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<th>Young capillary vessels rejuvenate aged pancreatic islets (Main article)</th>
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Pancreatic islets secrete hormones that play a key role in regulating blood glucose levels (glycemia). Age-dependent impairment of islet function and concomitant dysregulation of glycemia are major health threats in aged populations. However, the major causes of the age-dependent decline of islet function are still disputed. Here we demonstrate that aging of pancreatic islets in mice and humans is notably associated with inflammation and fibrosis of islet blood vessels but does not affect glucose sensing and the insulin secretory capacity of islet beta cells. Accordingly, when transplanted into the anterior chamber of the eye of young mice with diabetes, islets from old mice are revascularized with healthy blood vessels, show strong islet cell proliferation, and fully restore control of glycemia. Our results indicate that beta cell function does not decline with age and suggest that islet function is threatened by an age-dependent impairment of islet vascular function. Strategies to mitigate age-dependent dysregulation in glycemia should therefore target systemic and/or local inflammation and fibrosis of the aged islet vasculature.

Aging leads to progressive decline of various homeostatic processes in mammals, including a deteriorating regulation of blood glucose levels. Pancreatic islets are small organs composed of endocrine cells that secrete the major hormones insulin, glucagon, and somatostatin, which play a key role in regulating blood glucose levels. Age-dependent dysfunction of islets and the concomitant dysregulation of blood glucose levels increase the risk for type 2 diabetes (1), which in turn contributes to other age-related chronic diseases. In general, it has been assumed that aging causes an intrinsic dysfunction of the insulin-secreting beta cells through reduced proliferative capacity and/or defective insulin secretion (1–9). However, there have been numerous reports that age-dependent impairment of glucose homeostasis is not just a result of intrinsic, age-dependent dysfunction of islets but is also caused by systemic factors. For example, islet function may be compromised by age-related increases in adiposity (10, 11) and by bloodborne factors (12), or it could be affected indirectly by age-related deficiencies in vascular remodeling (13). Thus, the reductive decline of old pancreatic beta cells can be attributed to systemic factors (12). Recent studies identified factors present in young blood that reverse age-related cognitive impairments and induce vascular remodeling and regeneration in the brain and skeletal muscle (14–16), but so far it has not been feasible to discriminate systemic influences from aging factors intrinsic to islet endocrine cells. Here we address the long-standing question of whether the age-dependent impairment of glucose homeostasis is caused by intrinsic, age-dependent dysfunction of islets or by systemic aging factors.

Our strategy to discern age-related intrinsic changes in islet function was to study islets from young mature (2 mo) and aged (18 mo) mice and to follow these same groups of islets in three different environments: in vivo in the body of young and aged mice, in vitro after isolation, and again in vivo after transplantation into the anterior chamber of the eye in young mice (17). We also examined a large number of human islets from young mature and old pancreatic donors (17–65 y of age). We hypothesized that islets are affected by the systemic milieu, such that the effects the aged organism exerts on the islet can be rescued in a young organism. We characterized islet structure and function at the molecular, anatomic, and physiologic levels to distinguish intrinsic from systemic factors impinging on the islet as the organism ages. Our results reveal that aging of islets involves little intrinsic decline of beta cell function but is accompanied by malfunctioning blood vessels, suggesting that age-impaired glucose homeostasis is not caused by the intrinsic aging of beta cells but, rather, is a result of vascular aging that can be reversed by placing aged islets in a young environment.

Results

Beta Cell Secretory Function Is Robust in Aged Mice and Humans. How does aging affect beta cell secretory function? To answer this question, we used both mouse and human islets, as these differ in several aspects, such as cytoarchitecture, cellular plasticity, and turnover (18–20). Mouse islets were isolated from young mature (2 mo) and old (18 mo) virgin male C57BL/6 mice and function at the molecular, anatomic, and physiologic levels rescued in a young organism. We characterized islet structure and function in the body of young and aged mice, in vitro after isolation, and again in vivo after transplantation into the anterior chamber of the eye in young mice (17). We also examined a large number of human islets from young mature and old pancreatic donors (17–65 y of age). We hypothesized that islets are affected by the systemic milieu, such that the effects the aged organism exerts on the islet can be rescued in a young organism. We characterized islet structure and function at the molecular, anatomic, and physiologic levels to distinguish intrinsic from systemic factors impinging on the islet as the organism ages. Our results reveal that aging of islets involves little intrinsic decline of beta cell function but is accompanied by malfunctioning blood vessels, suggesting that age-impaired glucose homeostasis is not caused by the intrinsic aging of beta cells but, rather, is a result of vascular aging that can be reversed by placing aged islets in a young environment.

