mice

As apoA-I improves GSIS in Rat Insulinoma cell line-1E (Ins-1E) and Mouse Insulinoma 6 (MIN6) cells (14, 15), we asked whether this is also the case *in vivo*. β-DKO mice were treated with human apoA-I (8 mg/kg, i.p.) or PBS as described in Materials and Methods. The plasma concentration of human apoA-I was maximal at 4 h after injection (21.7 ± 2.8 μg/ml) and decreased to 9.4 ± 1.4 μg/ml by 24 h (**Fig. 2*A***). As the average plasma concentration of

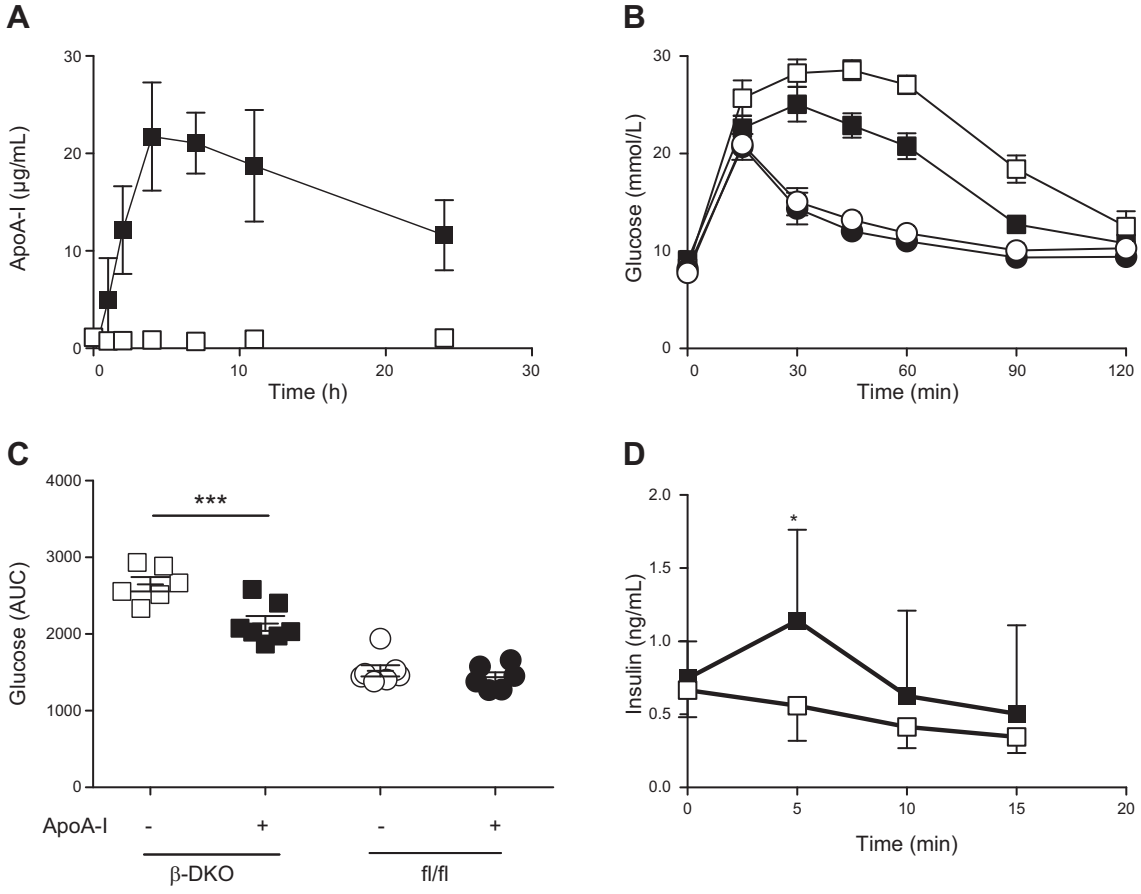

Figure 2. ApoA-I treatment improves glucose tolerance and GSIS in β-DKO mice. Twelve-week-old β-DKO mice and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice were treated intraperitoneally with apoA-I (8 mg/kg, i.p.) or PBS twice weekly for 4 wk (n = 4–8/group). At 24 h after the final injection, the mice had food withheld for 5 h and were then injected with glucose [2 g/kg, i.p., for glucose tolerance test (GTT), 3 g/kg, i.p., for GSIS]. *A*) Plasma levels of human apoA-I in β-DKO mouse plasma treated with apoA-I (closed symbols) or PBS (open symbols) as a function of time. *B*) Blood glucose in levels β-DKO mice (squares) and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice (circles) treated with apoA-I (black symbols) or PBS (open symbols) as a function of time. *C*) Incremental AUC (means ± SEM) for β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice treated with apoA-I or PBS. *D*) Plasma insulin levels at 5–15 min following a glucose challenge in β-DKO mice treated with apoA-I (closed symbols) or PBS (open symbols). Data were compared by 2-way ANOVA with Bonferroni's posttest. *$P < 0.05$, ***$P < 0.001$ for β-DKO mice treated with apoA-I *vs.* PBS.

endogenous apoA-I in wild-type mice is ~1.5 mg/dl, it follows that human apoA-I constituted only a minor proportion of the total circulating apoA-I in the apoA-I-treated β-DKO mice throughout the study. Nevertheless, the slow rate at which the injected human apoA-I was cleared from the circulation in β-DKO mice means that islets in these animals were exposed to human apoA-I for most of the treatment period.

Treatment with apoA-I improved glucose tolerance in the β-DKO mice [Fig. 2*B, C*, black squares, area under the curve (AUC) 2137 ± 97] compared with PBS-treated β-DKO mice (Fig. 2*B, C*, open squares, AUC 2649 ± 94), ($P < 0.001$). Glucose tolerance in the *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice was comparable irrespective of whether the animals were treated with PBS (Fig. 2*B, C*, open circles) or apoA-I (Fig. 2*B, C*, black circles). There was a small, nonsignificant increase in islet insulin levels in the apoA-I-treated β-DKO mice relative to apoA-I-treated *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice (18.6 ± 3.3 *vs.* 14.2 ± 3.0 ng/mg protein). Plasma insulin levels in apoA-I-treated. β-DKO mice, by contrast, increased rapidly during an intraperitoneal glucose tolerance test (Fig. 2*D*). At 5 min after glucose challenge, the plasma insulin concentration was 1.14 ± 0.62 *vs.* 0.56 ± 0.24 ng/ml in the apoA-I-treated and control β-DKO mice, respectively ($P < 0.05$) (Fig. 2*D*). As we have reported that insulin sensitivity is normal in β-DKO mice (25), it follows that the enhanced glycemic control in these animals most likely reflects an apoA-I-mediated improvement in β-cell function.

