The Expression of Aldolase B in Islets Is Negatively Associated With Insulin Secretion in Humans

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Context: Reduced β-cell mass, impaired islet function, and dedifferentiation are considered causal to development of hyperglycemia and type 2 diabetes. In human cohort studies, changes of islet cell–specific expression patterns have been associated with diabetes but not directly with *in vivo* insulin secretion.

Objective: This study investigates alterations of islet gene expression and corresponding gene variants in the context of *in vivo* glycemic traits from the same patients.

Methods: Fasting blood was collected before surgery, and pancreatic tissue was frozen after resection from 18 patients undergoing pancreatectomy. Islet tissue was isolated by laser capture microdissection. Islet transcriptome was analyzed using microarray and quantitative RT-PCR. Proteins were examined by immunohistochemistry and western blotting. The association of gene variants with insulin secretion was investigated with oral glucose tolerance test (OGTT)-derived insulin secretion measured in a large cohort of subjects at increased risk of type 2 diabetes and with hyperglycemic clamp in a subset.

Results: Differential gene expression between islets from normoglycemic and hyperglycemic patients was prominent for the glycolytic enzyme *ALDOB* and the obesity-associated gene *FAIM2*. The mRNA levels of both genes correlated negatively with insulin secretion and positively with HbA_{1c}. Islets of hyperglycemic patients displayed increased ALDOB immunoreactivity in insulin-positive

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Abbreviations: AUC, area under the curve; BMI, body mass index; GIIS, glucose-induced insulin secretion; GLP-1, glucagon-like peptide 1; GTEx, Genotype-Tissue Expression; HOMA2, updated homeostasis model assessment; LCM, laser capture microdissection; OGTT, oral glucose tolerance test; qRT-PCR, quantitative RT-PCR; SNP, single nucleotide polymorphism; TUEF, Tuebingen Family.

cells, whereas α- and δ-cells were negative. Exposure of isolated islets to hyperglycemia augmented ALDOB expression. The minor allele of the *ALDOB* variant rs550915 associated with significantly higher levels of C-peptide and insulin during OGTT and hyperglycemic clamp, respectively.

Conclusion: Our analyses suggest that increased ALDOB expression in human islets is associated with lower insulin secretion. ***(J Clin Endocrinol Metab* 103: 4373–4383, 2018)**

Appropriate insulin secretion and β-cell mass are prerequisites of the maintenance of glucose homeostasis. Insufficient insulin secretion is considered the ultimate cause for development of type 2 diabetes mellitus (1, 2). To date, multiple changes have been implicated in the development of insulin deficiency. The most prominent concepts involve defective insulin secretion, insufficient adaptation of insulin synthesis, reduced β-cell mass, and increased α-cell mass with inappropriate high glucagon secretion. These changes are triggered by metabolic stress factors (*i.e.*, hyperglycemia, hyperlipidemia, and other factors from the diabetogenic milieu) (3). Obesity accentuates metabolic stress and, when accompanied by a genetic predisposition, accelerates the development of type 2 diabetes mellitus. Several of the diabetes-associated single nucleotide polymorphisms (SNPs) are located within genes essential for islet function (4, 5).

Considerable efforts have been undertaken to decipher expressional changes in whole islets and individual islet cells (β-, α-, and δ-cells) in association with hyperglycemia and type 2 diabetes (6–10). Some of the genes differentially altered in type 2 diabetic islets are involved in glucose metabolism (*SLC2A2*), Ca^{2+} trafficking (*TMEM37*) mitochondrial metabolism (*GPD2* and *FXYD2*), cell cycling (P21/CIP), and fatty acid (*FFAR4*), insulin (*IR*), and IGF-1 (*IGF1R*) receptor signaling (6–8, 11).

Most human islets used for scientific purposes are obtained from deceased organ donors. In these studies, a potential bias could arise from *post mortem* alteration of mRNA levels. Furthermore, human islets and consequently the RNA preparations are usually analyzed after enzymatic digestion of pancreatic tissue. Although the enzymatic isolation of cells allows the separation of islets from exocrine tissue and their dissociation into single cells, such procedures are known to alter unstable mRNAs. Indeed, considerable differences between the expression profile of human type 2 diabetic islets isolated enzymatically or by laser capture microdissection (LCM) have been discussed recently (11). Specifically, LCM-collected islet tissue from patients with type 2 diabetes displayed lower mRNA levels of inflammatory markers and increased levels of *ALDOB* and *FAIM2* mRNA compared with enzyme-digested, isolated type 2 diabetic islets. The current study conducted a direct comparison between expressional changes in human islet tissue and *in vivo* insulin secretion. This goal was achieved by assessing the *in vivo* secretory capacity of insulin via a hyperglycemic clamp, a procedure that circumvents potential changes in blood glucose triggered by other mechanisms (*i.e.*, peripheral glucose handling). Glucagon-like peptide 1 (GLP-1) and arginine infusion on top of the hyperglycemic clamp further allows the evaluation of maximal secretory capacity.

Materials and Methods

Human pancreas biobank

Patients (n = 18) undergoing elective surgery for removal of operable pancreatic tumors participated in this ongoing study after providing written informed consent. For each patient, a brief clinical history was obtained. Fasting blood samples were collected prior to anesthesia and pancreatic surgery to assess metabolic traits. Tumor-free pancreatic tissue was dissected from surgical resections by a trained pathologist. The collection of human material and the study were approved by the Ethics Commission of the Medical Faculty of the University of Tuebingen (#697/2011BO1 and #355/2012BO2). Classification of normal glucose tolerance, prediabetes, or diabetes was performed according to clinical history and fasting glucose and HbA_{1c} levels using criteria of the American Diabetes Association (12). Clinical characteristics of the patients are summarized in Supplemental Table 1.

