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Loss-of-Function Mutations in *ABCA1* and Enhanced β-Cell Secretory Capacity in Young Adults

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Loss-of-function mutations affecting the cholesterol transporter ATP-binding cassette transporter subfamily A member 1 (ABCA1) impair cellular cholesterol efflux and are associated with reduced HDL-cholesterol (HDL-C) levels. ABCA1 may also be important in regulating β-cell cholesterol homeostasis and insulin secretion. We sought to determine whether loss-of-function *ABCA1* mutations affect β-cell secretory capacity in humans by performing glucose-potentiated arginine tests in three subjects homozygous for *ABCA1* mutations (age 25 ± 11 years), eight heterozygous subjects (28 ± 7 years), and eight normal control subjects pair-matched to the heterozygous carriers. To account for any effect of low HDL-C on insulin secretion, we studied nine subjects with isolated low HDL-C with no *ABCA1* mutations (age 26 ± 6 years) and nine pair-matched control subjects. Homozygotes for *ABCA1* mutations exhibited enhanced oral glucose tolerance and dramatically increased β-cell secretory capacity that was also greater in *ABCA1* heterozygous subjects than in control subjects, with no differences in insulin sensitivity. Isolated low HDL-C subjects also demonstrated an increase in β-cell secretory capacity but in contrast to those with *ABCA1* mutations, exhibited impaired insulin sensitivity, supporting β-cell compensation for increased insulin demand. These data indicate that loss-of-function mutations in *ABCA1* in young adults may be associated with enhanced β-cell secretory capacity and normal insulin sensitivity and support the importance of cellular cholesterol homeostasis in regulating β-cell insulin secretion.

Excessive islet cholesterol impairs insulin secretion in mouse models (1), yet normal insulin secretion is dependent on sufficient β-cell intracellular (2) and plasma membrane (3,4) cholesterol. Recent evidence also suggests that impairment of cellular cholesterol efflux and consequent cholesterol accumulation affects β-cell function and insulin secretion in vitro (5–7) and in mouse models (8,9). The ATP-binding cassette transporter subfamily A member 1 (ABCA1) is a membrane transporter that plays an important role in cholesterol efflux and HDL particle production. In mice, β-cell–specific loss of *ABCA1* leads to impaired glucose tolerance due to defective insulin secretion associated with increased islet cholesterol (8,9).

In humans, loss-of-function mutations in *ABCA1* are associated with reduced HDL-cholesterol (HDL-C) levels that are nearly absent in the rare homozygous state (Tangier disease) with accumulation of cholesterol in macrophages (10). Data in older subjects heterozygous for *ABCA1* mutations indicate an impaired first-phase insulin response to glucose (11); however, interpretation of these results is limited by disproportionately greater statin use in mutation carriers (12). We sought to determine whether loss-of-function mutations in *ABCA1* affect insulin secretion in humans by applying state-of-the-art

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methods to the study of subjects homozygous and heterozygous for *ABCA1* mutations and normal control subjects. Because HDL-C itself has been known to affect insulin secretion (7,13), to account for ABCA1-independent effects possibly attributable to low HDL-C, we similarly studied subjects with isolated low HDL-C levels (primary hypoalphalipoproteinemia) and no identifiable mutations in *ABCA1*.

RESEARCH DESIGN AND METHODS

Subjects with isolated low HDL-C levels (<25th percentile) were identified from the University of Pennsylvania Preventive Cardiovascular Program databases. Subjects with metabolic syndrome, as defined by the American Heart Association/National Heart, Lung, and Blood Institute guidelines (14), were excluded. If not already known, DNA of subjects with low HDL-C levels was isolated from blood, and the *ABCA1* gene was sequenced as previously described (15). Subjects with low HDL-C were classified by analysis for *ABCA1* loss-of-function mutations as homozygous (Tangier disease), heterozygous, or wild type (primary hypoalphalipoproteinemia). Normal control subjects were screened for normal levels of HDL-C and paired-matched by sex, race, age, BMI, and fasting glucose to an *ABCA1* heterozygous subject or to a subject with isolated low HDL-C (primary hypoalphalipoproteinemia). The University of Pennsylvania Institutional Review Board approved the study, and all subjects gave written informed consent to participate.

