Age-Dependent Decline in the Coordinated \([\text{Ca}^{2+}]\) and Insulin Secretory Dynamics in Human Pancreatic Islets

Matthew J. Westacott,1 Nikki L. Farnsworth,2 Joshua R. St. Clair,1 Greg Poffenberger,3,4,5 Audrey Heintz,1 Nurin W. Ludin,1 Nathaniel J. Hart,3 Alvin C. Powers,3,4,5 and Richard K.P. Benninger1,2

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Aging is associated with increased risk for type 2 diabetes, resulting from reduced insulin sensitivity and secretion. Reduced insulin secretion can result from reduced proliferative capacity and reduced islet function. Mechanisms underlying altered β-cell function in aging are poorly understood in mouse and human islets, and the impact of aging on intraislet communication has not been characterized. Here, we examine how β-cell \([\text{Ca}^{2+}]\) and electrical communication are impacted during aging in mouse and human islets. Islets from human donors and from mice were studied using \([\text{Ca}^{2+}]\) imaging, static and perifusion insulin secretion assays, and gap junction permeability measurements. In human islets, \([\text{Ca}^{2+}]\) dynamics were coordinated within distinct subregions of the islet, invariant with islet size. There was a marked decline in the coordination of \([\text{Ca}^{2+}]\) dynamics, gap junction coupling, and insulin secretion dynamics with age. These age-dependent declines were reversed by pharmacological gap junction activation. These results show that human islet function declines with aging, which can reduce insulin action and may contribute to increased risk of type 2 diabetes.

There is a significant decline in glucose tolerance and increased risk of type 2 diabetes with advancing age in humans (1). Reduced glucose tolerance in aging results from both reduced insulin sensitivity and reduced glucose-stimulated insulin secretion (2–5). β-Cell proliferation and proliferative capacity decline with age, which can partly explain the reduced insulin secretion and risk for type 2 diabetes (6,7). However, β-cell function is also altered upon aging (8,9). β-Cells respond to glucose by elevated mitochondrial respiration, membrane depolarization, increased intracellular free-calcium ([Ca$$^{2+}$$]), and insulin granule exocytosis. Several of these steps, including mitochondrial respiration (10–12) and [Ca$$^{2+}$$] handling (10,12,13), are altered in β-cells from aged mice and humans. However, there have been conflicting results. For example in human islets, improved insulin secretion upon aging or in senescent β-cells has been reported (11), yet others have reported declines in insulin secretion with age (9,10,12,14). Differences between mouse and human β-cell responses to aging have also been reported (10,13). Therefore, much remains to be understood regarding how β-cell function becomes altered with aging.

β-Cells within the islets of Langerhans do not function autonomously. There is extensive communication between β-cells and with other cell types (15–20) that is important for how β-cells function within the islet. Gap junctions formed from connexin36 (Cx36) electrically couple β-cells (21–23), which coordinates the oscillatory [Ca$$^{2+}$$] response to elevated glucose and regulates the dynamics of insulin secretion (21,22). A loss of Cx36 gap junction coupling disrupts first-phase and second-phase pulses and leads to glucose intolerance (24). Notably, gap junction coupling and coordinated [Ca$$^{2+}$$] dynamics are disrupted in models of obesity and type 2 diabetes (25–28), suggesting a role in the pathogenesis of diabetes (29). However, the effect of aging on intraislet communication and the regulation of insulin secretion, including that of gap junction coupling, has not been examined. Furthermore, the role of gap junction
coupling and coordinated [Ca\textsuperscript{2+}] in human islet function has been poorly characterized compared with rodent islets.