Isolation of pancreatic islet tissue by LCM

Islet tissue was isolated using a protocol previously described (11). Briefly, the freshly resected pancreatic tissue was embedded in cryomolds containing Tissue-Tek O.C.T. compound (Sakura Finetek GmbH, Staufen, Germany). The cryomolds were frozen in isopropyl alcohol precooled in a dry ice–ethanol mixture and stored at −80°C. For the LCM procedure, pancreatic slices (10-μm thick) were dehydrated in ethanol, incubated in xylene, and air dried for 5 minutes. The laser microdissection of islet tissue was performed with a PALM MicroBeam (Zeiss, Oberkochen, Germany). Islets of Langerhans were identified by means of autofluorescence. Islet area was selected with the "freehand" selection tool and microdissected using the laser beam. Islet tissue from 40 dehydrated cryosections/pancreatic samples was lysed in 100 μL extraction buffer (Arcturus Pico pure RNA isolation kit; Applied Biosystems, Foster City, CA) and the supernatant stored at −80°C.

RNA extraction and amplification

Total RNA of islet tissue from 18 patients was isolated using the same protocol as previously described using a commercial kit (Arcturus Pico pure RNA isolation kit) (7, 11). RNA integrity numbers were determined using a bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA samples with RNA integrity numbers >6.0 were processed in transcriptome analyses.

Transcriptome analysis

Affymetrix microarray

For this analysis, 10 to 25 ng of total RNA was amplified using the Ovation RNA Amplification System V2 and subsequently labeled with Biotin using the Encore™ Biotin Module (NuGEN Technologies, San Carlos, CA). The length distribution of the amplified cDNA products was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies). Hybridization of biotin-labeled cDNA to Affymetrix HG_U133Plus2.0 GeneChip microarrays (Affymetrix, Santa Clara, CA) was performed at ATLAS Biolabs GmbH (Berlin, Germany) as described in Solimena *et al.* (11).

Laboratory measurements and assessment of β-cell function *in vivo*

Fasting venous blood samples were obtained prior to pancreatic surgery. Glucose levels were measured using the hexokinase method. Plasma insulin and C-peptide were determined by an immunoassay with the ADVIA Centaur XP Immunoassay System (Siemens Healthineers, Eschborn, Germany). HbA_{1c} measurements were performed using the Tosoh glycohemoglobin analyzer HLC-723G8 (Tosoh Bioscience, Tokyo, Japan). β-cell function was computed from fasting glucose and C-peptide levels with the updated homeostasis model assessment (HOMA2) (13). Insulin secretion was expressed with the %B index adjusted for insulin resistance, also calculated as HOMA2 index (HOMA2-IR).

Immunohistochemistry

Paraffin-embedded, pancreatic serial sections from 12 to 17 patients were incubated with primary antibodies against insulin (1:1000; A0564; Dako, Carpinteria, CA) and glucagon (1:600; #13091; Santa Cruz Biotechnology, Santa Cruz, CA). The specific antibody binding was visualized using the Opti-View amplification system (Multimer Technology; Roche-Ventana, Tucson, AZ). Hematoxylin-eosin was used as counterstaining. Insulin- and glucagon-positive stained areas were used for quantification of α- and β-cell areas within the islets using the ImageJ software (National Institutes of Health, Bethesda, MD).

For immunofluorescence and confocal microscopy, the pancreatic sections and cultured human islet cells from organ donors were incubated with primary antibodies against insulin (1:200; mouse, #8304; Abcam, Cambridge, MA), glucagon (1:500; mouse, #10988; Abcam), somatostatin (1:1000; mouse, 14-9751-80; Invitrogen, Carlsbad, CA), and aldolase B (1:100; rabbit, #18065-1-AP; Proteintech, Rosemont, IL). The primary antibodies were visualized with goat anti-rabbit IgG-Alexa Fluor 488 (for aldolase B) and goat anti-mouse IgG-Alexa Fluor 546 (for insulin, glucagon, and somatostatin). Nuclei were stained with TO-PRO3.

Human subjects of the cross-sectional validation cohort (Tuebingen Family cohort) and hyperglycemic clamp study

The Tuebingen Family (TUEF) study currently includes >3000 healthy subjects from Southern Germany with an increased risk for type 2 diabetes as determined by a family history of diabetes, an impaired fasting glycemia, a body mass index (BMI) ≥27 kg/m^2, and/or previous gestational diabetes (14). From this study, 2645 participants with complete data sets of anthropometric assessment (sex, age, and BMI) and five-point oral glucose tolerance test (OGTT) including glucose, insulin, C-peptide measurements, and genotypes were selected for association analysis. In the hyperglycemic clamp study, participants underwent hyperglycemic clamps at 10 mM glucose with a stepwise GLP-1 infusion and an arginine bolus, as described earlier (15, 16). Genotyped participants were evaluated in the current analyses (n = 70). Both studies adhered to the Declaration of Helsinki and were approved by the Ethics Committee of the Eberhard Karls University of Tuebingen. Written informed consent was obtained from all participants.

Genotyping, association analysis, and data from open-access repositories

The TUEF participants were genotyped on a genome-wide scale using Illumina's Infinium® Global Screening Array-24 v1.0 BeadChip (Illumina, San Diego, CA) which was developed based on phase 3 data of the 1,000 Genomes Project and depicts 700,078 SNPs. Within the genomic region of *ALDOB* including 2 kb of 5′-flanking sequence, 21 SNPs were identified on the array. Among these SNPs, only two, rs550915 and rs533017, revealed minor allele frequencies >0.05 and were selected for association testing due to statistical power limitations of the study population. Both SNPs were successfully genotyped (call rates >99%), in the Hardy-Weinberg equilibrium ($P \geq 0.4$), and nonlinked ($R^2 = 0.34$).

Publicly available genotype-phenotype association data were accessed via the Type 2 Diabetes Knowledge Portal (accessed 16 March 2018; http://www.type2diabetesgenetics.org/variantInfo/variantInfo/rs550915#). We interrogated the Genotype-Tissue Expression (GTEx) project database for information on the association of a SNP of interest with gene transcription (17). Single-cell RNA-sequencing data in islet cells were analyzed from data shared from the work of Enge *et al.* (18).