Significance

The regulation of blood glucose is a homeostatic process that declines with age, but it is unknown whether this disturbance is a consequence of intrinsic dysfunction of the regulatory organ, the pancreatic islet. In marked contrast to the widely held notion that the insulin-producing pancreatic beta cell loses function with wear and tear, and thus causes age-related disturbances in glucose homeostasis, we show that mouse and human beta cells are fully functional at advanced age. The pancreatic islet as an organ, however, is threatened by vascular senescence. Replacing the islet vasculature in aged islet grafts rejuvenates the islet and fully restores glucose homeostasis, indicating that islet blood vessels should be targeted to mitigate frail glucose homeostasis associated with aging.

Author contributions: J.A., P.-O.B., A.C., and H.G.N. designed research; J.A. and J.M. performed research; M.H.A. contributed new reagents/analytic tools; J.A., R.A.e.D., W.B.J., and A.C. analyzed data; and J.A., A.C., and H.G.N. wrote the paper.

Conflict of interest statement: P.-O.B. is one of the founders of the biotech company Biocrine, which is going to use the anterior chamber of the eye as a commercial servicing platform. A.C. holds a patent on this servicing platform. M.H.A. is a consultant of Biocrine.

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The amount of insulin released by human islets did not show a statistically significant correlation with donor age ($r^2 = 0.041$ ($P = 0.08$) and $r^2 = 0.01$ ($P = 0.39$), respectively; Fig. 1 C and D and SI Appendix, Fig. S1). Even when pooling insulin secretion data from young human islets (donor age, 17–27 y; $n = 10$) and comparing these data with those from islets of aged humans (donor age, 50–60 y; $n = 25$), no significant differences were observed. We also found that islets from old mice are capable of healthy buffering of $[\text{Ca}^{2+}]$, in response to high glucose (SI Appendix, Fig. S1). These results indicate that as they age, mouse and human beta cells remain glucose-sensitive, produce adequate responses to glucose, and have robust insulin secretion.

Cytoarchitecture was similar in young and old mouse islets, with alpha cells in the periphery and beta cells in the core (SI Appendix, Fig. S2), and islets retained similar proportions of each cell type (SI Appendix, Fig. S2). We consistently observed that islets in aged mice were larger, which was a result of a simultaneous increase in beta cell number and size (SI Appendix, Fig. S2). Islet area, beta cell number, and size were not significantly different between islets of young (age 15–25 y) and old (age 50–60 y) humans, showing that beta cell mass is preserved in humans with advanced aging [SI Appendix, Fig. S2 (22)].

**Glucose Homeostasis Is Impaired in Old Mice.** The intrinsic ability of beta cells to produce and secrete insulin and the structural properties of islets did not deteriorate in old mice, but do aged mice have a normal glucose metabolism? We compared glucose metabolism in young and aged mice and found that old mice were insulin-resistant (Fig. 1E). Plasma insulin levels were twice as high in old mice (Fig. 1F), and blood glucose levels in nonfasting conditions were lower in old mice (SI Appendix, Fig. S1). These results suggest that old mice compensate for the increased demand for insulin, as indicated by insulin resistance, by increasing beta cell mass and beta cell secretory function (Fig. 1A and B and SI Appendix, Fig. S2). We further compared glucose tolerance in young and aged mice and found that glucose levels during glucose tolerance tests did not differ when challenged with a conventional glucose load (2 g/kg; Fig. 1G). However, when mice were challenged with a larger glucose load (4 g/kg; Fig. 1G) or with a conventional glucose load under stress conditions (Fig. 1H) (23), the recovery was delayed in old mice. These results indicate an age-dependent decline in glucose homeostasis in mice.