Here we examine the intraislet regulation of β-cell [Ca\textsuperscript{2+}] and insulin secretion in islets from mice and human donors without diabetes. We describe how gap junction coupling and coordinated [Ca\textsuperscript{2+}] are disrupted by aging, how this impacts the regulation of insulin secretion, and how gap junction modulation can restore changes in [Ca\textsuperscript{2+}] and insulin secretion. The results from this study provide further evidence for a decline in islet function with age in humans, which may contribute to increased risk of type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**Human Islets**

Human islets were obtained from the Integrated Islet Distribution Program (http://iidp.coh.org/) (Supplementary Table 1) during 2013–2016 (Ca\textsuperscript{2+}, static secretion, and gap junction measurements) or 2012–2016 (perifusion measurements). Islets were cultured in Connaught Medical Research Laboratories at 37°C, 5% CO\textsubscript{2}, for 24–48 h prior to imaging or secretion assays. All [Ca\textsuperscript{2+}] imaging, static secretion assays, and gap junction measurements were performed at the University of Colorado on islets from donors indicated in Supplementary Table 1A. All perifusion measurements were performed separately at Vanderbilt University on islets from donors indicated in Supplementary Table 1B, some of which were included in a previous study (30). Islets from a subset of donors were examined at both locations.

**Animal Care and Islet Culturing**

All animal use was approved by the University of Colorado Institutional Animal Care and Use Committee. Cx36\textsuperscript{-/-} mice were generated as described previously (31), and mouse islets were isolated under ketamine/xylazine anesthesia (80/16 mg/kg) with pancreas inflation using collagenase solution injected into the pancreatic duct. Islets were handpicked and cultured in RPMI with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin.

**[Ca\textsuperscript{2+}] Imaging**

Islets were mounted on 35-mm glass-bottom dishes using Cell-Tak (BD Biosciences). Fluoro4-AM was loaded at 4 μmol/L for 90 min at room temperature in imaging buffer (125 mmol/L NaCl, 5.7 mmol/L KCl, 2.5 mmol/L CaCl\textsubscript{2}, 1.2 mmol/L MgCl\textsubscript{2}, 10 mmol/L HEPES, 0.1% BSA, pH 7.4). Islets were imaged on an inverted Nikon Eclipse-TI widefield microscope using a 20× 0.75 NA objective, at 37°C. Images were acquired one frame per second using 490 nm/525 nm emission filter. Islets from batches in which cell viability was <80%, or islets with absent Fluoro4-AM signal or significant drift, were excluded from subsequent analysis.

**Fluorescence Recovery After Photobleaching**

Islets were mounted on 35-mm glass-bottom dishes using Cell-Tak. Rhodamine123 was loaded at 12.5 μmol/L for 30 min at 37°C. Islets were imaged on an inverted Zeiss LSM510 confocal microscope using a 40× 1.2 NA water objective at room temperature. Rhodamine123 was imaged and photobleached with a 488-nm Ar\textsuperscript{+} laser line at 2.88 mW/cm\textsuperscript{2} and 316.05 mW/cm\textsuperscript{2}, respectively (32). Three baseline images were acquired prior to photobleaching half the islet for 235.5 s until fluorescence was reduced 30–45%. Images were acquired every 15 s for 5 min to record fluorescence recovery. The recovery rate correlates with gap junction coupling and was calculated from the recovery curve for the bleached half of the islet.

**Insulin Secretion**

Insulin secretion was determined in static assays by incubating duplicates of 10 islets per tube in Krebs-Ringer buffer (128.8 mmol/L NaCl, 5 mmol/L NaHCO\textsubscript{3}, 5.8 mmol/L KCl, 1.2 mmol/L KH\textsubscript{2}PO\textsubscript{4}, 2.5 mmol/L CaCl\textsubscript{2}, 1.2 mmol/L MgSO\textsubscript{4}, 10 mmol/L HEPES, 0.1% BSA, pH 7.4) with 2 mmol/L glucose for 1 h, followed by 1 h incubation with either 2 or 20 mmol/L glucose. Supernatant (secreted fraction) was collected and then islets were lysed with 2% Triton X-100 and frozen overnight for analysis of insulin content. Insulin concentration was measured with a mouse ultrasensitive ELISA cross-reactive for human insulin, using human insulin standards (ALPCO and Chrystal Chem). Data are presented as insulin secretion normalized by content.