Culture of isolated human islets and western blotting

Isolated human islets obtained from the European Consortium for Islet Transplantation (Geneva, Switzerland and Milan, Italy) and dispersed human islet cells (19) were cultured for 72 hours with medium change every 24 hours in CMRL1066 medium supplemented with 5, 10, 15, or 20 mM glucose, 2 mM L-glutamine, 10 mM HEPES, and 10% fetal calf serum. At the end of the culture period, the islets were lysed for western blotting, and the islet cells were used for immunostaining (19). Protein lysates were subjected to SDS-PAGE, proteins transferred on nitrocellulose membranes (Hahnemühle, Dassel, Germany). Membranes were incubated for 1 hour in Tris-buffered saline–Tween supplemented with 5% milk, overnight with primary antibodies against aldolase B (Proteintech) or tubulin (Cell Signaling Technology, Danvers, MA), followed by 1-hour incubation with horseradish peroxidase–coupled secondary anti-rabbit IgG (GE Healthcare, München, Germany). Proteins were detected using ChemiDoc Touch Imaging System (Bio-Rad Laboratories, Hercules, CA). Analyses were performed using Bio-Rad Image laboratory software.

Statistics

The microarray data from the Affymetrix Human Genome Array HG_U133Plus2.0 were analyzed after quality control

and normalization. Differential gene expression was tested with the *limma* package in R. Linear models were fitted using robust fit, and the log-fold change was determined by empirical Bayes moderation of the SEs toward a common value. For the visualization of the correlation of gene expression with clinical traits, the relationship between two variables was plotted with GraphPad Prism (GraphPad Software Inc, La Jolla, CA) and Pearson correlation coefficients are provided. All other analyses were performed in R. The evaluation of SNP association with insulin secretion indices from the OGTT [area under the curve $(AUC)_{0–30min}$ C-peptide/$AUC_{0–30min}$ glucose and $AUC_{0–120min}$ C-peptide/$AUC_{0–120min}$ glucose] was carried out by multiple linear regression analysis (least squares method) adjusted for potential confounders (sex, age, BMI and insulin sensitivity). The series of insulin levels during the hyperglycemic clamp study was analyzed with a repeated-measures ANOVA approach using sex, age, and insulin sensitivity as covariates. The genotype was modeled using additive inheritance in all tests. Values ($P < 0.05$) were considered statistically significant.

Results

Alterations of islet gene expression associate with HbA_{1c} and *in vivo* insulin secretion

In the mRNA microarray data, several genes showed differential expression in patients with diabetes or increased HbA_{1c} >6% (Supplemental Fig. 1). The most highly differentially expressed genes showed good correlation with previously published data (Supplemental Fig. 2) (11). Among the top upregulated genes ($-1 \leq$ log fold change ≥ 1; Supplemental Fig. 1), only aldolase B (*ALDOB*) and Fas apoptotic inhibitory molecule 2 (*FAIM2*) displayed a substantial, negative correlation with *in vivo* insulin secretion (expressed as HOMA2%B; Fig. 1A and 1B). Moreover, *ALDOB* and *FAIM2* displayed a considerable positive correlation with HbA_{1c} (Fig. 1C and 1D). However, the expression level of *FAIM2* (mean $\log_2$ intensity 6.5) is much lower than that of *ALDOB* (mean $\log_2$ intensity 9.7) (Fig. 1).

Several other mRNAs, namely the glucose transporter *SLC2A2* (GLUT2), the fatty acid receptor 4 (*FFAR4*), and two transmembrane proteins *TMEM37* (Ca-channel inhibitory γ subunit) and *TMEM27*, associated negatively with HbA_{1c} (Fig. 1E–1H). These proteins are specifically expressed in β-cells and were reported to be decreased in type 2 diabetic islets (7, 11). However, the mRNA levels of *SLC2A2*, *FFAR4*, *TMEM37*, and *TMEM27* as well as the β-cell–specific isoform *ALDOA* displayed no correlation with HOMA2%B (data not shown).

ALDOB gene variation associates with insulin secretion in humans

If ALDOB plays a causal role in the impairment of insulin secretion during development of type 2 diabetes, genetic variation of *ALDOB* could associate with changes in