**Aged Pancreatic Islets Have Inflamed and Fibrotic Blood Vessels.** Given the lack of functional decline in beta cells of old mice, what, then, is leading to impaired glucose homeostasis? Islets are strongly vascularized, as their ability to sense blood glucose and release insulin depends on close contact with blood vessels. Because advanced age is associated with vascular alterations and chronic inflammation (24), we tested whether the age-associated impairment in glucose homeostasis is caused by islet blood vessel dysfunction. Blood vessel density did not differ between young and old mouse islets (14.5 ± 2.1% versus 14.8 ± 1.5%, respectively; $n = 3$ islets/pancreas, $n = 3$ pancreata/age). To examine the inflammatory status of the islet, we immunostained macrophages in pancreatic sections of young and old mice and humans (Fig. 2A and B and SI Appendix, Fig. S3) and found that islets in aged mice and humans contained twice as many macrophages (Fig. 2B). Macrophages were often associated with blood vessels expressing intercellular adhesion molecule 1 (ICAM-1) (Fig. 2A) and inflammatory marker (25) whose expression was increased in islets of aged mice (Fig. 2C). These findings indicate that blood vessels in aged islets are inflamed. This was further supported by increased expression of macrophage colony-stimulating factor receptor (CSFRI) and genes involved in immune cell recruitment such as ICAMI and vascular cell adhesion molecule 1 (VCAM1) in old islets (Fig. 2D).

FIG. 1. Beta cells in aged mice and humans are functionally robust, but glucose tolerance is fragile. (A) Insulin secretion from islets isolated from young (2 mo, green) and aged (18 mo, brown) C57BL/6 mice, stimulated with 11 mM glucose and KCl (25 mM; $n = 4$; insulin levels normalized to DNA concentration). (B) Total amount of insulin released during high glucose (area under the curve, 28,010 ± SEM) from young and old mouse islets (14.5 ± 2.1% versus 14.8 ± 1.5%, respectively; $n = 3$ islets/pancreas, $n = 3$ pancreata/age). (C) Total insulin secretion (C) denotes the peak of first-phase insulin secretion, and total insulin secretion (D) denotes the total amount of insulin released during 20 min glucose (expressed relative to responses to KCl). (E) Insulin tolerance tests performed with young and old mice (0.75 units insulin/kg body weight, i.p.; $n = 10$ mice; glycemia normalized to value at t0). (F) Fed plasma insulin concentration ($n = 10$ mice). (G and H) Glucose tolerance tests with young and aged mice with different glucose loads (G: 2 g/kg, $n = 10$ mice, open symbols; 4 g/kg, $n = 5$ mice, filled symbols; one-way ANOVA, *$P < 0.05$) or in restrained mice (H: 2 g/kg, $n = 7$–8 mice, area under the curve, 28,010 ± SEM) for young mice versus 36,350 ± 3399, *$P = 0.02$). Mean ± SEM are shown.
Fibrosis is a hallmark of aging in many organs (26, 27). We found that blood vessels in islets of aged mice contained more laminin, a biomarker of fibrosis (28) (Fig. 2F–H). In addition, expression of the matrix metalloproteinase genes MMP2 and MMP9, which are also involved in fibrosis and remodeling of the extracellular matrix, increased in islets of aged mice (Fig. 2E). This result is in line with the increased macrophage density in old mouse islets, as macrophages are key sources of matrix metalloproteinases (29). Extensive accumulation of fibrotic material was also observed in the extracellular matrix of islet blood vessels in pancreata from old human donors, as assessed by laminin immunostaining (SI Appendix, Fig. S3). Fibrosis and macrophage infiltration was even more evident in islets from an old donor with diabetes (SI Appendix, Fig. S3). Together, our data show that blood vessels in islets of aged mice and humans are inflamed and exhibit fibrosis.