**Data Analysis**

[Ca\textsuperscript{2+}] images were analyzed in MATLAB (MathWorks) to assess activity and coordination. A 4-pixel averaging filter was first applied, and peak detection (34) recorded the locations and amplitudes of oscillations across the islet image. Regions in which no significant peaks could be detected were determined to be inactive. Coordinated regions were segmented based on the coincidence of the time points of each detected peak. This was followed by cross-correlation analysis between time courses of each subregion. If the correlation coefficient was >85%, the two subregions were considered highly coordinated and merged into a larger region. Islet size was determined by the area with significant Fluo4 fluorescence above the background. FRAP (fluorescence recovery after photobleaching) recovery rates were calculated in ImageJ using the time series analyzer.

Perifusion time courses were analyzed in MATLAB. Time points for the addition/removal of 16.7 mmol/L glucose (±IBMX) were recorded and the fold change in insulin secretion calculated using the maximum secretion value in the stimulation period against all initial baseline measurements prior to 16.7 mmol/L glucose, or the previous four time points prior to 16.7 mmol/L glucose + IBMX. At the
end time point of 16.7 mmol/L glucose (±IBMX), an exponential decay \( a \exp(-b \cdot t) + c \) was fit, with \( b \) the decay rate.

Statistical analysis was performed in Prism6 (Graphpad). Data are presented as mean ± SEM or linear trend with 95% CIs. Student \( t \) test assessed statistical significance between groups; ANOVA was used for multiple comparisons followed by Tukey honestly significant difference post hoc analysis; and an \( F \) test assessed statistical significance in linear regression slopes. Outliers were identified using ROUT (Robust Regression and Outlier Removal) test (Q = 1%).

RESULTS
Islets Show a Range of Coordinated \([\text{Ca}^{2+}]\) Dynamics
We first examined \([\text{Ca}^{2+}]\) activity and \([\text{Ca}^{2+}]\) coordination in islets from wild-type (WT) and Cx36\(^{-/-}\) mice. Islets from WT mice exhibited coordinated \([\text{Ca}^{2+}]\) activity with synchronous oscillations across the entire islet (Fig. 1A, left). In islets from Cx36\(^{-/-}\) mice, \([\text{Ca}^{2+}]\) activity lacked any coordination (Fig. 1A, right), as previously described (21,22).

We next examined \([\text{Ca}^{2+}]\) activity and coordination in islets from human donors (Supplementary Table 1A, Colorado). Islets from 3 of 40 (~8%) donors showed behavior similar to islets from WT mice, where coordinated \([\text{Ca}^{2+}]\) activity occurred over the majority of the islet (Fig. 1B). In islets from the remaining 37 of 40 donors, \([\text{Ca}^{2+}]\) activity occurred over the majority of the islet, yet the coordination was restricted to subregions of the islet (Fig. 1C). Across all donors, the \([\text{Ca}^{2+}]\) activity, as expressed by the proportion of cells that responded to glucose by showing \([\text{Ca}^{2+}]\) oscillations, composed 63 ± 4% of the islet (Fig. 1D), consistent with the \( \beta \)-cell fraction in human islets (35,36). In contrast, the coordination of \([\text{Ca}^{2+}]\) dynamics, as expressed by the
largest fractional area, which showed coordinated synchronous \([\text{Ca}^{2+}]\) oscillations, was 31 ± 4% of the total islet area imaged (5,427 μm², ~36 cells) (Fig. 1E). This is substantially less than the area of \([\text{Ca}^{2+}]\) activity, and less than the \([\text{Ca}^{2+}]\) coordination in islets from WT mice \((P < 0.001)\). However, this \([\text{Ca}^{2+}]\) coordination was significantly larger than in islets from Cx36−/− mice \((P = 0.0032)\), indicating electrical coupling is present. The maximum coordinated area of \([\text{Ca}^{2+}]\) did not vary significantly with islet size (Fig. 1F, \(P = 0.31\)) and showed a nonsignificant trend to decline when normalized to islet size (Fig. 1G, \(P = 0.077\)). This indicates that the coordination of \([\text{Ca}^{2+}]\) dynamics is restricted to subregions of human islets, consistent with histological studies of cyto-architecture (35,36).