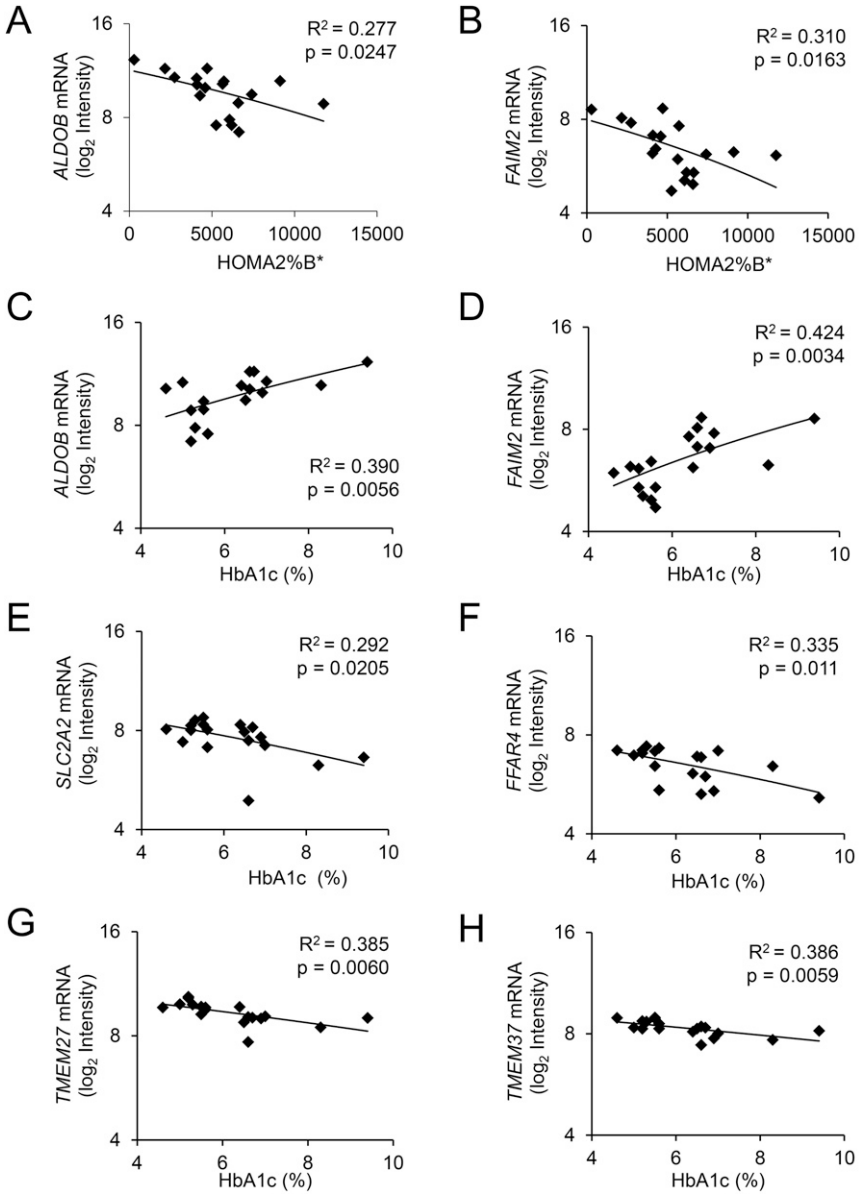

Figure 1. Correlation of mRNA levels with HbA_{1c} and *in vivo* insulin secretion (HOMA2%B*) in LCM-cut islet tissue. The mRNA levels determined by Affymetrix array of LCM-isolated islet tissue (n = 18), HbA_{1c}, fasting blood glucose, and *in vivo* insulin secretion of the same patients were assessed as described in *Materials and Methods*. Correlations of islet mRNAs with (A and B) insulin secretion expressed as HOMA2%B* or with (C–H) plasma HbA_{1c}. *Adjusted for insulin resistance (HOMA2-IR).