**Aged Islet Grafts Functionally Recover in Young Recipient Mice After a Prolonged Period.** Our results show that different tissue components in islets take separate aging paths: in old islets, blood vessels become inflamed and fibrotic, but beta cells remain functional. Because the vasculature is a systemic organ, we tested whether vascular defects observed in aged islets are caused by systemic or local pancreatic influences, rather than intrinsic aging of islets. To discriminate between these factors, we used a transplantation...
islets) can reverse diabetes and maintain glucose homeostasis (7). Because aged islets were able to rescue recipient mice from diabetes, similar to young islets, we examined the proliferative activity of aged and young islet grafts transplanted into the eye of young recipients. The young recipient mouse continued to grow and gain weight in the period from 3 to 7 mo after transplantation (SI Appendix, Fig. S4). During this period, both aged and young islet grafts grew similarly (Fig. 4 A and SI Appendix, Fig. S4). Growth of the islets grafts was in part a result of beta cell proliferation (Fig. 4 B–D and SI Appendix, Fig. S4). Aged islet grafts mounted a stronger proliferative response most likely caused by a higher glycemic pressure in the initial months after transplantation (Fig. 3 C and D) (32, 33).

Our results show that given enough time to recover in a young organism, aged islets can regulate glucose homeostasis, secrete insulin, and mount a proliferative response that is as strong as in young islet grafts. Thus, aged islets are not intrinsically old, but their age-dependent impairment can be rescued by systemic factors from a young host.

**Functional Recovery of Aged Islet Grafts in a Young Host Is Associated with Appearance of New Blood Vessels.** Although mice transplanted with aged islets recovered from diabetes, they took longer than recipients of young islets to return to normoglycemia (3 versus 2 mo; Fig. 3 C and D). In addition, mice transplanted with aged islets were less glucose-tolerant at 3 and 4.5 mo after transplantation, as shown by a delayed return to basal glycemic levels 120 min after glucose load (Fig. 3 E–G). Up to 7 mo after transplantation, aged islet recipients also had diminished plasma insulin levels (Fig. 3H).

Islets from aged mice secreted more insulin (Fig. 1 A and B). It was thus puzzling that islets from aged donors took longer to reverse diabetes and produced lower plasma insulin levels after transplantation. Because aged islets had defective blood vessels (Fig. 2), we monitored the engrafment of individual islets noninvasively and longitudinally (Fig. 5 A and B and SI Appendix, Fig. S5). We found that revascularization of aged islet grafts was delayed by 1 mo (SI Appendix, Fig. S5), which coincided with the delay in diabetes reversal (Fig. 3 C and D). Aged islets had lower vessel densities than young islets in the first month after transplantation, but they showed noticeable revascularization within the following month (SI Appendix, Fig. S5).

In addition to a slower initial revascularization, blood vessels in aged islet grafts were larger and did not branch out as much as blood vessels in young islet grafts (Fig. 5 B). The diameter of the capillaries at the end of the revascularization period in young islet grafts (7.5 ± 0.1 μm) was close to that measured in corrosion casts of islets in rat pancreata [6 μm (34)], indicating that blood vessels reached appropriate vascular sizes. Blood flow in larger vessels was faster and more turbulent than that in smaller capillaries (SI Appendix, Fig. S5 and Movies S1 and S2), thus

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**Fig. 4.** Old islet grafts in young recipient mice show strong proliferative activity. (A) Photograph of a mouse eye (*, pupil) showing two old islet grafts 3 and 9 mo after transplantation. A marked increase in islet graft size can be seen (scale bar, 200 μm). (B) Quantification of the fraction of proliferating beta cells (*P < 0.05; n = 6 young or old islet grafts). BrdU (1 mg/mL) was added for 21 d to the drinking water at 11 mo after transplantation. (C and D) Confocal images of a young (C) and an aged (D) islet graft showing BrdU-labeled, proliferating cells (green, BrdU; red, insulin; blue, DNA). Arrows point at beta cells, arrowhead indicates a nonbeta cell. (Scale bars, 50 μm.)