Subjects were admitted to the University of Pennsylvania Clinical and Translational Research Center and were required to fast overnight for 12 h before testing. Catheters were placed in an antecubital vein for infusions (where applicable) and in a warmed contralateral hand vein, retrograde when possible, for sampling arterialized venous blood. All metabolic tests were conducted on separate days.

Oral Glucose Tolerance Test

For the oral glucose tolerance test (OGTT), baseline blood samples were taken at −5 and −1 min before the ingestion of 75 g anhydrous glucose in solution over a 5-min period starting at t = 0. Additional samples were collected at t = 10, 20, 30, 60, 90, 120, 150, and 180 min after ingestion.

Glucose-Potentiated Arginine Test

The glucose-potentiated arginine (GPA) test followed established methodology for evaluation of β-cell function (16,17). Baseline blood samples were taken at −5 and −1 min before the injection of 5 g 10% arginine over a 1-min period starting at t = 0. Additional samples were collected at t = 2, 3, 4, and 5 min after injection. At t = 10 min, a hyperglycemic clamp technique (18) using a variable rate infusion of 20% glucose was performed to achieve a plasma glucose concentration of ∼230 mg/dL. After 45 min of the glucose infusion (at t = 55 min), a 5-g arginine pulse was injected again with identical sampling. Then, a 2-h period with no glucose infusion took place to allow glucose levels to return to baseline, after which a second hyperglycemic clamp of identical duration was performed to achieve a plasma glucose concentration of ∼340 mg/dL, and a 5-g arginine pulse was injected again with identical sampling.

Frequently Sampled Intravenous Glucose Tolerance Test

The method for the frequently sampled intravenous glucose tolerance (FSIGT) test and the calculations used to obtain FSIGT test–derived parameters are provided in the Supplementary Data.

Biochemical Analysis

Lipid parameters were assessed using standard laboratory procedures. Plasma glucose was measured in duplicate by the glucose oxidase method using an automated YSI 2300 glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin and C-peptide were measured in duplicate by double-antibody radioimmunoassays (Millipore, Billerica, MA). Samples from matched case subjects and normal control subjects were assayed simultaneously.

Calculations and Statistics

Oral glucose tolerance was evaluated as the incremental area under the curve for glucose values obtained during the OGTT, with similar calculations made for insulin.

The GPA parameters of acute insulin response (AIR_{arg}) and C-peptide response to arginine were calculated as the mean of the 2-, 3-, 4-, and 5-min values minus the mean of the baseline values (16–18). Acute responses during the 230 mg/dL glucose clamp enable determination of glucose potentiation of arginine-induced insulin (AIR_{pot}) and C-peptide release. Acute responses during the 340 mg/dL glucose clamp allow for determination of the maximum arginine-induced insulin (AIR_{max}) and C-peptide release (i.e., β-cell secretory capacity) (19). The mean glucose infusion rate required during the 230 mg/dL glucose clamp (M) was divided by the mean prestimulus insulin level (I) between 40 and 45 min of the glucose infusion to determine insulin sensitivity (M/I) (16). The disposition index (D_I) was then calculated as the product of AIR_{arg} and M/I, as previously validated (16).

All data are expressed as means ± SE unless specified. Comparison of test parameters between the *ABCA1* homozygous subjects and normal control subjects was performed with the unpaired Student t test, and comparisons between *ABCA1* heterozygous subjects and their matched normal control subjects and between isolated low HDL-C subjects and their matched normal control subjects were performed with the paired Student t test or Wilcoxon matched-pairs test, as appropriate (20). Analyses were conducted using Statistica software (StatSoft, Inc., Tulsa, OK), with significance considered at $P < 0.05$ (two-tailed).