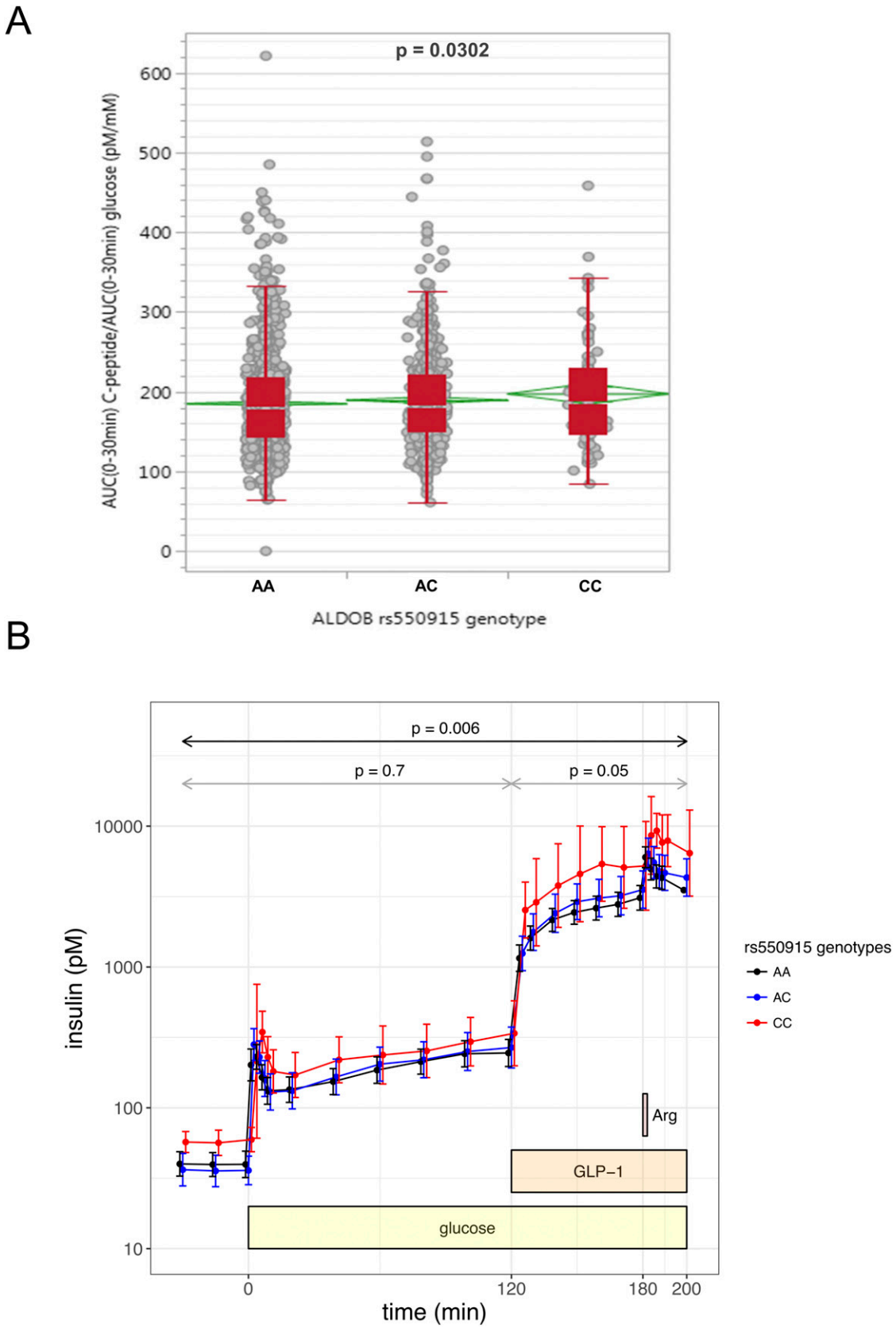

Figure 2. Effect of *ALDOB* SNP rs550915 A>C on insulin secretion derived from five-point OGTT and hyperglycemic clamp. (A) Insulin secretion is given as $AUC_{0–30min}$ C-peptide/$AUC_{0–30min}$ glucose adjusted for sex, age, BMI, and OGTT-derived insulin sensitivity by multiple linear regression modeling. The SNP was tested in the additive inheritance model. The red boxes indicate the interquartile range cut into two pieces by the median; the whiskers extend to the first quartile − 1.5 × (interquartile range) and to the third quartile + 1.5 × (interquartile range). The green diamonds indicate the mean and 95% CI of the mean. (B) The hyperglycemic clamp was started using a body weight–adapted intravenous bolus

insulin secretion. Therefore, we investigated the association of two *ALDOB* SNPs in 2645 genotyped individuals of our TUEF cohort with insulin secretion assessed from 5-point OGTT. The minor allele of rs550915 in the *ALDOB* gene region associated with a significantly higher insulin secretion (Fig. 2A). Similarly, when insulin secretion was assessed from hyperglycemic clamp to circumvent the effects of glucose handling, insulin secretion was higher in minor allele carriers of rs550915 (n = 70; Fig. 2B). The effect was most prominent during the last phase of the clamp involving GLP-1 and arginine administration. From seven SNPs of the *FAIM2* gene identified in our TUEF cohort, none was associated with alterations in insulin secretion (data not shown). These results endorse the prior assumption that ALDOB directly interferes with insulin secretion.

The expression of aldolase B in β-cells is augmented by glucose

To elucidate the cell type expressing ALDOB, pancreatic sections were immunostained for ALDOB, insulin, somatostatin, and glucagon (Fig. 3A–3C). In pancreatic islets from patients with HbA_{1c} <6%, ALDOB immunostaining was undetectable. In tissue slices of patients with HbA_{1c} >6%, ALDOB was detected in some cells within the islets. The ALDOB-positive cells displayed insulin immunoreactivity and were glucagon and somatostatin negative (Fig. 3A–3C). Of note, there was a poor overlapping between ALDOB and insulin signals, which suggests no colocalization of ALDOB and insulin within these cells (Fig. 3C and Supplemental Fig. 3A). The same distribution pattern of ALDOB was observed in isolated human islet β-cells from organ donors upon exposure to 20 mM glucose for 48 hours (Supplemental Fig. 3B). These results suggest that hyperglycemia induces expression of ALDOB in islet β-cells. In agreement, previous studies reported an elevation of *AldoB* mRNA in rodent islets exposed to high glucose concentrations (20). To confirm these findings, we examined whether high glucose alters the expression of ALDOB in human islets isolated from organ donors. ALDOB protein was already detectable in the islets cultured at 5 mM glucose, and its level was significantly increased upon islets exposure to 15 mM or 20 mM glucose (Fig. 3D and 3E).

LCM-cut islet tissue is enriched in β-cells

The composition of LCM-cut tissue samples with respect to endocrine cell ratio was evaluated by analyzing insulin, glucagon, and somatostatin mRNA levels using quantitative RT-PCR (qRT-PCR) (Table 1). The relative insulin mRNA level was 2500-fold higher than that of the housekeeping gene *RPS13* and ~100-fold higher than glucagon and somatostatin mRNA levels. This proportion differs from the one observed in cultured human islets isolated from organ donors (19). The insulin mRNA level of LCM-cut islet tissue, determined by the same qRT-PCR method, was 10-fold higher than that of isolated islets from organ donors, whereas the mRNA level of glucagon was 10-fold lower. This observation implies an enrichment of β-cells over α-cells when using the LCM as method for islet isolation.

Endocrine cell differentiation markers and α/β-cell areas do not correlate with HbA_{1c}

β-cell dedifferentiation is a mechanism proposed to be, at least partly, responsible for reduced β-cell mass and impaired β-cell function (21). The mRNA level of the β-cell differentiation marker *PDX1* as well as other β- and α-cell–specific markers (*MAFB*, *NEUROD1*, and *FOXO1*) was remarkably similar between individuals and did not correlate with HbA_{1c} (Supplemental Fig. 4).

To examine a possible association of β- and α-cell mass with glycemia, we assessed the area of insulin- and glucagon-immunostained islet cells in the pancreatic resections. Insulin immunostaining varied between 39% and 76% of total islet area, but there was no correlation with either HbA_{1c} or BMI (61.06 ± 3.4% of total islet area in n = 10 subjects with HbA_{1c} <6% and 60.56 ± 2.7% of total islet area in n = 7 subjects with HbA_{1c} >6%) (Fig. 4A and 4B). Similarly, glucagon immunostaining varied between 13% and 39% of islet area, but there was no correlation between α-cell area and HbA_{1c} or BMI (Fig. 4C and 4D). The average α-cell area was 23.8 ± 3.1% of total islet area in n = 9 subjects with HbA_{1c} <6% and 28.7 ± 1.7% of total islet area in n = 9 subjects with HbA_{1c} > 6%. Furthermore, the highly variable mRNA levels of insulin, glucagon, and somatostatin did also not correlate to HbA_{1c}, in our subjects at least (Fig. 4E–4G). These results suggest that dedifferentiation and loss of β-cell mass are not major causes of the development of frequent hyperglycemic episodes (*i.e.*, increased HbA_{1c}).