This was addressed directly in β-DKO mice that were treated twice weekly for 4 wk with apoA-I or PBS. Islets were isolated immediately after the animals were euthanized, and GSIS was assessed as described in Materials and Methods. Insulin secretion in islets isolated from the apoA-I-treated (**Fig. 3**, black symbols) and PBS-treated β-DKO (Fig. 3, open symbols) was comparable under low glucose (2.8 mM) conditions. Under high glucose (25 mM) conditions, insulin secretion increased by 7.2 ± 1.1-fold in the islets isolated from apoA-I-treated β-DKO mice, compared with 2.9 ± 0.4-fold for the PBS-treated β-DKO mice (Fig. 3, $P < 0.001$).

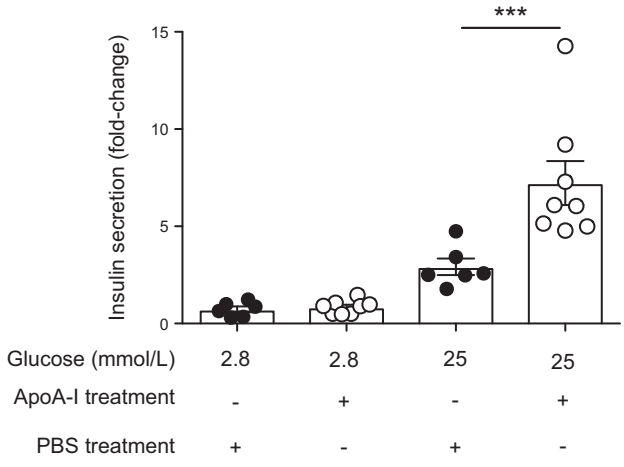

Figure 3. ApoA-I treatment improves GSIS in isolated β-DKO mouse islets. Twelve-week-old β-DKO mice were treated with PBS (black circles) or apoA-I (8 mg/kg, i.p., open symbols) twice weekly for 4 wk and euthanized 24 h after the final injection. Islets were isolated and incubated for 1 h with 2.8 or 25 mM glucose. GSIS was quantified by radioimmunoassay. Insulin levels in the culture medium were normalized to total islet insulin content (n = 6–8/group). Values are expressed as the percentage insulin secreted relative to total islet insulin content and normalized to 1 for mice maintained under basal glucose (2.8 mM) conditions. Fold change in insulin secretion in response to 25 mM glucose is shown. Data were compared by 2-way ANOVA with Bonferroni's posttest. ***$P < 0.001$.

ApoA-I treatment does not affect plasma lipid, plasma lipoprotein, or islet cholesterol levels in β-DKO mice

As we have previously reported that total cholesterol levels in β-DKO mouse islets are increased 2-fold relative to total cholesterol levels in *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets (25), we next asked whether the increased GSIS in islets isolated from the apoA-I-treated β-DKO mice could be explained in terms of excess cholesterol being exported to apoA-I in an ABCA1/ABCG1-independent manner. Islet cholesterol levels in the apoA-I- and PBS-treated β-DKO mice were comparable (20.5 ± 1.4 μg cholesterol/μg protein for apoA-I-treated β-DKO mice *vs.* 25.5 ± 5.2 μg cholesterol/μg protein for PBS-treated β-DKO mice). There were also no statistically significant differences in plasma total cholesterol, unesterified cholesterol, triglyceride, and HDL cholesterol levels between the β-DKO mice and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice, irrespective of whether they were treated with apoA-I or PBS (Supplemental Table S1). The distribution of cholesterol in plasma lipoproteins was also comparable for the β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice (Supplemental Fig. S1). Collectively, these results indicate that the mechanism by which apoA-I improves GSIS in β-DKO mice is not related to changes in islet cholesterol levels.

Differential gene expression in β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets

To determine whether conditional deletion of ABCA1 and ABCG1 in β cells affects key metabolic pathways in islets, isolated islets from β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mice were subjected to microarray and principal component analysis. *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ (blue dots) and β-DKO mouse islets (red dots) had distinct gene expression profiles as shown on the 3-dimensional principal component analysis plot (Supplemental Fig. S2*A*). A total of 4216 genes (2617 down-regulated and 1599 up-regulated, fold change ≥1.2, $P < 0.05$) were differentially expressed in β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets (Supplemental Fig. 2*B*). Supplemental Tables S2 and S3 show selected up- and down-regulated genes, respectively, with an absolute fold change ≥1.5 and $P < 0.05$.

Functional enrichment analysis of differentially expressed genes in the β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets indicated that the ABCA1 and ABCG1 in β cells regulate pathways involved in cholesterol

metabolism, with the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (*Hmgcr*), low-density lipoprotein receptor (*Ldlr*), insulin-induced gene 1 (*Insig1*), and sterol regulatory element binding factor 2 (*Srebf2*) genes all being significantly down-regulated (**Table 2**). Genes involved in glucose metabolism, including cGMP-dependent protein kinase type I (*Prkg1*); solute carrier family 2, member 2 (*Glut2*); and insulin receptor substrate 1 (*Irs1*), were also down-regulated, whereas *Glut1* was up-regulated (Table 2). Genes related to inflammation, including *Il-1β*, guanylate binding protein 11 (*Gbp11*), and surfactant-associated protein D (*Sftpd*), were also up-regulated in β-DKO mouse islets relative to islets from $Abca1^{fl/fl}Abcg1^{fl/fl}$ mice (Table 2). Differential expression of these genes was validated by real-time PCR (**Figs. 4–6**).