**Age-Dependent Decline in \([\text{Ca}^{2+}]\) and Gap Junction Function in Human Islets**

Age-dependent changes occur in several aspects of β-cell function, but changes in intraislet communication with aging have not been reported. Given the substantial variability we observed in human islet \([\text{Ca}^{2+}]\), we tested how \([\text{Ca}^{2+}]\) activity and coordination change as a function of donor age. \([\text{Ca}^{2+}]\) activity was variable and the decreasing trend with age was not significant (Fig. 2A, \(P = 0.11\)). When donors were segmented by age (median = 40.0 years for \([\text{Ca}^{2+}]\) imaging experiments), \([\text{Ca}^{2+}]\) activity in the older group was lower but not significantly different than the younger group: 56 ± 4% compared with 71 ± 4%, respectively (Fig. 2A, \(P = 0.092\)). The coordination of \([\text{Ca}^{2+}]\) dynamics showed a significant decline with age, as measured by the size of the largest coordinated area normalized by size of the islet (Fig. 2B, \(P = 0.051\)) and the absolute size of the largest coordinated area (Fig. 2C, \(P = 0.0076\)). Similarly, when segmented by age, the older group had significantly reduced coordination compared with the younger group: 24 ± 3% and 3,874 ± 485 μm² (~26 cells) compared with 40 ± 4% and 7,437 ± 1,111 μm² (~50 cells) (Fig. 2B, \(P = 0.030\), and Fig. 2C, \(P = 0.0062\)). Consistent with these data, there was a significant correlation between \([\text{Ca}^{2+}]\) activity and the coordination of \([\text{Ca}^{2+}]\) dynamics over each donor (Supplementary Fig. 1A, \(P < 0.001\)). The mean size of islets studied did not vary with age (Supplementary Fig. 1B, \(P = 0.60\)), excluding a size effect. The mean duty cycle (plateau fraction) of the largest coordinated area did not show variation with age (43 ± 2%) (Fig. 2D, \(P = 0.59\)). Similarly, there was no change in the mean oscillatory period with age (Supplementary Fig. 1C, \(P = 0.22\)). Across donors in which \([\text{Ca}^{2+}]\) was measured, there was no correlation between BMI and age (Supplementary Fig. 2A, \(P = 0.85\)), indicating age-dependent changes were not a result of increased BMI. In support of this, similar age-dependent changes in \([\text{Ca}^{2+}]\) coordination were observed for donors with BMI <30 and BMI >30 (Supplementary Fig. 2B–D).

Age-dependent decreases in \([\text{Ca}^{2+}]\) coordination were also observed across race (Supplementary Fig. 3).

We next examined how Cx36 gap junction coupling varied with age in islets from human donors (Supplementary Table 1A, Colorado). Cx36 gap junction coupling, as measured by FRAP, showed a decrease with increased age (Fig. 3A, \(P = 0.051\)). When segmented by age (40.0 years), the older group had significantly reduced Cx36 gap junction coupling, as measured by FRAP, showed a decrease with increased age (Fig. 2A, \(P = 0.051\)). When segmented by age (40.0 years), the older group had significantly reduced Cx36 gap junction coupling, as measured by FRAP, showed a decrease with increased age (Fig. 2A, \(P = 0.051\)).
coupling compared with the younger group (Fig. 3A, \( P = 0.035 \)). Islets that showed increased Cx36 gap junction coupling showed a nonsignificant trend to increased \([Ca^{2+}]\) coordination (Fig. 3B, \( P = 0.10 \)). Donors with islet gap junction coupling above the median value showed a significant approximately twofold increase in \([Ca^{2+}]\) coordination compared with donors with islet gap junction coupling below the median value (Fig. 3B, \( P = 0.031 \)). This indicates that increased gap junction function contributes to greater \([Ca^{2+}]\) coordination. Therefore, there exists an age-dependent decline in human islet \([Ca^{2+}]\) coordination, which results in part from an age-dependent decline in gap junction coupling.