Furthermore, no mRNA levels examined correlated with BMI, suggesting that obesity had no major impact on β-cell–specific gene expression in our cohort

Figure 2. (Continued). of 20% glucose solution at min 0 and a continuous infusion adjusted to a target glucose level of 10 mM. GLP-1 infusion (bolus 4.5 pmol/kg, followed by a continuous administration with 1.5 pmol kg^{-1} min^{-1}) was started at min 120. An arginine bolus (5 g, injected over 45 s) was administered at 180 min. Insulin was assessed from venous blood samples at the respective time points. Shown are geometric means with 95% CIs for n = 70 (n_{AA}= 42, n_{AC} = 25, and n_{CC} = 3) individuals.

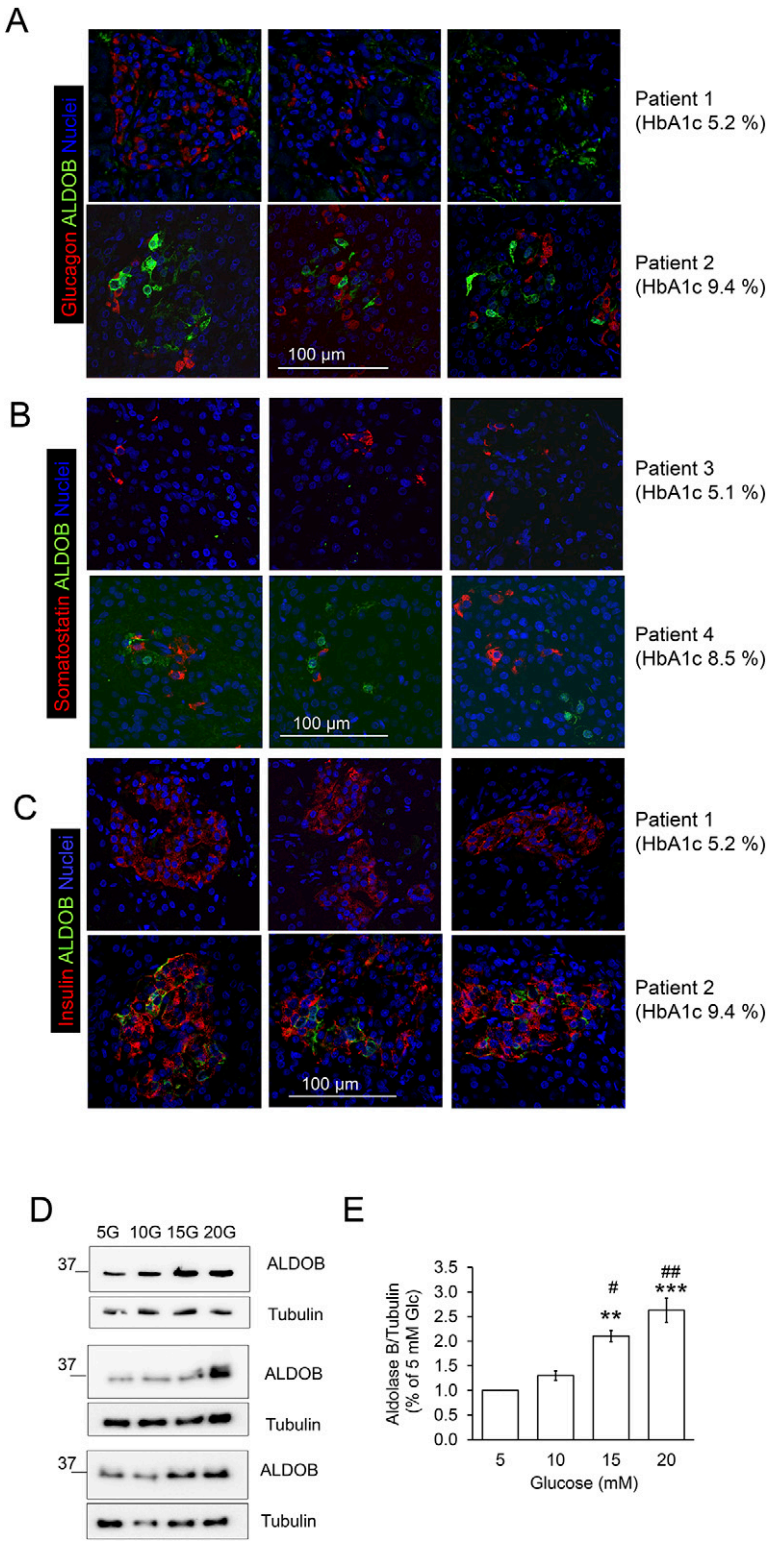

Figure 3. Expression of aldolase B protein is increased by hyperglycemia in human islets. (A–C) Human pancreatic slices from the samples analyzed by Affymetrix array were immunostained for ALDOB, insulin, glucagon, and somatostatin as described in *Materials and Methods*. Representative confocal pictures of (A) ALDOB (green), glucagon (red), and nuclei (blue), (B) ALDOB (green), somatostatin (red), and nuclei (blue), and (C) ALDOB (green), insulin (red), and nuclei (blue) in two patients with HbA_{1c} <5.5% and HbA_{1c} >8%. (D and E) Isolated human islets were cultured for 72 h in the presence of different glucose concentration as indicated and described in *Materials and Methods*. (D) Western blots of ALDOB and (E) quantitative analysis presented as mean ± SEM of n = 3 independent experiments. $^{**}P < 0.005$; $^{***}P < 0.001$ vs 5 mM glucose; $\#P < 0.05$; $\#\#P < 0.005$ vs 10 mM glucose.

(Supplemental Fig. 5). Because vitamin D may modulate insulin secretion (22), we also assessed plasma concentrations of 25-hydroxy-vitamin D. No correlation of vitamin D with any of the examined parameters was found (data not shown).

In summary, ALDOB expression is increased in islets from hyperglycemic subjects, an event that is associated with reduced insulin secretion.

