Loss of Both ABCA1 and ABCG1 Results in Increased Disturbances in Islet Sterol Homeostasis, Inflammation, and Impaired β-Cell Function

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Cellular cholesterol homeostasis is important for normal β-cell function. Disruption of cholesterol transport by decreased function of the ATP-binding cassette (ABC) transporter ABCA1 results in impaired insulin secretion. Mice lacking β-cell ABCA1 have increased islet expression of ABCG1, another cholesterol transporter implicated in β-cell function. To determine whether ABCA1 and ABCG1 have complementary roles in β-cells, mice lacking ABCG1 and β-cell ABCA1 were generated and glucose tolerance, islet sterol levels, and β-cell function were assessed. Lack of both ABCG1 and β-cell ABCA1 resulted in increased fasting glucose levels and a greater impairment in glucose tolerance compared with either ABCG1 deletion or loss of ABCA1 in β-cells alone. In addition, glucose-stimulated insulin secretion was decreased and sterol accumulation increased in islets lacking both transporters compared with those isolated from knockout mice with each gene alone. Combined deficiency of ABCA1 and ABCG1 also resulted in significant islet inflammation as indicated by increased expression of interleukin-1β and macrophage infiltration. Thus, lack of both ABCA1 and ABCG1 induces greater defects in β-cell function than deficiency of either transporter individually. These data suggest that ABCA1 and ABCG1 each make complimentary and important contributions to β-cell function by maintaining islet cholesterol homeostasis in vivo. *Diabetes* **61:659–664, 2012**

Type 2 diabetes is characterized by both progressive β-cell dysfunction and loss of β-cell mass. Recently, abnormalities of cholesterol metabolism have emerged as a potential contributor to β-cell dysfunction (1,2). In humans, low levels of HDL, which is the predominant acceptor of cellular cholesterol, is a risk factor for the development of type 2 diabetes (3) and is associated with β-cell dysfunction (4). Although multiple mechanisms may explain the protective properties of HDL on β-cell dysfunction, enhancement of cellular cholesterol efflux seems to play a crucial role. Cholesterol accumulation in islets compromises β-cell function and reduces insulin secretion in mice (5–8). In vitro data indicate that HDL-mediated increase in insulin secretion is dependent on the ATP-binding cassette (ABC) transporters ABCA1 and ABCG1 (9). We previously demonstrated that ABCA1 plays a critical role in β-cell cholesterol homeostasis and β-cell function in mice (5). Deletion of ABCA1 specifically in β-cells leads to markedly impaired glucose tolerance, defective insulin secretion, and cholesterol accumulation in islets (5). Islets lacking β-cell ABCA1 have increased expression of the related cholesterol transporter ABCG1, perhaps as a compensatory mechanism to maintain islet cholesterol homeostasis.

ABCG1 promotes cholesterol efflux to HDL and acts sequentially with ABCA1 to remove cellular cholesterol (10). Mice with deletion of ABCG1 also have impaired glucose-induced insulin secretion (11). ABCA1 and ABCG1 have complementary roles in macrophage function (12,13). In addition to massive lipid accumulation, loss of both ABCA1 and ABCG1 in macrophages leads to increased expression of proinflammatory cytokines and enhanced susceptibility to apoptosis (13). However, the relative importance of ABCA1 and ABCG1 in islets is thus far unknown.

In this study, we examined whether ABCA1 and ABCG1 have a complimentary role in mediating cholesterol efflux in β-cells. To determine whether loss of both cholesterol transporters induces an exacerbated phenotype compared with loss of either transporter alone, we assessed cholesterol accumulation, glucose tolerance, insulin secretion, and islet inflammation in mice with β-cells deficient in both ABCA1 and ABCG1.