Abca1 and *Abcg1* mRNA levels were decreased by 45 ± 4% (Fig. 4*A*) and 35 ± 2.6% (Fig. 4*B*), respectively, in β-DKO mouse islets relative to $Abca1^{fl/fl}Abcg1^{fl/fl}$ mouse islets ($P < 0.01$ for both). The residual *Abca1* and *Abcg1* mRNA in β-DKO mouse islets likely reflects expression in endocrine pancreas cells other than β cells. Validation of differentially expressed cholesterol metabolism genes in β-DKO and $Abca1^{fl/fl}Abcg1^{fl/fl}$ mouse islets revealed a 58± 4.8% decrease in *Hmgcr* mRNA levels (Fig. 4*C*, $P < 0.01$), a 52 ± 4.5% decrease in *Ldlr* mRNA levels (Fig. 4*D*, $P < 0.01$), a 55 ± 4.5% decrease in *Insig1* mRNA levels (Fig. 4*E*, $P < 0.01$), a 57 ± 1.7% decrease in *Srebf2* mRNA levels (Fig. 4*F*, $P < 0.01$), and a 35 ± 8% decrease in fatty acid synthase (*Fasn*) mRNA levels (Fig. 4*G*, $P < 0.05$).

Differentially expressed genes involved in glucose metabolism were also validated in β-DKO and $Abca1^{fl/fl}Abcg1^{fl/fl}$ mouse islets. There was a 63 ± 7.9% decrease in Peroxisome proliferator-activated receptor gamma coactivator 1-α (*Ppargc1a*) mRNA levels (Fig. 5*A*), a 61 ± 3.9% decrease in *Prkg1* mRNA levels (Fig. 5*B*), a 45 ± 7.9% decrease in *Glut2* mRNA levels (Fig. 5*C*), and a 37 ± 5.4% decrease in phosphoinositide-3-kinase regulatory subunit 1 (*Pik3r1*) mRNA levels (Fig. 5*D*) ($P < 0.01$ for all). Thymoma viral proto-oncogene 1 (*Akt1*) mRNA levels increased by 76 ± 11% (Fig. 5*E*, $P < 0.01$), and there was an 88 ± 23% increase in *Glut1* mRNA levels (Fig. 5*F*, $P < 0.05$). There was an apparent decrease in *Irs1* mRNA levels (Fig. 5*H*) in the β-DKO mouse islets relative to the $Abca1^{fl/fl}Abcg1^{fl/fl}$ mouse islets, but this did not reach statistical significance. β-DKO mice and $Abca1^{fl/fl}Abcg1^{fl/fl}$ mouse islets had comparable forkhead box A2 (*Foxa2*) (Fig. 5*I*) and *Glut4* (Fig. 5*G*) mRNA levels. Glucagon and somatostatin mRNA levels were also unchanged (data not shown).

The most highly up-regulated genes involved in inflammation, *Sftpd* and *Gbp11*, and *Il-β* were also validated. *Il1β* mRNA levels in the β-DKO mouse islets were increased by 136 ± 17% compared with $Abca1^{fl/fl}Abcg1^{fl/fl}$ mouse islets (Fig. 6*A*, $P < 0.01$). *Sftpd* mRNA levels were increased by 10,000 ± 27% in β-DKO mouse islets compared with $Abca1^{fl/fl}Abcg1^{fl/fl}$ mouse islets (Fig. 6*B*,

TABLE 2. *Differential expression of selected genes in islets from β-DKO and* $Abca1^{fl/fl}Abcg1^{fl/fl}$ *mice*

Pathway	Gene	Fold change	*P*	Regulation	Gene description
Cholesterol metabolism pathway	*Hmgcr*	−1.66	1.50E−02	Down	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
	Ldlr	−1.53	1.87E−02	Down	LDL receptor
	Abca1	−1.48	8.49E−03	Down	ATP-binding cassette, subfamily A (ABC1), member 1
	Insig1	−1.45	4.01E−02	Down	Insulin-induced gene 1
	Srebf2	−1.39	4.04E−02	Down	Sterol regulatory element binding factor 2
	Abcg1	−1.34	2.27E−02	Down	ATP-binding cassette, subfamily G (WHITE), member 1
	Fasn	−1.21	1.49E−02	Down	Fatty acid synthase
Glucose metabolism pathway	*Ppargc1a*	−1.86	1.54E−03	Down	Peroxisome proliferator activated receptor γ coactivator 1 α
	Prkg1	−1.77	6.37E−03	Down	Protein kinase, cGMP-dependent, type I
	Glut2	−1.43	1.52E−03	Down	Solute carrier family 2 (facilitated glucose transporter), member 2
	Irs1	−1.28	9.78E−03	Down	Insulin receptor substrate 1
	Pik3r1	−1.25	3.38E−03	Down	PI3K, regulatory subunit, polypeptide 1
	Foxa2	−1.24	4.99E−03	Down	Forkhead box A2
	Glut4	1.22	3.30E−02	Up	Solute carrier family 2 (facilitated glucose transporter), member 4
	Akt1	1.29	3.64E−02	Up	Thymoma viral proto-oncogene 1
	Glut1	1.48	3.62E−02	Up	Solute carrier family 2 (facilitated glucose transporter), member 1
Inflammation pathway	*Il1b*	1.56	4.79E−02	Up	IL-1 β
	Sftpd	6.79	1.98E−02	Up	Surfactant-associated protein D
	Gbp11	10.56	2.18E−02	Up	Guanylate binding protein 11

Islets were collected from 16-wk-old β-DKO mice and $Abca1^{fl/fl}Abcg1^{fl/fl}$ mice ($n = 5$/group). Total RNA was extracted and subjected to Affymetrix GeneChip Mouse Gene ST 2.0 analysis. Whole-genome microarray data are presented. Fold change ≥1.2-fold, $P < 0.05$.