**Age-Dependent Decline in Insulin Secretion Dynamics**

Cx36 gap junction coupling and coordinated \([Ca^{2+}]\) responses are important for regulating insulin secretion dynamics (21,24,37). The response time for insulin secretion to revert to basal levels after lowering of glucose is markedly slower upon loss of Cx36 in mice (37). However, insulin secretion levels at elevated glucose do not vary upon loss of Cx36 in mice (15). Therefore, we measured the decline kinetics of islets from human donors (Supplementary Table 1B, Vanderbilt) during perifusion measurements after elevated glucose (9 or 30 min) and glucose + IBMX (9 min) (Fig. 4A). There was no variation in the fold change in insulin secretion upon elevated glucose with age (Fig. 4B, \( P = 0.61 \)), as previously reported (30). Baseline and peak secretion levels or cumulative secretion levels (area under the curve) during elevated glucose also lacked age dependence (Supplementary Fig. 4A–C). Addition of IBMX amplified the fold change in insulin secretion (Fig. 4C, \( P < 0.001 \)), which was independent of age. Under elevated glucose, the rise rate lacked age dependence (Supplementary Fig. 4D, \( P = 0.69 \)). After glucose elevation, the rate of decline in insulin secretion after resumption of basal glucose showed a significant reduction with age, indicating a slower responsiveness of the islet to glucose changes (Fig. 4D, \( P = 0.028 \)). These findings showing normal secretion levels but altered secretion kinetics during aging are consistent with reduced gap junction coupling occurring with age. In contrast, after glucose elevation with IBMX, the rate of decline in insulin secretion after resumption of basal glucose showed no significant change with age (Fig. 4E, \( P = 0.42 \)). In islets from younger donors (<40.0 years), the rate of decline after glucose stimulation showed no difference with or without IBMX (Fig. 4E, \( P = 0.59 \)). However, in islets from older donors (>40.0 years), the rate of decline showed a significant increase with IBMX (Fig. 4E, \( P = 0.0044 \)), reaching the rate observed in younger donors. Thus, IBMX acutely restores the age-dependent decline in insulin secretion dynamics, which is consistent with overcoming the effects of an age-dependent decline in gap junction coupling.

The first phase of insulin secretion upon elevated glucose is also reduced upon loss of Cx36 in mice (24). By including a longer duration of elevated glucose, we measured first-phase insulin secretion 0–9 min after glucose elevation and second-phase insulin secretion 9–30 min after glucose elevation. The first-phase and second-phase levels did not change significantly with age (Supplementary Fig. 4E). However, the ratio of first-phase to second-phase secretion, which factors out donor variability, significantly declined with age (Supplementary Fig. 4F, \( P = 0.0097 \)).

We further examined the impact of age on insulin secretion through static assays using islets from human donors (Supplementary Table 1A, Colorado), where age-dependent declines in secretion have been reported (9,10,12,14). Insulin secretion at 2 mmol/L glucose showed a nonsignificant trend to decrease with age (Fig. 5A, \( P = 0.12 \)). Insulin secretion at 20 mmol/L glucose significantly declined with age (Fig. 5B, \( P = 0.028 \)), which when segmented, showed an ~42% decline in secretion in older donors compared with younger donors. However, there was no change in the stimulation index with age (Fig. 5C, \( P = 0.97 \)). Insulin content also did not vary with age (Fig. 5D, \( P = 0.30 \)). Elevations in \([Ca^{2+}]\) trigger insulin secretion, and age-dependent declines in \([Ca^{2+}]\) activity significantly correlated with insulin secretion at 20 mmol/L glucose (Supplementary Fig. 5A, \( P = 0.0064 \)). However, neither \([Ca^{2+}]\) coordination nor Cx36
gap junction function correlated with insulin secretion (Supplementary Fig. 5B, *P = 0.23*, and Supplementary Fig. 5C, *P = 0.33*). As insulin secretion levels under static assays do not vary upon loss of Cx36 in mice, the lack of correlation between insulin secretion levels and \([\text{Ca}^{2+}]\) coordination are still consistent with reduced gap junction coupling occurring with age (15,21,24).