RESEARCH DESIGN AND METHODS

Animals. ABCA1$^{fl/fl}$;Rip-Cre mice (5) and ABCG1$^{-/-}$ mice (Deltagen, San Mateo, CA), both on pure C57Bl/6 backgrounds, were crossed to generate F1 heterozygotes. Heterozygote F1 animals were crossbred to obtain the following mice: ABCA1$^{fl/fl}$;ABCG1$^{+/+}$ (designated in text as "control"), ABCA1$^{fl/fl}$; Rip-Cre;ABCG1$^{+/+}$ (ABCA1$^{-P/-P}$), ABCA1$^{fl/fl}$;ABCG1$^{-/-}$ (ABCG1$^{-/-}$), and ABCA1$^{fl/fl}$;Rip-Cre;ABCG1$^{-/-}$ (ABCA1$^{-P/-P}$;ABCG1$^{-/-}$) mice. We have previously reported that under our experimental conditions, mice with floxed ABCA1 alleles (ABCA1$^{fl/fl}$) or mice transgenic for the RIP-Cre transgene do not have altered glucose homeostasis compared with ABCA1$^{+/+}$ mice (5). All mice were 3–4 months of age. All studies were approved by the University of British Columbia Animal Care Committee.

Physiological and metabolic studies. Intraperitoneal glucose tolerance tests (GTTs) were performed on 4 h–fasted mice injected with 2 g/kg glucose as previously described (5). Insulin secretion during static incubation and perifusion were performed on hand-picked islets isolated after intraductal collagenase injection (5,7). Plasma cholesterol was determined by enzymatic assay (Thermo Electron Corporation). Islet cholesterol, desmosterol, lathosterol, cholestanol, campesterol, and sitosterol were determined by gas-liquid chromatography-mass spectrometry (14).
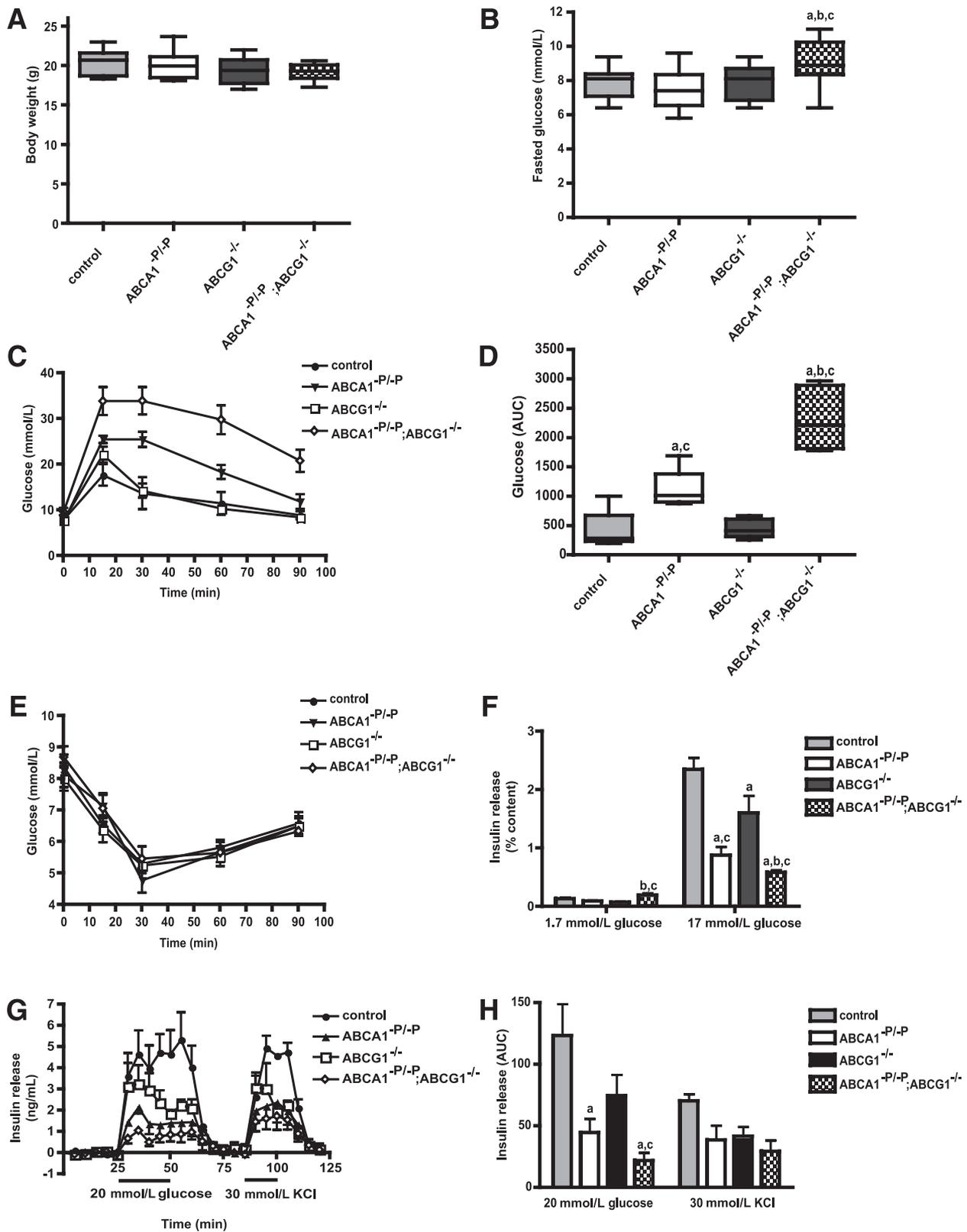
FIG. 1. Loss of β-cell ABCA1 and ABCG1 exacerbates glucose intolerance and β-cell dysfunction. *A*: Body weight of 3-month-old mice (*n* = 8–12 per group). *B*: Fasted plasma glucose levels (*n* = 10–14 per group). *C*: Plasma glucose levels during intraperitoneal GTT (*n* = 5–9). Values represent mean ± SEM. *D*: Area under curve of GTT. *E*: Plasma glucose levels during intraperitoneal insulin tolerance test (*n* = 5–9). Values represent mean ± SEM. *F*: Insulin secretion from isolated islets during static incubation. Islets were cultured overnight and then stimulated for 1 h in the conditions indicated. Values represent pooled data from three separate experiments, each consisting of pooled islets from two mice per genotype. *G*: Insulin release during islet perifusion experiments (*n* = 3 per group). Values represent mean ± SEM. *H*: Area under the curve of insulin release during islet perifusion experiments. [a]$P < 0.05$ versus control; [b]$P < 0.05$ versus ABCA1$^{-P/-P}$; [c]$P < 0.05$ versus ABCG1$^{-/-}$.