RESULTS

Subject Characteristics

Three subjects homozygous and eight heterozygous for mutations in *ABCA1* and nine with isolated low HDL-C but

with no identifiable mutations in *ABCA1* were enrolled in the study. Normal control subjects were recruited to match by design the sex, ethnicity, age, BMI, and fasting glucose of the *ABCA1* heterozygous and the low HDL-C subjects (Table 1). All subjects carrying *ABCA1* mutations had a lipid profile consistent with loss-of-function mutations. Of note, *ABCA1* homozygous subjects had lower LDL-C levels and higher fasting insulin levels, and the isolated low HDL-C group exhibited elevated triglyceride levels relative to their respective control groups.

OGTT

Oral glucose tolerance was enhanced in the *ABCA1* homozygous subjects compared with the control subjects ($P < 0.05$), a finding associated with higher peak insulin levels for two of the *ABCA1* homozygous subjects (Fig. 1*A* and *C*). Samples from the third homozygous subject were affected by gross hemolysis that invalidated the insulin assay. Oral glucose tolerance was not different between the *ABCA1* heterozygous subjects and their control subjects (Fig. 1*A*) or between the isolated low HDL-C subjects and their control subjects (Fig. 1*B*). Similarly, the corresponding insulin responses (Fig. 1*C* and *D*) were not different.

GPA Test

In the *ABCA1* homozygous subjects, AIR_{arg} and AIR_{pot} tended to be greater ($P \leq 0.1$) and AIR_{max} was significantly greater than in control subjects ($P < 0.05$; Table 2 and Fig. 2*A*). In *ABCA1* heterozygous subjects, AIR_{arg} was not different; however, AIR_{pot} and AIR_{max} were significantly greater than in the control group ($P < 0.05$; Table 2 and Fig. 2*C*). In subjects with isolated low HDL-C, AIR_{arg}, AIR_{pot}, and AIR_{max} were all significantly greater than in control subjects ($P < 0.05$; Table 2 and Fig. 2*E*). Similar results were seen with C-peptide (Table 2 and Fig. 2*B*, *D*, and *F*).

Insulin sensitivity (M/I) was not different between the *ABCA1* mutation homozygotes or heterozygotes and their normal control subjects, but M/I was significantly less in isolated low HDL-C subjects than in control subjects ($P < 0.01$; Table 2). The resulting D_I was not different between the *ABCA1* mutation groups or the isolated low HDL-C group and their respective control groups.

Findings from the FSIGT test showed no differences in the first-phase insulin release to glucose in *ABCA1* mutation carriers and overall were consistent with those obtained during the GPA test (Supplementary Data).

DISCUSSION

These results demonstrate enhanced β-cell secretory capacity in young adults heterozygous for loss-of-function mutations in the gene encoding the cholesterol transporter ABCA1. This effect was even more dramatic in homozygous subjects with Tangier disease, suggesting a gene-dose effect as also seen on HDL-C levels. Importantly, insulin sensitivity was not impaired in the subjects with mutations in *ABCA1*, indicating that the effect of loss of ABCA1 function on augmenting insulin secretion is independent from insulin sensitivity. Significantly increased insulin secretion was only revealed under hyperglycemic clamp conditions that are necessary to demonstrate the reserve capacity for insulin release. In the homozygous subjects, increased insulin secretion was