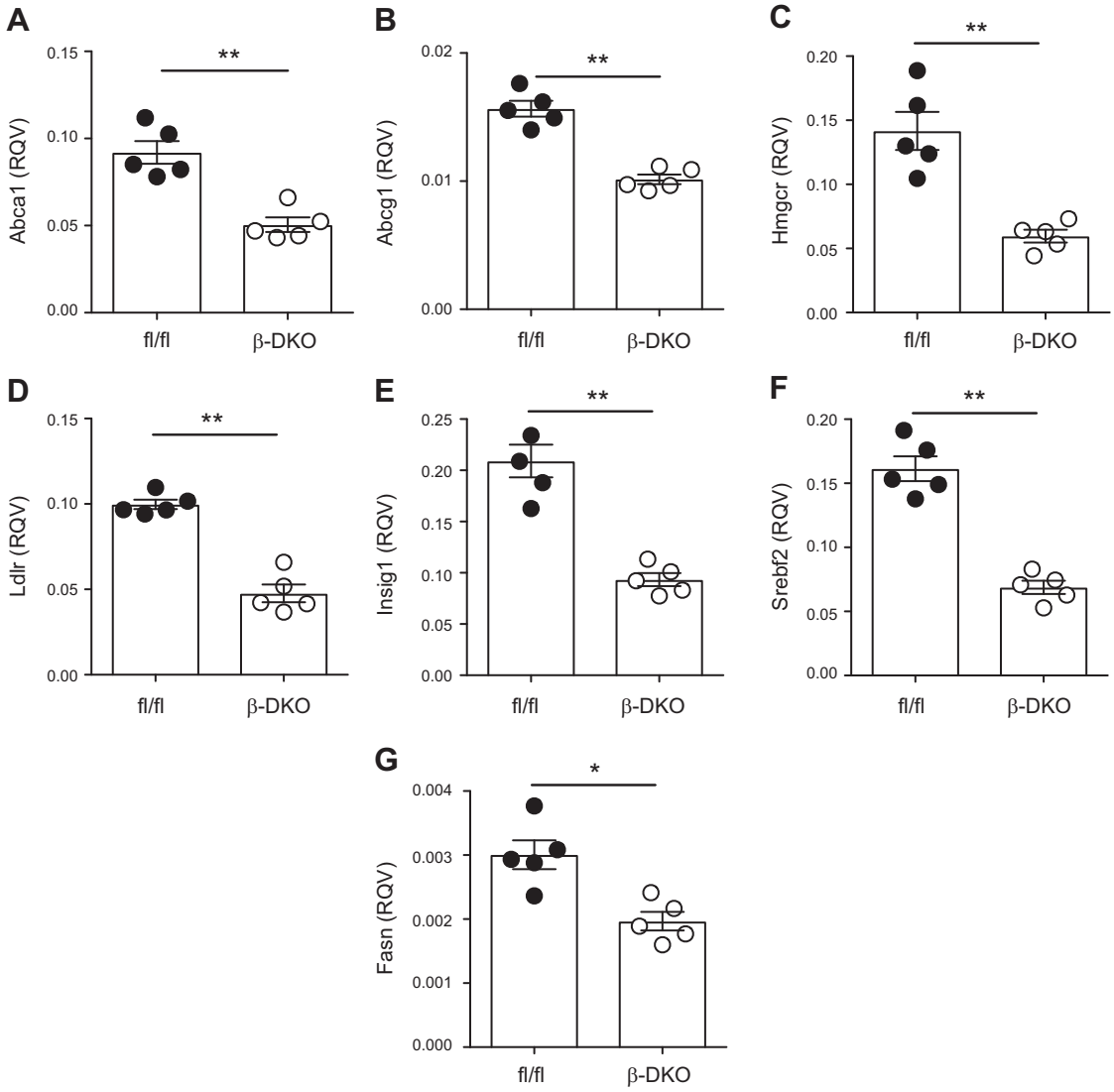

Figure 4. Differential expression of cholesterol metabolism genes in β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets. Islets were isolated from 16-wk-old *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ and β-DKO mice and total RNA was extracted. *Abca1* (*A*), *Abcg1* (*B*), *Hmgcr* (*C*), *Ldlr* (*D*), *Insig1* (*E*), *Srebf2* (*F*), and *Fasn* (*G*) mRNA levels were validated by real-time PCR. All data (means ± SEM) are shown as RQVs normalized to *Gapdh* (n = 4–5/group). Data were compared by Mann-Whitney test. *$P < 0.05$, **$P < 0.01$.

$P < 0.01$), whereas *Gbp11* mRNA levels increased by 84,000 ± 487% (Fig. 6C). The increased inflammation in isolated islets from β-DKO mice is consistent with what has been previously reported in ABCG1 knockout mice with conditional deletion of ABCA1 in β cells (29).

Treatment of β-DKO mice with apoA-I did not significantly change expression of any of the up- or down-regulated genes involved in cholesterol metabolism (Supplemental Table S4), glucose metabolism (Supplemental Table S5), or inflammation (Supplemental Table S6) in isolated islets.

DISCUSSION

This study establishes apoA-I as a treatment that improves glycemic control and β-cell function in β-DKO mice and increases *ex vivo* GSIS in isolated islets. It also establishes that expression of key genes involved in cholesterol metabolism, glucose metabolism, and inflammation are differentially regulated in β-DKO mice relative to *Abca1*$^{fl/fl}$ *Abcg1*$^{fl/fl}$ control mice. The results further reveal that treatment with apoA-I does not affect plasma lipid or lipoprotein levels in β-DKO mice.

One of the most unexpected findings to emerge from this study is that treatment of β-DKO mice for 4 wk with apoA-I markedly increases GSIS without significantly altering islet cholesterol levels. Whether this is also the case for β-DKO mice treated acutely with a single injection of apoA-I remains to be determined. However, because a single 14 mg/kg apoA-I injection has been reported to improve GSIS in normal and high-fat fed C57BL6 mice (16), it seems reasonable to assume that apoA-I may have a similar effect in β-DKO mice.