Quantitative PCR. Real-time PCR for C/EBP homologous protein (CHOP), interleukin-1β (IL-1β), and F4/80 was performed as previously described (5). Primer sequences are available upon request.

β-Cell mass and immunofluorescence. β-Cell mass was determined from five evenly spaced, paraffin-embedded pancreatic sections as previously described (5). Macrophage staining was performed with antibodies to F4/80 (Cedarlane) and insulin (DAKO) and quantified using ImagePro software (MediaCybernetics). All islets in three sections from different areas of the pancreas were analyzed.

Statistical analysis. Differences between groups were calculated by Kruskal-Wallis test with Conover post-test (for four groups) with a P value of 0.05 considered significant.

RESULTS

Loss of β-cell ABCA1 and ABCG1 exacerbates glucose intolerance and β-cell dysfunction. In order to determine the effects of combined deficiency of ABCA1 and ABCG1 on β-cell function, we crossed mice with β-cell–specific ABCA1 deficiency (ABCA1$^{-P/-P}$) with globally deficient ABCG1 (ABCG1$^{-/-}$) mice to generate ABCA1$^{-P/-P}$;ABCG1$^{-/-}$ double knockout animals. Double knockout mice showed no changes in body weight (Fig. 1A), but had increased fasting glucose levels (Fig. 1B) compared with single knockout mice, which had similar fasting glucose levels as control animals. Lack of ABCA1 specifically in β-cells resulted in glucose intolerance (Fig. 1C and D), as previously reported (5). Although deletion of ABCG1 alone had no significant effect on glucose tolerance, combined deletion of ABCG1 and ABCA1 greatly exacerbated glucose intolerance compared with either ABCG1$^{-/-}$ or ABCA1$^{-P/-P}$ mice alone. Insulin sensitivity was similar in all groups (Fig. 1E).