Discussion

The current study shows that islet *ALDOB* mRNA levels negatively correlate with *in vivo* insulin secretion in humans. A direct effect of ALDOB on insulin secretion is suggested by the association of a common variant in *ALDOB* with insulin secretion evaluated using OGTT and hyperglycemic clamp studies in humans. Hyperglycemic clamp is the gold standard for the assessment of insulin secretion, allowing the estimation of insulin secretion independently from endogenous changes of blood glucose levels.

An upregulation of *ALDOB* and *FAIM2* expression with hyperglycemia has been documented previously (11). Despite the limited sample size in our study, the expressional alterations mirror recent data obtained from a larger set (n = 103) of LCM-cut islet tissue (Supplemental Fig. 2) (11).

In agreement with the transcriptome analysis of LCM-cut islet tissue, chronic exposure to high glucose augmented ALDOB protein in human islets isolated from organ donors. Similarly, in mature, functional rodent β-cells, *AldoB* expression is repressed, but strongly upregulated by hyperglycemia (7, 20, 23). The confocal laser scanning imaging of immunostained pancreatic tissue and isolated human islet cells suggests that ALDOB-expressing islet cells are β-cells, as ALDOB and insulin signals were detected in the same cells. Single islet cell

Table 1. Relative mRNA Levels Assessed by Quantitative PCR of Insulin (*INS*), Glucagon (*GCG*), and Somatostatin (*SST*) in 12 RNA Samples From LCM-Isolated Islet Tissue

	Relative mRNA Level (ΔCt)		
Gene Symbol	**Mean**	**SEM**	**n**
INS	2466.15	349.86	12
GCG	35.31	5.76	12
SST	18.43	2.62	12
RPS13	1.00	—	12

The housekeeping gene RPS13 was set to 1.

Abbreviation: Ct, threshold cycle.

transcriptomics data suggest that only a minor fraction of β-cell population expresses *ALDOB* (Supplemental Fig. 6) (7, 18). However, an increased expression of *ALDOB* was also detected in β-, δ-, and γ- cells from subjects with type 2 diabetes (Supplemental Fig. 7) (7). That dedifferentiation of β-cells underlies this process is unlikely, because the mRNA levels of β-cell–specific genes (*PDX1*, *MAFB*, *INS*, and *NEUROD1*), as well as β- and α-cell mass, were not different between patients with high and low HbA_{1c}. The transcripts of *NANOG* and *POU5F1B* were only modestly elevated in the hyperglycemic islets (data not shown), which, similarly to previous studies, suggests no considerable dedifferentiation of type 2 diabetic β-cells (9, 11).

Our observations suggest that increased expression of *ALDOB* in β-cells affects *in vivo* insulin secretion. A recent study with a β-cell–specific *Raptor* knockout mouse showed increased expression of *AldoB* in the islets and impaired glucose-induced insulin secretion (GIIS) and ATP production (24). Similarly, β-cell–specific *NeuroD1* knockout mice display impaired GIIS and upregulated *AldoB* in their β-cells (25). Noteworthy, the juvenile, glucose-unresponsive β-cells express high levels of ALDOB and have high glycolytic flux and low oxidative metabolism (23, 26). Thus, a chronic upregulation of ALDOB in adult β-cells might shift the glucose metabolism away from ATP production, an effect that could impair GIIS. ALDOB catalyzes the cleavage of fructose 1,6-bisphosphate and fructose 1-phosphate. The glycolytic intermediates DHAP and GA3P are primary sources for methylglyoxal production (27). ALDOB seems to be involved in glucose-dependent overproduction of methylglyoxal (28). Methylglyoxal accumulation predicts endothelial dysfunction in type 2 diabetes (29). In β-cells, methylglyoxal-induced protein glycation and reactive oxygen species accumulation impair mitochondrial function and insulin secretion (30). Further experiments are required to elucidate whether ALDOB mediates these events in human β-cells of subjects with type 2 diabetes.

The association of rs550915 in *ALDOB* with altered insulin secretion both in OGTT and hyperglycemic clamp studies indicates that ALDOB modulates insulin secretion (Fig. 2A and 2B). Data from the GTEx project indicate that this SNP is an expression quantitative trait locus. The minor allele of rs550915 is significantly associated with lower *ALDOB* expression in the tibial nerve ($P = 0.000015$; normalized effect size = −0.39). Islets are not specifically sampled in the GTEx project, but an association with expression in pancreas was not seen. However, rs550915 is a proposed multitissue expression quantitative trait locus in GTEx data, suggesting that it regulates gene expression of *ALDOB* in several tissues. In genome-wide association studies (with results accessed from www.type2diabetesgenetics.org), only one GWAS showed a nominal association of rs550915 with type 2 diabetes (31). The discrepancy between effects on insulin secretion and type 2 diabetes prevalence could derive from compensatory glycemic effects of the variant in other tissues. *ALDOB* is prominently expressed in liver and kidney, two organs involved in glucose disposal and reabsorption. Effects of *ALDOB* in islets could be potentially offset by effects in these organs (32, 33). In our study, the endogenous glucose handling was circumvented using a hyperglycemic clamp, which enabled us to investigate alterations of *in vivo* insulin secretion (Fig. 2B).

The other prominently upregulated gene in hyperglycemic patients was the obesity-related gene *FAIM2* (34). *FAIM2* expression in healthy islet cells is low (7). Recent publications reported the upregulation and increased methylation of *FAIM2* in type 2 diabetic islets, but its function in β-cells is currently unknown (11, 34, 35). We also found no association between SNPs of *FAIM2* and insulin secretion in our cohorts. As corroborated by the low expression level, this observation prompted us to attribute a rather minor role to FAIM2 in insulin secretion.