Cholesterol accumulation in mice with conditional deletion of ABCA1 in β cells is associated with impaired exocytosis of insulin secretory granules (30). As ABCG1 maintains cholesterol homeostasis in insulin secretory

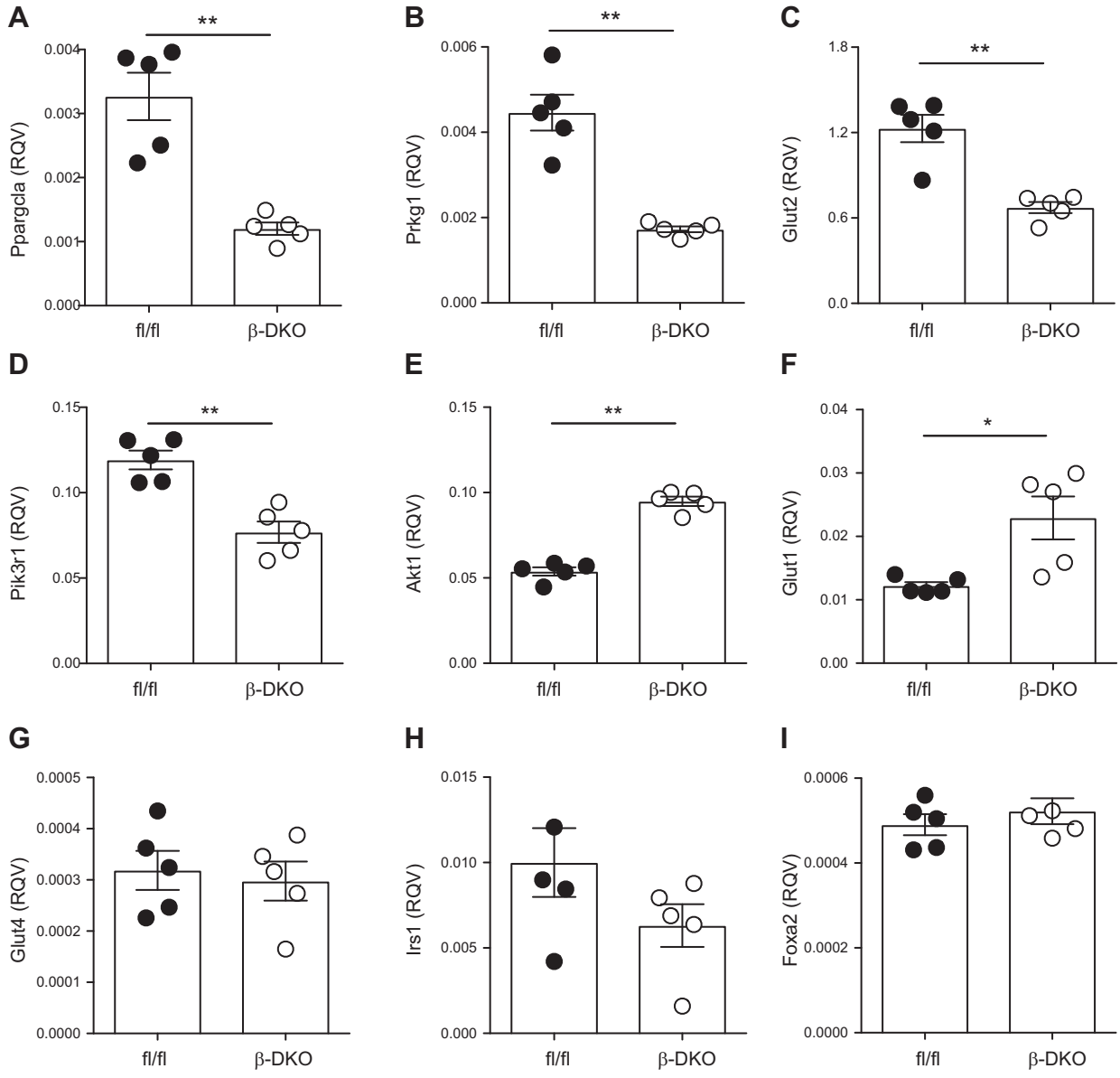

Figure 5. Differential expression of glucose metabolism genes in β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets. Islets were isolated from *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ and β-DKO mice and mRNA levels were quantified as described in the caption to Fig. 4. *Ppargc1a* (*A*), *Prkg1* (*B*), *Glut2* (*C*), *Pik3r1* (*D*), *Akt1* (*E*), *Glut1* (*F*), *Glut4* (*G*), *Irs1* (*H*), and *Foxa2* (*I*) were validated by real-time PCR. RQVs normalized to *Gapdh* (means ± SEM) are shown (n = 4–5/group). Data were compared by Mann-Whitney test. *$P < 0.05$, **$P < 0.01$.

granules, and granule cholesterol levels and maturation are decreased in its absence, granule exocytosis is likely to be further impaired when ABCA1 and ABCG1 are both conditionally deleted from β cells, as we have reported in β-DKO mice (23). A potential explanation for the increased GSIS in isolated islets from apoA-I-treated β-DKO mice is that, as has been reported for endothelial cells (31), apoA-I transcytosed across the β-cell membrane and acquired cholesterol from intracellular organelles *via* aqueous diffusion. Delivery of the newly acquired cholesterol from the

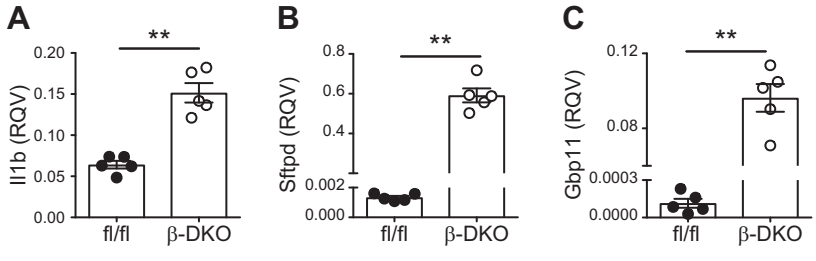

Figure 6. Differential expression of proinflammatory genes in β-DKO and *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ mouse islets. Islets were isolated from *Abca1*$^{fl/fl}$*Abcg1*$^{fl/fl}$ and β-DKO mice, and mRNA levels were quantified as described in the Fig. 4 legend. *Il1b* (*A*), *Sftpd* (*B*), and *Gbp11* (*C*) were validated by real-time PCR. RQVs normalized to *Gapdh* (means ± SEM) are shown (n = 5/group). Data were compared by Mann-Whitney test. **$P < 0.01$.

transcytosed apoA-I to the granule membrane could override the loss of ABCG1 by restoring cholesterol homeostasis and correcting granule insulin secretory function.

We have previously reported that apoA-I increases insulin secretion in the Ins-1E rat insulinoma cell line by translocating FoxO1 out of the nucleus and derepressing insulin gene transcription (15). However, cellular cholesterol levels were not elevated in that study, and the increase in insulin secretion was dependent on ABCA1 expression. This suggests that the mechanism by which apoA-I improves insulin secretion in the current study is distinct from what we previously described and warrants further investigation, as does the possibility that other insulin secretagogues also mediate this effect.