As reported previously (5), islets lacking β-cell ABCA1 showed decreased glucose-induced insulin secretion during static incubation (Fig. 1F), as well as perifusion experiments (Fig. 1G and H). Both first and second-phase, glucose-stimulated insulin secretion were reduced. Glucose-stimulated insulin secretion was significantly but less markedly decreased in ABCG1$^{-/-}$ mice (Fig. 1F). Notably, islets lacking both ABCA1 and ABCG1 had an even greater reduction in glucose-stimulated insulin secretion compared with islets from mice lacking either transporter (Fig. 1F, G, and H).

Loss of β-cell ABCA1 and ABCG1 exacerbates islet sterol accumulation. As loss of both ABCA1 and ABCG1 leads to massive sterol accumulation in several tissues (15), we measured sterol levels in isolated islets. In agreement with our previous findings (5), loss of β-cell ABCA1 resulted in significant islet cholesterol accumulation (Table 1). Although ABCG1 deletion alone had no effect on cholesterol accumulation, combined loss of both transporters resulted in increased islet cholesterol levels compared with control, ABCA1$^{-P/-P}$, and ABCG1$^{-/-}$ islets (Table 1). Other sterols, such as desmosterol, cholestanol, campesterol, and sitosterol, were also increased in islets lacking both transporters (Table 1). Importantly, plasma cholesterol levels were similar in all groups (control mice, 2.26 ± 0.08 mmol/L; ABCA1$^{-P/-P}$ mice, 2.37 ± 0.07 mmol/L; ABCG1$^{-/-}$ mice, 2.36 ± 0.12 mmol/L; ABCA1$^{-P/-P}$;ABCG1$^{-/-}$ mice, 2.19 ± 0.23 mmol/L).

Increased CHOP expression, but no differences in β-cell mass, in islets lacking ABCA1 and ABCG1. Cholesterol accumulation in macrophages leads to activation of the unfolded protein response and CHOP-induced apoptosis (16). CHOP is an unfolded protein response–induced transcription factor that links endoplasmic reticulum stress to β-cell dysfunction and apoptosis in animal models of type 2 diabetes (17). We found that β-cell–specific deletion of ABCA1 caused a significant increase in CHOP expression in isolated islets, whereas ABCG1 deficiency had no effect (Fig. 2A). Lack of both transporters led to a further increase in CHOP expression (Fig. 2A), but this was not associated with any change in β-cell mass at the time point studied (Fig. 2B).

Loss of both β-cell ABCA1 and ABCG1 leads to macrophage infiltration and increased IL-1β expression. Islet inflammation is emerging as an important contributor to type 2 diabetes (18). Recent studies have shown that both ABCA1 and ABCG1 modulate inflammation (19). Thus, we examined mRNA levels of IL-1β, a proinflammatory cytokine that plays a central role in modulating islet chemokine release and impairs islet function (18). IL-1β mRNA was significantly increased in islets lacking both ABCA1 and ABCG1, but not in islets lacking either ABCA1 or ABCG1 alone (Fig. 3A). To assess islet macrophage infiltration, we analyzed expression of the macrophage marker F4/80 in islets. Both ABCA1$^{-P/-P}$ and ABCA1$^{-P/-P}$;ABCG1$^{-/-}$ islets were found to have increased mRNA levels of the macrophage marker F4/80 (Fig. 3B). In support of these data, increased numbers of F4/80-positive cells were observed, by immunostaining, to be present in islets of ABCA1$^{-P/-P}$ and ABCA1$^{-P/-P}$;ABCG1$^{-/-}$ mice (Fig. 3C).