Table 1—Subject characteristics

	ABCA1			Low HDL-C	
	Homozygous	Heterozygous	Control subjects	Case subjects	Control subjects
Sex (*n*)					
Male	2	5	5	7	7
Female	1	3	3	2	2
Race (*n*)					
Caucasian	3	7	7	7	7
African American	0	1	1	2	2
Age (years)	25 ± 11	28 ± 7	28 ± 6	26 ± 6	26 ± 4
BMI (kg/m^2)	25 ± 4	25 ± 4	25 ± 3	25 ± 2	25 ± 2
Glucose (mg/dL)	95 ± 9	86 ± 10	89 ± 9	89 ± 4	88 ± 7
Insulin (μU/mL)	13.8 ± 1.2*	10.3 ± 4.7	8.4 ± 3.5	9.1 ± 6.7	7.1 ± 2.2
Total cholesterol (mg/dL)	74 ± 21†	157 ± 24	181 ± 27	158 ± 36	175 ± 27
Triglyceride (mg/dL)	127 ± 59	115 ± 59	75 ± 32	134 ± 44*	84 ± 45
HDL-C (mg/dL)	2 ± 0†	27 ± 7‡	65 ± 17	30 ± 4‡	65 ± 10
LDL-C (mg/dL)	57 ± 26*	105 ± 22	97 ± 20	97 ± 24	94 ± 21
VLDL-C (mg/dL)	16 ± 6	24 ± 22	19 ± 8	30 ± 17	17 ± 10
ApoA-I (mg/dL)	8 ± 3‡	83 ± 12†	159 ± 38	100 ± 13†	155 ± 20
ApoB (mg/dL)	76 ± 14	86 ± 16	68 ± 15	83 ± 20	66 ± 19

Data are means ± SD. ApoA-I, apoprotein A-I; ApoB, apoprotein B. *$P < 0.05$ case vs. control subjects. †$P < 0.001$ case vs. control subjects. ‡$P < 0.0001$ case vs. control subjects.