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**Fig. 5.** Revascularization of old islet grafts. (A) Longitudinal in vivo images of blood vessels in an old islet graft. Capillaries with smaller diameters appear in newly formed regions. (Scale bars, 100 μm.) (B) Distribution curves of the diameters of blood vessels in young and old islet grafts at 2 wk and 2 and 10 mo after transplantation. (C and D) Quantification of the fractional area of laminin immunostaining (C) and of the number of macrophages per islet area (mm², D) in young and old islet grafts 11 mo after transplantation (n = 3 islet grafts/eye, n = 2 recipient mice/age).
diminishing the efficiency of transcapillary exchange (35). During the last 4 mo, however, capillaries with small diameters appeared in newly formed regions of aged islet grafts (Fig. 5 A and B and SI Appendix, Fig. S5). We conclude that the dysfunctional vascular phenotype of aged islet grafts, characterized by larger diameters and turbulent blood flow, contributed to lower plasma insulin levels and glucose intolerance during the first 7 mo after transplantation (Fig. 3 E–H), and appearance of small capillaries in the following months likely favored the functional recovery of aged islets.

Because aged islets in the pancreas displayed an inflamed and fibrotic vascular phenotype (Fig. 2), we compared these phenotypes in blood vessels formed in young and aged islet grafts. Expression levels of laminin were similar in young and old islet grafts 11 mo after transplantation (Fig. 5C), in contrast to the age-related differences we observed in the pancreas (Fig. 2H). Laminin expression in old islet grafts was heterogeneous, with smaller vessels containing less laminin (SI Appendix, Fig. S6). The number of macrophages in young and aged islet grafts was not significantly different at this stage (Fig. SD). These results show that the young environment reverses the inflamed and fibrotic nature of blood vessels, even though the actual age of old islet grafts at the end was 29 mo.

Discussion

Our study demonstrates that the ability of beta cells to secrete insulin does not decline with age in mice and humans (Fig. 1 A–D). The most notable feature of islets in aged mice and humans was a deranged vasculature, characterized by increased inflammation and fibrosis of islet blood vessels (Fig. 2 and Fig. S3), which likely led to dysfunction of aged islets (Fig. 1 E–H). After prolonged residence in a young host, islets from aged mice display new blood vessels without inflammatory and fibrotic phenotypes and function similar to islets from young mice. This observation is striking, given that the aged islets under examination were 29 mo old, which is near the end of the mouse’s life span [equivalent to an 80-y-old human (36); Fig. 3B]. Our results clearly indicate that beta cells in mice and humans show little functional decline, but islets undergo an age-dependent decline in their vascular function.

All organisms experience a slow physiological decline and increased risk with age. Our results show that pancreatic islets in mice are affected by systemic aging, and aged mice exhibit age-dependent deterioration of glucose homeostasis, despite beta cells being fully competent in advanced age. In particular, aged mice were insulin-resistant and glucose-intolerant, although intolerance was only observed when aged mice were forced to release more insulin; for example, after a higher glucose load (37, 38). Our results concur with those of several previous studies (3, 5, 6, 39) but contrast with studies reporting insulin secretory defects with age (1, 2, 4, 8). The discrepancies in the literature may be explained by the confounding influence of the systemic environment (10). For example, aging is associated with an increase in visceral fat, with higher levels of circulating proinflammatory cytokines secreted by adipocytes, and with increased inflammation of tissues such as the local pancreatic environment that contribute to insulin resistance and disturb beta cell proliferation and function (40, 41). However, when systemic effects are compensated for, as we did here, aged islets function similar to young islets, indicating there is little intrinsic age-related decline in beta cell function.

Diabetic predisposition and added risk factors and epigenetic regulation, however, may trump the resiliency of the beta cell (9, 42). Our study now shows that the islet as an organ seems to be threatened by factors that affect vascular function. The increased functional demand aged islets face may lead to increased islet blood flow (43, 44), which in turn may trigger increased capillary pressure and compromise islet microcirculation (45). In addition, with aging, the vascular phenotype makes a proinflammatory shift that contributes to endothelial dysfunction (46). As demand for insulin increases with age, beta cells also cosecrete more ATP and other molecules that are potentially proinflammatory and that stimulate cytokine secretion and innate immune responses (47–49). The fibrosis associated with blood vessels in the aged islet likely diminishes hormone diffusion through the interstitial space and disrupts hormone release into the circulation (35), delaying insulin delivery to target tissues and causing the fragile glucose tolerance we observed in aged mice (Fig. 1 E–H). Of note, local islet inflammation and fibrosis are further increased in type 2 diabetes [SI Appendix, Fig. S3 (50)]. A dysfunctional vasculature also helps explain why there is a discrepancy between in vitro data showing no deterioration of insulin secretion with age (5) and in vivo studies reporting lower plasma insulin levels in response to hyperglycemia in aged mice (6, 10).