DISCUSSION

β-Cell cholesterol homeostasis is an emerging factor that has been shown to influence β-cell function and insulin secretion (1,2). Islet cholesterol accumulation due to impaired cholesterol efflux or hypercholesterolemia leads to decreased insulin secretion (5–8). The current study shows that two major cellular cholesterol efflux transporters, ABCA1 and ABCG1, play complementary roles in mediating cholesterol efflux from β-cells and protecting against islet sterol accumulation and resultant impairment of insulin secretion and islet inflammation.

TABLE 1
Islet sterol levels are increased in ABCA1$^{-P/-P}$;ABCG1$^{-/-}$ mice

	Control	ABCA1$^{-P/-P}$	ABCG1$^{-/-}$	ABCA1$^{-P/-P}$;ABCG1$^{-/-}$
Cholesterol (μg/mg protein)	33.2 ± 3.5	52.8 ± 2.9[a,c]	38.3 ± 5.3	118.8 ± 4.8[a,b,c]
Lathosterol (ng/mg protein)	18 ± 4	8 ± 3	15 ± 4	10 ± 2
Desmosterol (ng/mg protein)	249 ± 50	268 ± 48	151 ± 44	466 ± 36[a,b,c]
Cholestanol (ng/mg protein)	463 ± 47	636 ± 36[a,c]	481 ± 55	1,760 ± 200[a,b,c]
Campesterol (ng/mg protein)	400 ± 43	543 ± 76	478 ± 78	1,876 ± 325[a,b,c]
Sitosterol (ng/mg protein)	428 ± 71	520 ± 30	536 ± 72	990 ± 253[a,b,c]

Data are mean ± SEM. [a]P < 0.05 versus control. [b]P < 0.05 versus ABCA1$^{-P/-P}$. [c]P < 0.05 versus ABCG1$^{-/-}$ (n = 4–6 in each group).
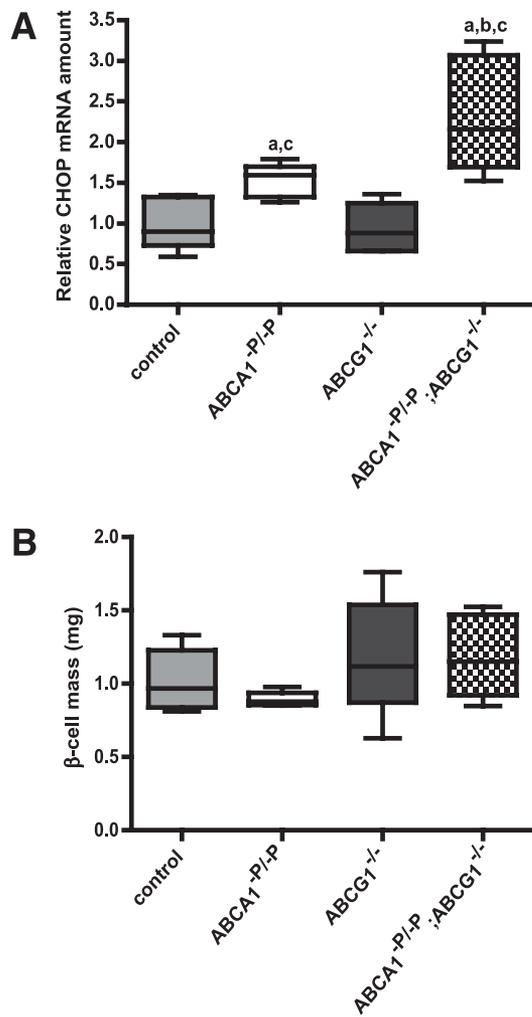
FIG. 2. Increased CHOP expression, but normal β-cell mass in ABCA1$^{-P/-P}$ and ABCA1$^{-P/-P}$;ABCG1$^{-/-}$ islets. *A*: Relative CHOP mRNA levels in isolated islets (*n* = 5–7 per group). *B*: β-Cell mass (*n* = 4–8 per group). $^{a}P < 0.05$ versus control; $^{b}P < 0.05$ versus ABCA1$^{-P/-P}$; $^{c}P < 0.05$ versus ABCG1$^{-/-}$.