The present study establishes that conditional deletion of ABCA1 and ABCG1 in β cells regulates expression of genes in islets that are involved in cholesterol metabolism, glucose metabolism, and inflammation. Expression of the cholesterol metabolism genes, *Hmgcr*, *Ldlr*, *Insig-1*, *Fasn*, and *Srebf2*, was significantly decreased. As *Hmgcr* and *Ldlr* are key genes in the cholesterol biosynthesis pathway, their down-regulation was expected in β-DKO mice with elevated islet cholesterol levels. This is also in agreement with what has been reported for mice with conditional β-cell deletion of ABCA1 (24). Studies of transgenic and knockout mice have shown that sterol regulatory element-binding transcription factor 2 (SREBP2) activation plays a key role in regulation of cholesterol synthesis and metabolism (32) and increases cell cholesterol levels by up-regulating transcription of target genes including *Hmgcr*, *Ldlr*, *Insig-1*, *Srebf2*, and *Fasn*. However, high intracellular cholesterol levels can lead to SREBP2 retention in the endoplasmic reticulum and inhibition of *Srebf2* gene transcription (33). This indicates that the reduced expression of SREBP2 and its target genes in the current study may be a direct consequence of the increased islet cholesterol content in β-DKO mice.

Deletion of ABCA1 and ABCG1 in β cells was also accompanied by reduced expression of the glucose metabolism genes, *Ppargc1a*, *Prkg1*, *Glut2*, and *Pik3r1*, in β-DKO mouse islets. *Ppargc1a* encodes for peroxisome proliferator activated receptor γ coactivator 1 α which regulates mitochondrial genes that increase oxidative phosphorylation and ATP production (34). Decreased peroxisome proliferator activated receptor γ coactivator 1 α mRNA levels have been reported in islets from patients with type 2 diabetes mellitus and in rats with impaired insulin secretion (35). *Prkg1* encodes for the cGMP-dependent protein kinase type I, which is involved in the NO/cGMP-dependent protein kinase type I signaling pathway and the pathogenesis of T2D (36).

GLUT2 mediates glucose uptake into pancreatic β cells in mice (37). GLUT2 knockout mice have impaired glucose signaling and reduced insulin biosynthesis and secretion (38). However, β cells in GLUT2 knockout mice have normal glucokinase (Gck) expression, indicating that GLUT2 is not essential for Gck function (38). This is consistent with the present results, in which *Gck* mRNA levels in β-DKO mouse islets were unaffected (data not shown) despite a reduction in *Glut2* mRNA levels.

PIK3R1, the p85 α regulatory subunit of PI3K, has been implicated in the development of T2D (39). Reduced *Pik3r1* gene expression ameliorates insulin resistance and macrophage accumulation in adipose tissue in obese mice (40). Deletion of the *Pik3r1* gene also improves insulin signaling and activation of IRS1 in obese, insulin-resistant mice (40). When taken together, these results suggest that the decreased expression of *Ppargc1a*, *Prkg1*, *Glut2*, and *Pik3r1* may act to reduce glucose intolerance and impaired insulin secretion in β-DKO mice. These results do not provide insights into the relative contributions of these genes to impaired glycemic control in β-DKO mice. However, they do raise the possibility that at least some of these genes, and their encoded proteins, may act synergistically to regulate β-cell function.

The increased expression of the *Il1β*, *Sftpd*, and *Gbp11* genes in β-DKO mouse islets is also of interest. IL-1β is a proinflammatory cytokine that contributes to glucotoxity in islets (41). Increased expression of this gene in β-DKO mouse islets is consistent with what has been reported in islets from ABCG1 knockout mice with conditional β-cell deletion of ABCA1 (29). It also indicates that β-DKO mice may have insulitis, as can occur in patients with T1D and T2D. *Sftpd* and *Gbp11* were the most highly differentially expressed proinflammatory genes in β-DKO mouse islets. *Sftpd* encodes for surfactant pulmonary-associated protein D, which contributes to surfactant homeostasis and pulmonary immunity (42). *Gbp11* encodes for Gbp11, which contributes to interferon responses in a variety of organisms (43). As previous reports have not implicated expression of either of these genes in β-cell inflammation, further examination of this relationship may provide novel insights into the origins and mechanistic basis of the β-cell inflammation that has been reported in people with diabetes (1).

In summary, this study establishes for the first time that conditional deletion of ABCA1 and ABCG1 in mouse pancreatic β cells perturbs islet cholesterol metabolism and glucose metabolism and increases expression of genes that cause inflammation. It also establishes that treatment with apoA-I improves glucose tolerance in β-DKO mice and increases GSIS in β-DKO mouse islets. Whether these beneficial effects of apoA-I reflect the restoration of β-cell cholesterol homeostasis in β-DKO mouse islets remains to be determined. FJ