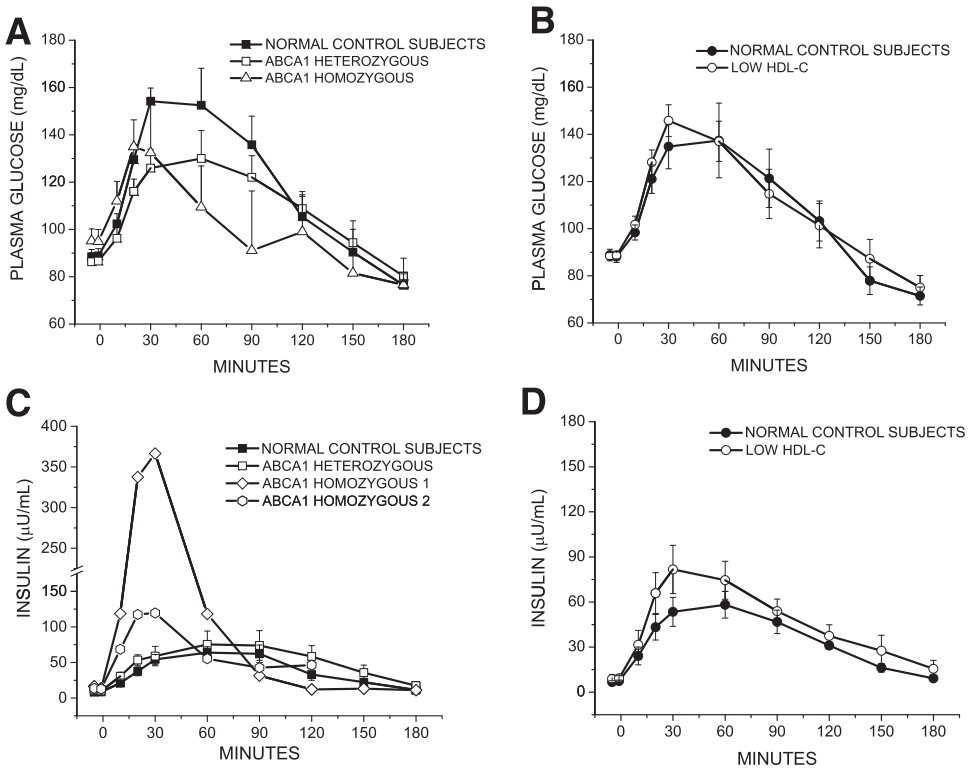

Figure 1—Plasma glucose (*A* and *B*) and insulin (*C* and *D*) levels during the 75-g OGTT in *ABCA1* homozygous ($n = 3$), *ABCA1* heterozygous ($n = 8$), and matched normal control ($n = 8$) subjects (*A* and *C*) and in isolated low HDL-C ($n = 9$) and matched normal control ($n = 9$) subjects (*B* and *D*). The incremental area under the curve (AUC) for glucose was 1,255 ± 1,543, 4,369 ± 1,115, and 5,565 ± 914 mg/dL · min for *ABCA1* homozygous, heterozygous, and control subjects, respectively ($P < 0.05$ for *ABCA1* homozygous vs. control subjects), and 4,060 ± 1,154 and 3,686 ± 1,205 mg/dL · min for the isolated low HDL-C subjects and their control subjects. The AUC for insulin was 7,786 ± 1,951 and 5,811 ± 763 μU/mL · min for the *ABCA1* heterozygous subjects and their control subjects and 7,062 ± 1,221 and 5,195 ± 629 μU/mL · min for the isolated low HDL-C subjects and their control subjects. Not shown are the insulin levels of one *ABCA1* homozygous subject whose samples were affected by gross hemolysis that invalidated the insulin assay.

also apparent during oral glucose administration and associated with enhanced oral glucose tolerance. Because cellular cholesterol is required for the formation of insulin secretory granules (2), these results highlight the importance of cellular cholesterol homeostasis for β-cell function.

The current study was designed to assess the relevance in humans of data obtained in a mouse model of

Table 2—GPA test parameters

	ABCA1			Low HDL-C	
	Homozygous $n = 3$	Heterozygous $n = 8$	Control subjects $n = 8$	Case subjects $n = 9$	Control subjects $n = 9$
AIR_{arg} (μU/mL)	62 ± 32[a]	33 ± 8	28 ± 7	58 ± 10*	27 ± 6
AIR_{pot} (μU/mL)	350 ± 228[a]	195 ± 33*	122 ± 20	278 ± 45†	134 ± 14
AIR_{max} (μU/mL)	426 ± 153*	219 ± 30*	173 ± 34	304 ± 66†	143 ± 20
ACR_{arg} (ng/mL)	3.47 ± 0.86*	2.03 ± 0.36*	1.43 ± 0.26	3.07 ± 0.29*	1.64 ± 0.25
ACR_{pot} (ng/mL)	14.17 ± 6.24[a]	8.31 ± 1.70*	6.30 ± 0.97	9.70 ± 1.06†	5.24 ± 0.52
ACR_{max} (ng/mL)	14.08 ± 6.01*	8.39 ± 1.16*	5.77 ± 0.55	10.45 ± 1.66*	5.60 ± 0.65
M (mg/kg/min)	9.8 ± 0.1	9.6 ± 0.5	9.4 ± 0.7	8.7 ± 0.4	10.3 ± 1.0
I (μU/mL)	52 ± 25	45 ± 11	28 ± 4	50 ± 10	34 ± 6
M/I (mg/kg per min/μU/mL)	0.30 ± 0.12	0.29 ± 0.05	0.36 ± 0.03	0.22 ± 0.03†	0.37 ± 0.06
D_I (mg/kg/min)	11.0 ± 1.8	9.0 ± 2.6	9.7 ± 2.3	11.0 ± 1.5	10.4 ± 3.6

Data are means ± SE. ACR_{arg}, acute C-peptide response to arginine under fasting conditions; ACR_{max}, acute C-peptide response to arginine under ~340 mg/dL glucose clamp conditions; ACR_{pot}, acute C-peptide response to arginine under ~230 mg/dL glucose clamp conditions. [a]$P \leq 0.1$ case subjects vs. control subjects. *$P < 0.05$ case subjects vs. control subjects. †$P < 0.01$ case subjects vs. control subjects.