The dramatic sterol accumulation in islets lacking both ABCA1 and ABCG1 confirmed the important role of these transporters in lipid metabolism in β-cells. Importantly, the degree of sterol accumulation, as well as glucose intolerance in mice deficient for both transporters, was more than additive, indicating that these two transporters have a synergistic effect on islet sterol homeostasis. Islets lacking ABCA1 and ABCG1 not only accumulate cholesterol, the cholesterol metabolite cholestanol, and the cholesterol precursor desmosterol, but also plant sterols such as campesterol and sitosterol, underlining the importance of these transporters in sterol efflux. Previously, it was suggested that ABCG1 does not mediate cholesterol efflux in β-cells (11), as ABCG1 deficiency did not impact cholesterol levels or cholesterol efflux in β-cells. Cholesterol levels in insulin vesicles was decreased in ABCG1 knockdown β-cells, which led to the suggestion that ABCG1 in β-cells primarily regulates subcellular cholesterol distribution (11). Although we similarly observed that ABCG1 deficiency alone does not result in islet cholesterol accumulation, a role for ABCG1 in β-cell cholesterol export was unmasked in the absence of β-cell ABCA1.

In the current study, we used β-cell–specific ABCA1-deficient mice crossed with the global ABCG1$^{-/-}$ mice to
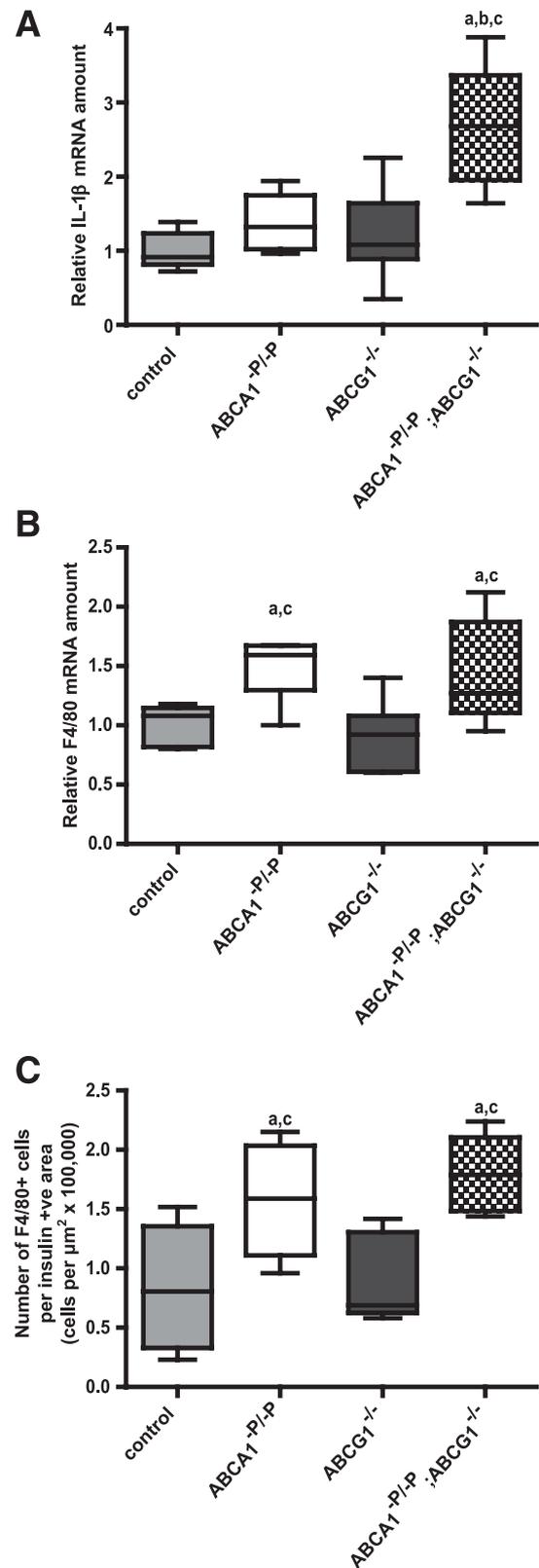