To further examine the link between insulin secretion dynamics and \([\text{Ca}^{2+}]\) dynamics, we examined perfusion measurements from a subset of donors (Supplementary Table 1) in which static secretion assays and \([\text{Ca}^{2+}]\) imaging were also performed (Supplementary Fig. 6A–I). Perfusion and static measurements of the stimulation index did not show a significant trend in correlation (Supplementary Fig. 6A, *P = 0.14*), in accordance with their age independence. However, the decay rate and \([\text{Ca}^{2+}]\) coordination showed a trend in correlation (Supplementary Fig. 6H, *P = 0.06*), supporting that changes in \([\text{Ca}^{2+}]\) coordination impact insulin secretion dynamics with aging.

**Cx36 Gap Junction Activation Recovers Age-Dependent \([\text{Ca}^{2+}]\) Decline**

Acute IBMX treatment reversed age-dependent declines in insulin secretion dynamics, where IBMX can elevate mouse islet Cx36 gap junction coupling (32). To further test whether age-dependent declines in \([\text{Ca}^{2+}]\) resulted from altered gap junction coupling, we used modafinil, a compound that activates electrical coupling in the central nervous system via CaMKII, and which can also activate Cx36 gap junction coupling in the islet (32,38). We tested if acute modafinil treatment could restore the declines in Cx36 gap junction coupling and \([\text{Ca}^{2+}]\) coordination. Treatment with modafinil for 1 h significantly increased \([\text{Ca}^{2+}]\) activity in islets from older (>40.0 years) donors (Fig. 6A, *P = 0.034*), and with a nonsignificant trend to increased activity in islets from younger (<40.0 years) donors (*P = 0.063*). \([\text{Ca}^{2+}]\) coordination was also significantly increased in islets from older donors (Fig. 6B, *P = 0.051*, and Fig. 6C, *P = 0.024*). However, no significant increase was observed for each measurement in islets from younger donors (Fig. 6B and C). Notably, modafinil increased the \([\text{Ca}^{2+}]\) coordination in islets from older donors to the level in islets from younger donors (Fig. 6B and C). Similarly, modafinil significantly elevated gap junction coupling in islets from older donors (Fig. 6D, *P = 0.023*) but showed no significant increase in islets from younger donors. Thus, acute gap junction activation restores the age-dependent decline in \([\text{Ca}^{2+}]\) coordination and gap junction coupling.

**DISCUSSION**

In this study, we characterized the \([\text{Ca}^{2+}]\) activity and coordinated dynamics in mouse and human islets and tested how aging impacts islet \([\text{Ca}^{2+}]\). Our key findings were that the coordinated \([\text{Ca}^{2+}]\) response at elevated glucose is disrupted in human islets from aged donors. This disruption correlated with reduced gap junction coupling and disrupted insulin secretion dynamics. Notably, this age-dependent disruption could be restored by activators of gap junction coupling.