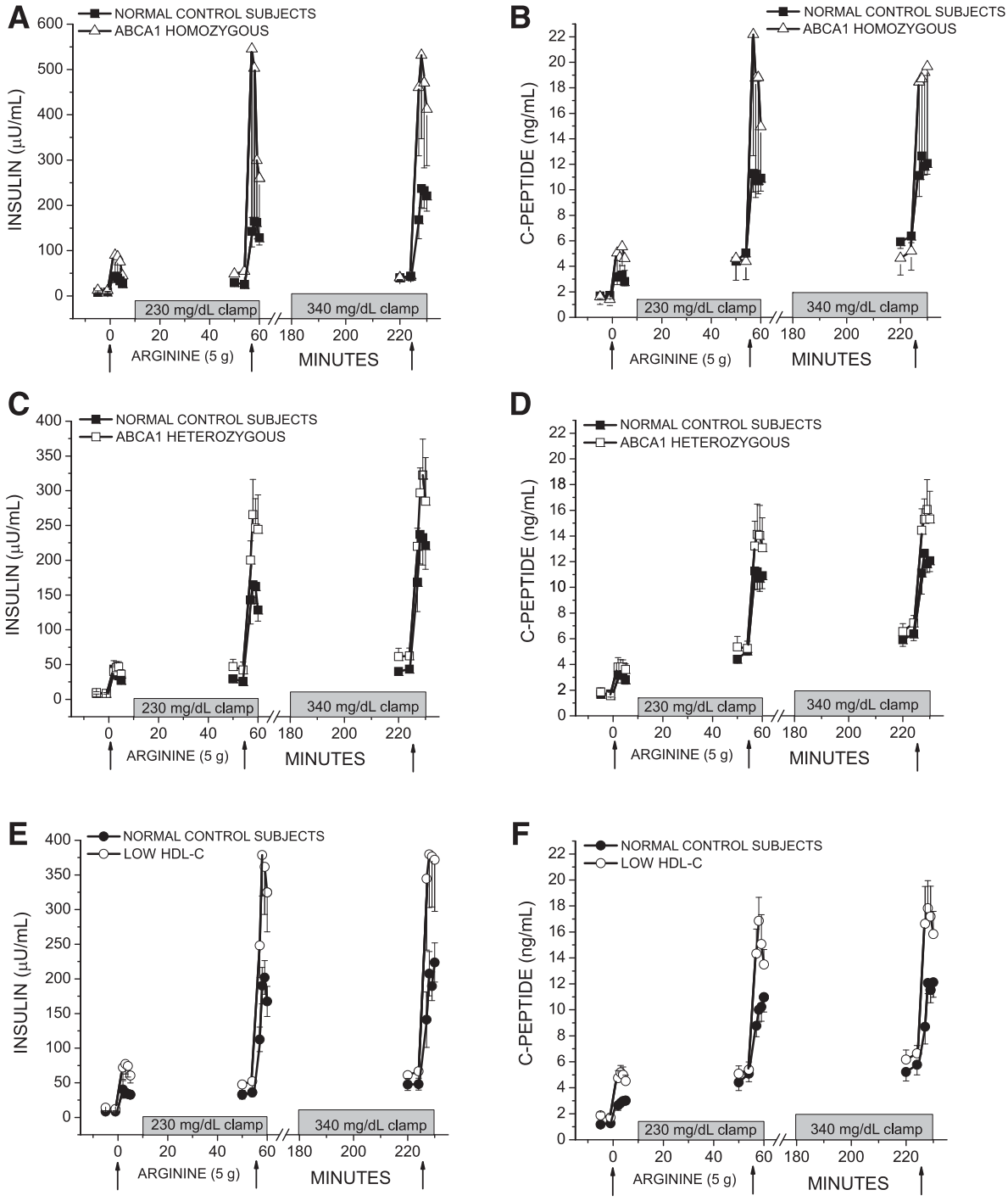

Figure 2—Plasma insulin (*A*, *C*, and *E*) and C-peptide (*B*, *D*, and *F*) levels in response to bolus injections of arginine (arrows) administered under fasting, ~230 mg/dL hyperglycemic clamp, and ~340 mg/dL hyperglycemic clamp conditions in *ABCA1* homozygous (n = 3) (*A* and *B*), *ABCA1* heterozygous (n = 8) (*C* and *D*), and matched normal control (n = 8) subjects and in isolated low HDL-C (n = 9) and matched normal control (n = 9) subjects (*E* and *F*).