FIG. 3. Loss of β-cell ABCA1 and ABCG1 results in islet inflammation. *A*: Relative IL-1β mRNA levels in isolated islets (*n* = 5–7 per group). *B*: Relative mRNA levels of the macrophage marker F4/80 in isolated islets (*n* = 5–7 per group). *C*: Number of F4/80-positive cells per insulin-positive area in pancreas isolated from control, ABCA1$^{-P/-P}$, ABCG1$^{-/-}$, and ABCA1$^{-P/-P}$;ABCG1$^{-/-}$ mice (*n* = 4–6 per group). $^{a}P < 0.05$ versus control; $^{b}P < 0.05$ versus ABCA1$^{-P/-P}$; $^{c}P < 0.05$ versus ABCG1$^{-/-}$.

determine whether ABCA1 and ABCG1 have complementary roles in β-cells. We chose not to use mice with global ABCA1 deficiency that have very low plasma cholesterol levels (20), as we previously showed that β-cells lacking ABCA1 are susceptible to dysfunction in vivo only in the presence of sufficient levels of plasma cholesterol (5). An obvious limitation of the current study is that the loss of ABCG1 is not β-cell specific and we can therefore not exclude effects of ABCG1 deficiency in other cell types mediating the phenotype reported here.

Absence of both ABCA1 and ABCG1 in β-cells resulted in no overt alteration in β-cell mass in 4-month-old mice. In contrast, glucose-stimulated insulin secretion was further impaired in islets lacking both transporters, indicating that impaired β-cell function and not β-cell loss resulted in the hyperglycemia in the double knockout mice. It remains to be determined whether β-cell mass will be diminished in older mice, since we did notice a marked increase in expression of the endoplasmic reticulum stress–related apoptosis factor CHOP in islets lacking both ABCA1 and ABCG1. In keeping with our previous findings (5), β-cell dysfunction was characterized by loss of both glucose- and KCl-stimulated insulin secretion, consistent with a defect in granule exocytosis associated with cholesterol accumulation (21).

Loss of both ABCA1 and ABCG1 resulted in increased IL-1β expression and increased macrophage infiltration in islets. Islet inflammation has been reported in type 2 diabetes (22). Elevated glucose and free fatty acids increase expression of proinflammatory cytokines in islets (22). Our results suggest that decreased activity of ABCA1 and ABCG1 in β-cells could also lead to islet inflammation. Loss of both transporters in macrophages results in an exaggerated cytokine response to toll-like receptor (TLR) stimuli (19). Although the precise mechanism is unknown, recent data suggest that macrophages lacking ABCA1 exhibit increased trafficking of TLRs to lipid rafts, which leads to enhanced signaling (23). The IL-1 receptor, which appears to be critical for inflammatory responses in β-cells (18), also depends on lipid rafts for signaling (24). Therefore, lack of both ABCA1 and ABCG1 in β-cells could potentially lead to enhanced TLR or IL-1 receptor signaling, resulting in islet inflammation. Furthermore, cholesterol accumulation could lead to the formation of cholesterol crystals, which have been shown to induce inflammation by stimulating the NLRP3 inflammasome in macrophages (25). Our data provide an important link between islet cholesterol accumulation and islet inflammation, which both potentially contribute to increased islet dysfunction in the absence of ABCA1 and ABCG1.

In summary, our data show that ABCA1 and ABCG1 have complementary roles in protecting against islet cholesterol accumulation, inflammation and impaired insulin secretion. These data add further support to the concept that regulation of β-cell cholesterol homeostasis is essential for normal islet function, and suggest that upregulating the activity of ABCA1 and ABCG1 could be a promising approach to decrease islet cholesterol accumulation, decrease islet inflammation, and improve β-cell function in type 2 diabetes.

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