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AUTHOR CONTRIBUTIONS

L. Hou, B. J. Cochran, F. Tabet, and K.-A. Rye were responsible for the experimental design and data analysis; L. Hou carried out the experiments; S. Tang, B. J. Wu, K.-L. Ong, and B. J. Cochran assisted with the experiments and data analysis; M. Westerterp, P. J. Barter, F. Tabet, K.-A. Rye, and F. Tabet and K.-A. Rye participated in manuscript preparation; and B. J. Cochran, F. Tabet, and K.-A. Rye are the guarantors of this work and, as such, have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1. Atkinson, M. A., Eisenbarth, G. S., and Michels, A. W. (2014) Type 1 diabetes. *Lancet* **383**, 69–82
2. Xu, G., Liu, B., Sun, Y., Du, Y., Snetselaar, L. G., Hu, F. B., and Bao, W. (2018) Prevalence of diagnosed type 1 and type 2 diabetes among US adults in 2016 and 2017: population based study. *BMJ* **362**, k1497
3. Maahs, D. M., West, N. A., Lawrence, J. M., and Mayer-Davis, E. J. (2010) Epidemiology of type 1 diabetes. *Endocrinol. Metab. Clin. North Am.* **39**, 481–497
4. Haffner, S. M., Lehto, S., Rönnemaa, T., Pyörälä, K., and Laakso, M. (1998) Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N. Engl. J. Med.* **339**, 229–234
5. Prentki, M., and Nolan, C. J. (2006) Islet beta cell failure in type 2 diabetes. *J. Clin. Invest.* **116**, 1802–1812
6. Brun, T., and Maechler, P. (2016) Beta-cell mitochondrial carriers and the diabetogenic stress response. *Biochim. Biophys. Acta* **1863**, 2540–2549
7. Gerber, P. A., and Rutter, G. A. (2017) The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. *Antioxid. Redox Signal.* **26**, 501–518
8. Hasnain, S. Z., Prins, J. B., and McGuckin, M. A. (2016) Oxidative and endoplasmic reticulum stress in β-cell dysfunction in diabetes. *J. Mol. Endocrinol.* **56**, R33–R54
9. Poitout, V., and Robertson, R. P. (2008) Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr. Rev.* **29**, 351–366
10. Brunham, L. R., Kruit, J. K., Hayden, M. R., and Verchere, C. B. (2010) Cholesterol in beta-cell dysfunction: the emerging connection between HDL cholesterol and type 2 diabetes. *Curr. Diab. Rep.* **10**, 55–60
11. Ishikawa, M., Iwasaki, Y., Yatoh, S., Kato, T., Kumadaki, S., Inoue, N., Yamamoto, T., Matsuzaka, T., Nakagawa, Y., Yahagi, N., Kobayashi, K., Takahashi, A., Yamada, N., and Shimano, H. (2008) Cholesterol accumulation and diabetes in pancreatic beta-cell-specific SREBP-2 transgenic mice: a new model for lipotoxicity. *J. Lipid Res.* **49**, 2524–2534
12. Hao, M., Head, W. S., Gunawardana, S. C., Hasty, A. H., and Piston, D. W. (2007) Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction. *Diabetes* **56**, 2328–2338
13. Di Angelantonio, E., Sarwar, N., Perry, P., Kaptoge, S., Ray, K. K., Thompson, A., Wood, A. M., Lewington, S., Sattar, N., Packard, C. J., Collins, R., Thompson, S. G., and Danesh, J.; Emerging Risk Factors Collaboration. (2009) Major lipids, apolipoproteins, and risk of vascular disease. *JAMA* **302**, 1993–2000
14. Fryirs, M. A., Barter, P. J., Appavoo, M., Tuch, B. E., Tabet, F., Heather, A. K., and Rye, K. A. (2010) Effects of high-density lipoproteins on pancreatic beta-cell insulin secretion. *Arterioscler. Thromb. Vasc. Biol.* **30**, 1642–1648
15. Cochran, B. J., Bisoendial, R. J., Hou, L., Glaros, E. N., Rossy, J., Thomas, S. R., Barter, P. J., and Rye, K. A. (2014) Apolipoprotein A-I increases insulin secretion and production from pancreatic β-cells via a G-protein-cAMP-PKA-FoxO1-dependent mechanism. *Arterioscler. Thromb. Vasc. Biol.* **34**, 2261–2267
16. Domingo-Espín, J., Lindahl, M., Nilsson-Wolanin, O., Cushman, S. W., Stenkula, K. G., and Lagerstedt, J. O. (2016) Dual actions of apolipoprotein A-I on glucose-stimulated insulin secretion and insulin-independent peripheral tissue glucose uptake lead to increased heart and skeletal muscle glucose disposal. *Diabetes* **65**, 1838–1848
17. Cochran, B. J., Ryder, W. J., Parmar, A., Tang, S., Reilhac, A., Arthur, A., Charil, A., Hamze, H., Barter, P. J., Kritharides, L., Meikle, S. R., Gregoire, M. C., and Rye, K. A. (2016) *In vivo* PET imaging with [(^{18})F]FDG to explain improved glucose uptake in an apolipoprotein A-I treated mouse model of diabetes. *Diabetologia* **59**, 1977–1984
18. Drew, B. G., Duffy, S. J., Formosa, M. F., Natoli, A. K., Henstridge, D. C., Penfold, S. A., Thomas, W. G., Mukhamedova, N., de Courten, B., Forbes, J. M., Yap, F. Y., Kaye, D. M., van Hall, G., Febbraio, M. A., Kemp, B. E., Sviridov, D., Steinberg, G. R., and Kingwell, B. A. (2009) High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. *Circulation* **119**, 2103–2111
19. Barter, P. J., Rye, K. A., Tardif, J. C., Waters, D. D., Boekholdt, S. M., Breazna, A., and Kastelein, J. J. (2011) Effect of torcetrapib on glucose, insulin, and hemoglobin A1c in subjects in the investigation of lipid level management to understand its impact in atherosclerotic events (ILLUMINATE) trial. *Circulation* **124**, 555–562
20. Bowman, L., Hopewell, J. C., Chen, F., Wallendszus, K., Stevens, W., Collins, R., Wiviott, S. D., Cannon, C. P., Braunwald, E., Sammons, E., and Landray, M. J.; HPS3/TIMI55–REVEAL Collaborative Group. (2017) Effects of anacetrapib in patients with atherosclerotic vascular disease. *N. Engl. J. Med.* **377**, 1217–1227
21. Oram, J. F., Lawn, R. M., Garvin, M. R., and Wade, D. P. (2000) ABCA1 is the cAMP-inducible apolipoprotein receptor that mediates cholesterol secretion from macrophages. *J. Biol. Chem.* **275**, 34508–34511
22. Kennedy, M. A., Barrera, G. C., Nakamura, K., Baldán, A., Tarr, P., Fishbein, M. C., Frank, J., Francone, O. L., and Edwards, P. A. (2005) ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. *Cell Metab.* **1**, 121–131
23. Sturek, J. M., Castle, J. D., Trace, A. P., Page, L. C., Castle, A. M., Evans-Molina, C., Parks, J. S., Mirmira, R. G., and Hedrick, C. C. (2010) An intracellular role for ABCG1-mediated cholesterol transport in the regulated secretory pathway of mouse pancreatic beta cells. *J. Clin. Invest.* **120**, 2575–2589
24. Brunham, L. R., Kruit, J. K., Pape, T. D., Timmins, J. M., Reuwer, A. Q., Vasanji, Z., Marsh, B. J., Rodrigues, B., Johnson, J. D., Parks, J. S., Verchere, C. B., and Hayden, M. R. (2007) Beta-cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment. *Nat. Med.* **13**, 340–347
25. Cochran, B. J., Hou, L., Manavalan, A. P., Moore, B. M., Tabet, F., Sultana, A., Cuesta Torres, L., Tang, S., Shrestha, S., Senanayake, P., Patel, M., Ryder, W. J., Bongers, A., Maraninchi, M., Wasinger, V. C., Westerterp, M., Tall, A. R., Barter, P. J., and Rye, K. A. (2016) Impact of perturbed pancreatic β-cell cholesterol homeostasis on adipose tissue and skeletal muscle metabolism. *Diabetes* **65**, 3610–3620; erratum: 66, 560
26. Weisweiler, P. (1987) Isolation and quantitation of apolipoproteins A-I and A-II from human high-density lipoproteins by fast-protein liquid chromatography. *Clin. Chim. Acta* **169**, 249–254
27. Iverius, P. H., and Laurent, T. C. (1967) Precipitation of some plasma proteins by the addition of dextran or polyethylene glycol. *Biochim. Biophys. Acta* **133**, 371–373
28. Kritharides, L., Jessup, W., Gifford, J., and Dean, R. T. (1993) A method for defining the stages of low-density lipoprotein oxidation by the separation of cholesterol- and cholesteryl ester-oxidation products using HPLC. *Anal. Biochem.* **213**, 79–89
29. Kruit, J. K., Wijesekara, N., Westwell-Roper, C., Vanmierlo, T., de Haan, W., Bhattacharjee, A., Tang, R., Wellington, C. L., LütJohann, D., Johnson, J. D., Brunham, L. R., Verchere, C. B., and Hayden, M. R. (2012) Loss of both ABCA1 and ABCG1 results in increased disturbances in islet sterol homeostasis, inflammation, and impaired β-cell function. *Diabetes* **61**, 659–664
30. Kruit, J. K., Wijesekara, N., Fox, J. E., Dai, X. Q., Brunham, L. R., Searle, G. J., Morgan, G. P., Costin, A. J., Tang, R., Bhattacharjee, A., Johnson, J. D., Light, P. E., Marsh, B. J., Macdonald, P. E., Verchere, C. B., and Hayden, M. R. (2011) Islet cholesterol accumulation due to loss of ABCA1 leads to impaired exocytosis of insulin granules. *Diabetes* **60**, 3186–3196
31. Cavelier, C., Ohnsorg, P. M., Rohrer, L., and von Eckardstein, A. (2012) The β-chain of cell surface F(0)F(1) ATPase modulates apoA-I and HDL transcytosis through aortic endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **32**, 131–139