β-cell–specific loss of *ABCA1* (8), characterized by impaired glucose tolerance due to defective insulin secretion associated with increased islet cholesterol (8). Vergeer et al. (11) recently reported a study in heterozygous carriers of mutations in *ABCA1*. Similar to our results, they demonstrated normal insulin sensitivity; however, in contrast to our results, they exhibited impaired first-phase insulin release and normal instead of enhanced β-cell secretory capacity (11). Our cohort of relatively young heterozygous subjects differs from the cohort reported by Vergeer et al. (11), who were older and had higher levels of LDL-C. These findings may

represent an interaction between more advanced age and/or higher levels of LDL-C in older carriers of *ABCA1* mutations on β-cell function, whereby decades of enhanced secretion, accumulation of intracellular cholesterol, and/or LDL-C exposure may eventually predispose to islet dysfunction. In addition, many of these older subjects were treated with hydroxymethylglutaryl-CoA reductase inhibitors (statins) that increase the risk of diabetes in older individuals (12). Future testing of the influence of age and systemic cholesterol availability on the effect of *ABCA1* mutations on insulin secretion is warranted.

The mechanism for the increased insulin secretion with ABCA1 loss of function is not known. Because ABCA1 is important in the maintenance of cholesterol homeostasis, it is possible that intracellular or membrane cholesterol concentration plays an important role in insulin secretion (1–4). Importantly, human β-cells contain lipid-storage vesicles that may enable the accommodation of excess cholesterol not possible in rodent islets (21). We hypothesize that although ABCA1 deficiency may lead to a slight increase in cellular cholesterol, this does not become excessive in the context of the low concentration of plasma LDL-C as found in our subjects with Tangier disease and enables the establishment of a new set point for cellular cholesterol homeostasis. This new set point may be advantageous for the function of cells with high secretory demand, such as the β-cell, and likely explains the enhanced β-cell secretory capacity reported here.

We observed increased insulin secretion and impaired insulin sensitivity in our cohort of subjects with primary isolated low HDL-C. These subjects displayed appropriate β-cell adaptation for the impaired insulin sensitivity, as indicated by increased insulin responses under fasting conditions such that the D_I remained normal. The mechanism for impaired insulin sensitivity this group is unknown, although the absence of this finding in subjects with *ABCA1* mutations supports its independence from ABCA1 function.

In conclusion, loss-of-function mutations affecting the cellular cholesterol transporter ABCA1 results in enhanced β-cell secretory capacity in young adults, possibly by altering the cellular set point of cholesterol availability for secretory granule formation and function. Further investigation is required to determine whether the initial hypersecretion leads to β-cell secretory dysfunction with aging. Through its association with effects on insulin secretion, the targeting of cellular cholesterol homeostasis will likely lead to novel approaches for the prevention and treatment of diabetes.

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Author Contributions. M.R.R. designed and conducted the study, researched data, and wrote the manuscript. E.S.G. participated in the conduct of the study, researched data, and revised the manuscript critically for important intellectual content. C.F., C.L., and A.M.B. participated in the conduct of the study, researched data, and reviewed and edited the manuscript. N.M.D. contributed to discussion and reviewed and edited the manuscript. R.A.H. researched data and revised the manuscript critically for important intellectual content. M.C. designed and conducted the study, researched data, and wrote the manuscript. M.R.R. and M.C. are the guarantors of this work and, as such, take full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

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References

1. Hao M, Head WS, Gunawardana SC, Hasty AH, Piston DW. Direct effect of cholesterol on insulin secretion: a novel mechanism for pancreatic beta-cell dysfunction. Diabetes 2007;56:2328–2338