In contrast to the upregulation of *ALDOB* and *FAIM2*, expression of the β-cell proteins *SLC2A2, FFAR4, TMEM37*, and *TMEM27* correlated negatively with hyperglycemia, confirming recent observations in type 2 diabetic islets (6, 11). The reduced *SLC2A2* mRNA level points to a functional defect of β-cells (36). Nonetheless, we found no correlation of *SLC2A2* with *in vivo* insulin secretion. *FFAR4*, a long-chain fatty acids receptor, is expressed in β-, α-, and δ-cells and augments glucagon and somatostatin secretion (7, 37, 38). Again, we found no correlation with insulin secretion. Although a reduced expression of *TMEM37*, the regulatory γ subunit of the voltage-gated Ca^{2+} channel, was found in type 2

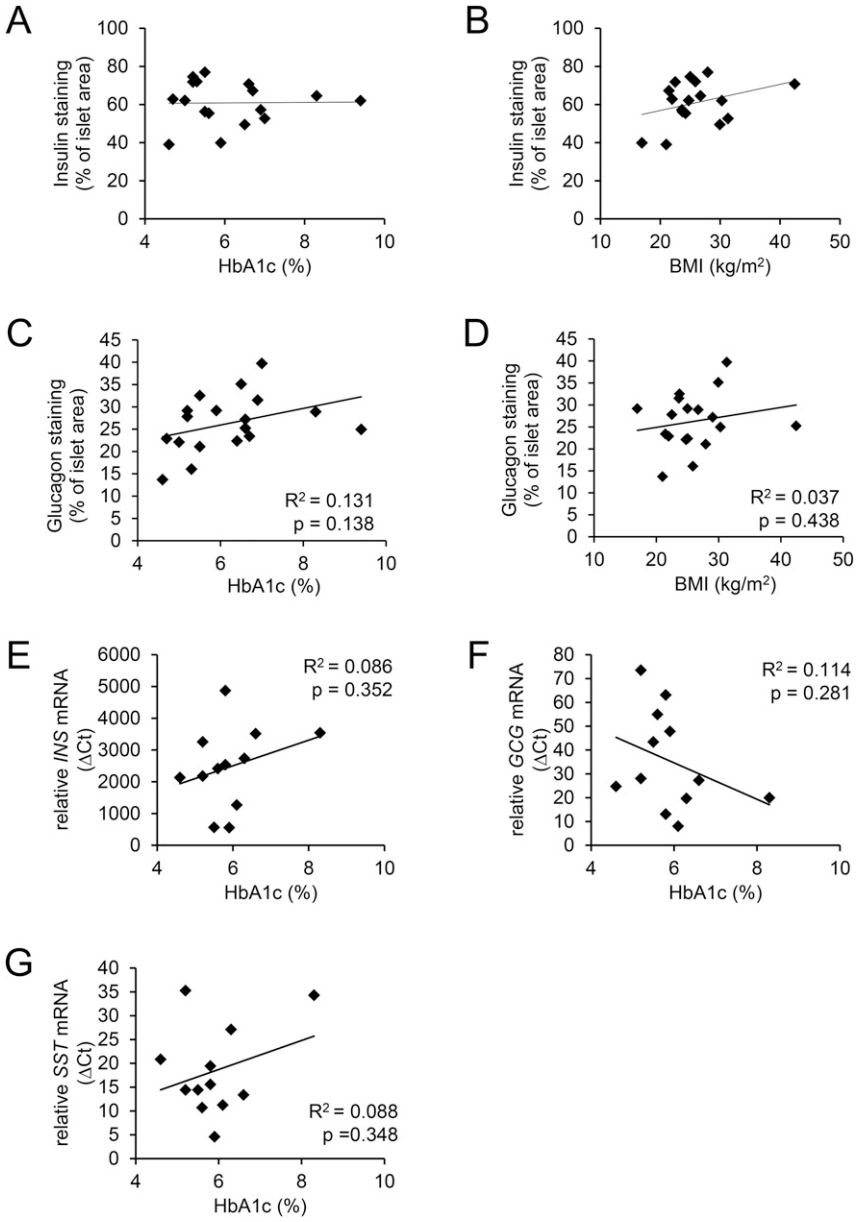

Figure 4. β-Cell and α-cell areas and the expression level of the islet hormones did not correlate with either HbA_{1c} or BMI. (A and B) β-cell area (expressed as percentage of total islet area) determined by insulin immunostaining in n = 17 pancreatic resections. (C and D) α-Cell area (expressed as percentage of total islet area) determined by glucagon immunostaining in n = 18 pancreatic resections as described in *Materials and Methods*. HbA_{1c} and BMI were from the same patients. The relative mRNA levels of (E) insulin (*INS*), (F) glucagon (*GCG*), and (G) somatostatin (*SST*) were assessed by qRT-PCR as described in *Materials and Methods* in n = 12 LCM-isolated islet tissue samples.

diabetic islets, TMEM37 downregulation in INS-1E cells augmented insulin secretion (11). The negative correlation between *TMEM27* and HbA_{1c} confirms previous reports (11, 39). TMEM27 supports GIIS and was proposed as a marker of β-cell mass (7, 40). This study did not uncover a correlation of *TMEM27* with insulin secretion, nor was β-cell area reduced in patients with high HbA_{1c}.

In conclusion, our data suggest that an upregulation of *ALDOB* in human β-cells occurs upon the development of hyperglycemia and may contribute to impairment of insulin secretion in humans.

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patients and obtained tissue and blood samples. F.G., H.S., A.M.S., E.L.-G., G.K., M.P., S.H., M. Schütz., M. Stadion, A.S., F.M., M.I., T.F., P.P.N., S.U., and R.W. performed experiments and analyzed data. F.G., H.S., S.U., and R.W. prepared figures and wrote the manuscript. F.G., B.A.J., H.S., A.M.S., E.L.-G., G.K., M.P., S.H., M.H., M. Schütz, M. Stadion, A.S., F.M., M.I., B.S., F.F., T.F., P.P.N., A.K., S.N., S.W., A.P., A.F., D.R., M. Solimena, H.-U.H., S.U., and R.W. approved the final version of the manuscript. M. Solimena, H.-U.H., S.U., and R.W. conceived and designed the study.

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