The damaging effects of inflamed and fibrotic blood vessels were exacerbated in the context of transplantation. Indeed, the aged islet showed delayed islet graft revascularization, likely because the inflamed and fibrotic vascular cells are transferred together with the donor islet and participate in early processes of blood vessel formation (30, 51). With time, the young systemic environment reverses these effects by replacing the vasculature with healthy blood vessels with diameters close to those of pancreatic capillaries (5–7 μm) and with regular blood flow [Fig. 5 and Fig. S5 (34)], coinciding with restored glucose tolerance (Fig. 3).

In mice, aged islets are able to adapt to increased demand for insulin caused by insulin resistance, possibly through increased islet size and increased insulin secretory capacity (Fig. 1 A and B and SI Appendix, Fig. S2), in agreement with previous reports (5). This was accompanied by increased beta cell numbers, indicating some beta cell proliferation during aging (SI Appendix, Fig. S2). In the transplantation experiment, we observed that aged islet grafts mounted an even stronger proliferative response to accommodate for the organismal growth and increased insulin demand of the young recipient mouse. These results are remarkable because a diminished proliferative response is considered a hallmark of islet aging (7). However, our study concurs with recent studies (32, 33) showing that beta cells in old mice can increase their proliferative rate under particular circumstances.

Most studies on islet aging have focused on the age-related loss of regenerative capacity of beta cells, presumably because of its therapeutic implications. Our results indicate that the functional properties of beta cells in humans, as well as in mice, change little as the adult organism ages. Instead, bloodborne factors, low-grade chronic inflammation, and other factors affecting vascular function may represent larger threats to islet health and glucose homeostasis. Potential strategies for mitigating age-related impairment in islet function should therefore target systemic or local inflammation and fibrosis within the islet. Although expanding beta cell mass may be desirable for future therapies, improving the local environment of the otherwise healthy aged beta cell could prevent age-associated deterioration in glucose tolerance and promote healthy aging.

Methods

We used young mature (2 mo old) and old (18 mo old) virgin C57BL/6 mice from the National Institute on Aging. Human islets (n = 82 preparations; age range: 17–65 y) were obtained from the Integrated Islet Distribution Program of the National Institute of Diabetes and Digestive and Kidney Diseases. Human pancreatic tissue used was from young (15–25 y) and old (50–60 y) donors.

Islet isolation, transplantation into the anterior chamber of the mouse eye, and in vivo islet imaging were performed as previously described (17, 31). Blood vessels were labeled by tail vein injection of 150,000 Da Dextran-FITC. Diabetes was induced in young mice with streptozotocin (200 mg/kg, i.v.) before 200 mouse islet equivalents from young and old donors were
transplanted. BrdU (1 ng/mL) was added to the drinking water for 21 d at the end of the study.

Assessment of Islet Function in Vivo. Islet function was monitored by measuring glycemia and plasma insulin under fed conditions, as well as during glucose and insulin tolerance tests (17).

Assessment of Islet Function in Vitro. Perfusion and [Ca
+2]], were performed as previously described (49). Insulin secretion from isolated mouse and human islets (100 islets per column) was stimulated with 11 mM glucose or 25 mM KCl. Insulin content was normalized for DNA.

Statistical Analyses. Statistical tests were performed with Prism 5.0 software (GraphPad Software). Significance was considered when P < 0.05 (unpaired Student t test or one-way ANOVA). Data presented as mean ± SEM. A complete description of materials and methods is available in the SI Appendix.

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