2. Xia F, Xie L, Mihic A, et al. Inhibition of cholesterol biosynthesis impairs insulin secretion and voltage-gated calcium channel function in pancreatic beta-cells. Endocrinology 2008;149:5136–5145

3. Vikman J, Jimenez-Feltström J, Nyman P, Thelin J, Eliasson L. Insulin secretion is highly sensitive to desorption of plasma membrane cholesterol. FASEB J 2009;23:58–67

4. Xia F, Gao X, Kwan E, et al. Disruption of pancreatic beta-cell lipid rafts modifies Kv2.1 channel gating and insulin exocytosis. J Biol Chem 2004;279: 24685–24691

5. Wijesekara N, Zhang LH, Kang MH, et al. miR-33a modulates ABCA1 expression, cholesterol accumulation, and insulin secretion in pancreatic islets. Diabetes 2012;61:653–658

6. Kang MH, Zhang LH, Wijesekara N, et al. Regulation of ABCA1 protein expression and function in hepatic and pancreatic islet cells by miR-145. Arterioscler Thromb Vasc Biol 2013;33:2724–2732

7. Fryirs MA, Barter PJ, Appavoo M, et al. Effects of high-density lipoproteins on pancreatic beta-cell insulin secretion. Arterioscler Thromb Vasc Biol 2010;30: 1642–1648

8. Brunham LR, Kruit JK, Pape TD, et al. Beta-cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment. Nat Med 2007;13:340–347

9. Kruit JK, Wijesekara N, Westwell-Roper C, et al. Loss of both ABCA1 and ABCG1 results in increased disturbances in islet sterol homeostasis, inflammation, and impaired β-cell function. Diabetes 2012;61:659–664

10. Assmann G, von Eckardstein A, Brewer HB. Familial analphalipoproteinemia: Tangier disease. In *The Metabolic and Molecular Bases of Inherited Disease*. Scriver CR Beaudet AL, Sly WS, Valle D, Eds. New York: McGraw-Hill, Inc., 2001, p. 2937–2960

11. Vergeer M, Brunham LR, Koetsveld J, et al. Carriers of loss-of-function mutations in ABCA1 display pancreatic beta-cell dysfunction. Diabetes Care 2010;33:869–874

12. Sattar N, Preiss D, Murray HM, et al. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. Lancet 2010;375:735–742

13. Drew BG, Duffy SJ, Formosa MF, et al. High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. Circulation 2009; 119:2103–2111

14. Grundy SM, Cleeman JI, Daniels SR, et al.; American Heart Association; National Heart, Lung, and Blood Institute. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;112:2735–2752

15. Wang J, Burnett JR, Near S, et al. Common and rare ABCA1 variants affecting plasma HDL cholesterol. Arterioscler Thromb Vasc Biol 2000;20:1983–1989

16. Guldstrand M, Ahrén B, Adamson U. Improved beta-cell function after standardized weight reduction in severely obese subjects. Am J Physiol Endocrinol Metab 2003;284:E557–E565

17. Larsson H, Ahrén B. Glucose-dependent arginine stimulation test for characterization of islet function: studies on reproducibility and priming effect of arginine. Diabetologia 1998;41:772–777

18. Ward WK, Halter JB, Beard JC, Porte D Jr. Adaptation of B and A cell function during prolonged glucose infusion in human subjects. Am J Physiol 1984;246:E405–E411

19. Seaquist ER, Robertson RP. Effects of hemipancreatectomy on pancreatic alpha and beta cell function in healthy human donors. J Clin Invest 1992;89: 1761–1766

20. Bland JM, Altman DG. Matching. BMJ 1994;309:1128

21. Cnop M, Grupping A, Hoorens A, Bouwens L, Pipeleers-Marichal M, Pipeleers D. Endocytosis of low-density lipoprotein by human pancreatic beta cells and uptake in lipid-storing vesicles, which increase with age. Am J Pathol 2000;156:237–244