**Human Islet \([\text{Ca}^{2+}]\) Coordination Is Restricted to Subpopulations of Cells**

Despite wide variability, we generally observed \([\text{Ca}^{2+}]\) to be coordinated over a limited range in human islets, where a similar maximal number of cells were coordinated irrespective of islet size (Fig. 1). This is consistent with prior \([\text{Ca}^{2+}]\) measurements in human islets where \([\text{Ca}^{2+}]\) oscillations
were reported over subregions of the islet (35,39) and is also consistent with histological studies showing fewer \(\beta\)-cell to \(\beta\)-cell contacts in human islets that would reduce coordinated activity (35,36,40). The absence of significant increases in Cx36 gap junction coupling or [Ca\(^{2+}\)] coordination in younger donors upon gap junction activation (Fig. 6) indicates an upper limit to the coordination achievable in human islets, which is suggestive of functional subdivisions of coupled \(\beta\)-cells. However, human islet architecture has also been suggested to consist of folded two-dimensional “sheets” of \(\beta\)-cells and \(\alpha\)-cells. Our findings are similar to measurements of [Ca\(^{2+}\)] in two-dimensional clusters of \(\beta\)-cells where coordinated activity is restricted to subregions (41). Overall, human islet [Ca\(^{2+}\)] coordination is consistent with the electrical coupling between \(\beta\)-cells being governed by so-called “small-world” principles, where electrical connections between small numbers of cells dominate the electrical behavior of the islet, as shown in mouse islets (42,43).

Studying human islets is challenging given human heterogeneity, donor health, variability in processing, and culture time and shipment, and we observed substantial variability within our measurements likely resulting from these factors. Nevertheless, by studying islets from large numbers (32–76) of donors, we were able to observe consistent age-dependent effects in [Ca\(^{2+}\)] coordination, gap junction coupling, and insulin secretion dynamics, as well gap junction–mediated restoration in these factors.

**Age-Dependent Decline in Gap Junction Coupling Disrupts [Ca\(^{2+}\)] Coordination and Insulin Secretion Dynamics in Humans**

Cx36 gap junction channels coordinate [Ca\(^{2+}\)] dynamics within mouse islets. Therefore, the decline in coordinated [Ca\(^{2+}\)] dynamics with age (Fig. 2) likely results from the reduced gap junction coupling with age (Fig. 3). This is supported by the recovery in coordinated [Ca\(^{2+}\)] dynamics by gap junction activation (Fig. 6). The maximum age-dependent decline in gap junction coupling we measure (30%) is less than the loss in [Ca\(^{2+}\)] coordination (60%). In mouse islets, modeling studies predict that beyond a certain level of gap junction coupling, subsequent small decreases in coupling can cause large disruptions to [Ca\(^{2+}\)] coordination (22,41). The architecture of human islets results in fewer \(\beta\)-cell \(\beta\)-cell neighbors, which may explain our results and suggests a greater susceptibility of human islets to [Ca\(^{2+}\)] disruption upon loss of gap junction coupling.

Restoring the age-dependent decline in coordinated [Ca\(^{2+}\)] oscillations and insulin secretion dynamics in human islets was achieved acutely by activators of gap junction coupling, modafinil or IBMX. This recovery occurred in \(\leq 1\) h, indicating that the age-dependent decline to electrical coordination in human islets is unlikely to be due to transcriptional changes. This is consistent with the action of modafinil to regulate electrical coupling via CaMKII-dependent channel gating or trafficking (38). This is also consistent with IBMX-induced cAMP elevations regulating electrical coupling via
PKA-dependent channel gating or trafficking (44,45), although IBMX may also blunt the means by which age-dependent decline in gap junction coupling occurs. In human islets, Cx36 transcription increases (and Cx43 decreases) during maturity (18 years), as in mouse islets (46), but there is no significant change in Cx36 or Cx43 transcription during aging (18–40 compared with >40 years) (K. Kaestner, personal communication). Therefore, the age-dependent decline in gap junction coupling likely results from posttranslational regulation and may include altered phosphorylation, which reduces Cx36 gap junction coupling in other systems (47,48). However, determining the cause of age-dependent Cx36 dysregulation, including oxidative stress, and the underlying mechanism involved requires further study.