32. Horton, J. D., Shah, N. A., Warrington, J. A., Anderson, N. N., Park, S. W., Brown, M. S., and Goldstein, J. L. (2003) Combined analysis of oligonucleotide microarray data from transgenic and knockout mice identifies direct SREBP target genes. *Proc. Natl. Acad. Sci. USA* **100**, 12027–12032
33. Bommer, G. T., and MacDougald, O. A. (2011) Regulation of lipid homeostasis by the bifunctional SREBF2-miR33a locus. *Cell Metab.* **13**, 241–247
34. Handschin, C., and Spiegelman, B. M. (2006) Peroxisome proliferator-activated receptor gamma coactivator 1 coactivators, energy homeostasis, and metabolism. *Endocr. Rev.* **27**, 728–735
35. Ling, C., Del Guerra, S., Lupi, R., Rönn, T., Granhall, C., Luthman, H., Masiello, P., Marchetti, P., Groop, L., and Del Prato, S. (2008) Epigenetic regulation of PPARGC1A in human type 2 diabetic islets and effect on insulin secretion. *Diabetologia* **51**, 615–622
36. Kaneko, Y., Ishikawa, T., Amano, S., and Nakayama, K. (2003) Dual effect of nitric oxide on cytosolic Ca^{2+} concentration and insulin secretion in rat pancreatic beta-cells. *Am. J. Physiol. Cell Physiol.* **284**, C1215–C1222
37. Thorens, B., Sarkar, H. K., Kaback, H. R., and Lodish, H. F. (1988) Cloning and functional expression in bacteria of a novel glucose transporter present in liver, intestine, kidney, and beta-pancreatic islet cells. *Cell* **55**, 281–290
38. Guillam, M. T., Dupraz, P., and Thorens, B. (2000) Glucose uptake, utilization, and signaling in GLUT2-null islets. *Diabetes* **49**, 1485–1491
39. Barroso, I., Luan, J., Middelberg, R. P., Harding, A. H., Franks, P. W., Jakes, R. W., Clayton, D., Schafer, A. J., O'Rahilly, S., and Wareham, N. J. (2003) Candidate gene association study in type 2 diabetes indicates a role for genes involved in beta-cell function as well as insulin action. *PLoS Biol.* **1**, E20; erratum: 445
40. McCurdy, C. E., Schenk, S., Holliday, M. J., Philp, A., Houck, J. A., Patsouris, D., MacLean, P. S., Majka, S. M., Klemm, D. J., and Friedman, J. E. (2012) Attenuated Pik3r1 expression prevents insulin resistance and adipose tissue macrophage accumulation in diet-induced obese mice. *Diabetes* **61**, 2495–2505
41. Maedler, K., Sergeev, P., Ris, F., Oberholzer, J., Joller-Jemelka, H. I., Spinas, G. A., Kaiser, N., Halban, P. A., and Donath, M. Y. (2002) Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *J. Clin. Invest.* **110**, 851–860
42. Kishore, U., Greenhough, T. J., Waters, P., Shrive, A. K., Ghai, R., Kamran, M. F., Bernal, A. L., Reid, K. B., Madan, T., and Chakraborty, T. (2006) Surfactant proteins SP-A and SP-D: structure, function and receptors. *Mol. Immunol.* **43**, 1293–1315
43. Vestal, D. J., and Jeyaratnam, J. A. (2011) The guanylate-binding proteins: emerging insights into the biochemical properties and functions of this family of large interferon-induced guanosine triphosphatase. *J. Interferon Cytokine Res.* **31**, 89–97

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