A loss of gap junction coupling and coordinated [Ca\(^{2+}\)] dynamics in mice disrupts pulsatile insulin secretion dynamics and glucose tolerance (24), with the decline in insulin secretion after glucose reduction being markedly slower (24,37) and the first-phase secretion being reduced (24). We were unable to measure insulin secretion dynamics from single islets to assess pulsatility. However, perfusion results demonstrating altered secretory kinetics including a slower decline in insulin secretion indicate a reduced glucose responsivenes of insulin secretion. This suggests that a significant disruption to pulse amplitude may also be present. Indeed, a disruption to pulse amplitude in vivo, both on the time scale of second-phase pulsatility as well as ultradian pulses, has been shown to occur during aging (5). These altered secretion dynamics could contribute to the reduced glucose tolerance that occurs in aged humans. For instance, reduced pulsatility can reduce insulin action (49), which would lead to greater glucose excursions and blunt any compensatory changes in type 2 diabetes. Gap junction coupling is also protective against β-cell apoptosis (50); therefore, reduced gap junction coupling could exacerbate β-cell decline. Overall, these age-dependent changes to intraislet communication are summarized in Fig. 7.

**Age-Dependent Decline in Insulin Secretion in Humans**

We also observed a decline in insulin secretion levels with age in human islets under static assays. Our results demonstrate reduced insulin secretion levels but maintained stimulation index, which are consistent with published findings (3,8,10,30). The decline in secretion correlated with a decline in [Ca\(^{2+}\)] activity but not declines in [Ca\(^{2+}\)] coordination or gap junction coupling (Supplementary Fig. 5). In mouse islets, deficiency in Cx36 gap junction coupling...
does not impact the amount of insulin secretion but rather disrupts insulin secretion dynamics under stimulatory conditions (15). This includes first-phase secretion, insulin pulsatility, and the decline in secretion after resumption of basal glucose, causing glucose intolerance. Our findings therefore suggest that other pathways beyond Ca^2+ gap junction coupling and [Ca^{2+}] coordination are also disrupted during aging that impact insulin secretion levels. β-Cell glucose metabolism, mitochondrial function, and ATP production are disrupted with aging in human islets (9,10). Given the importance of ATP production for membrane depolarization, [Ca^{2+}] activity, and insulin secretion, disrupted glucose metabolism is consistent with the decline in insulin secretion levels with age and its correlation with [Ca^{2+}] activity, which occur independent of [Ca^{2+}] coordination or gap junction coupling. A disruption to glucose metabolism with aging would also reduce amplifying pathways and impact second-phase insulin secretion (36,51). We did not observe a significant decline in second-phase secretion within 30 min of glucose elevation. However, a decline over longer timescales may result from disrupted amplification. This could explain why perfusion analysis does not show an age-dependent decline in secretion levels (30), yet static assays, which assess secretion accumulated over a greater duration, do show a decline (8–10). Nevertheless, the role of gap junctions in human islet function is poorly understood, and we cannot exclude that the age-dependent decline in gap junction coupling and [Ca^{2+}] coordination may weakly impact [Ca^{2+}] activity, given the correlation between [Ca^{2+}] coordination and activity (Supplementary Fig. 1C). This may also be explained by subpopulations of β-cells that can disproportionately affect islet function (43,52,53) being lost during aging (54). Therefore, the decline in intra-islet communication upon aging may have a broader impact on human islet function, and the resultant loss in secretion could blunt compensatory changes in type 2 diabetes.

In summary, we observed a decline in gap junction function and coordinated [Ca^{2+}] within human islets during aging. This decline disrupts insulin secretion dynamics and the responsiveness of the islet to acute glucose changes. The age-dependent disruption to gap junction coupling, [Ca^{2+}] coordination, and insulin secretion dynamics can be restored acutely by activators of gap junction coupling. These results provide further understanding to the decline in islet function with age in humans that causes glucose intolerance and predisposes to increased risk for diabetes.

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