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Review Article

Rodent pancreatic islet models for testing bioactive substances: Isolation quality, β-cell function, and translational relevance

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Abstract

Primary pancreatic islets offer a practical way to study β-cell function *ex vivo* while retaining much of the native endocrine tissue context. They are widely used to test bioactive substances, including natural products, purified plant compounds, peptides, and synthetic small molecules that may support insulin secretion, stress resistance, or β-cell survival. This review examines rat and mouse islet models with a focus on how preparation quality affects the interpretation of compound effects. We summarize the main stages of rodent islet preparation, including ductal enzyme delivery, collagenase digestion, purification, manual picking, recovery culture, and quality control. We also discuss how species differences, enzyme properties, digestion endpoints, purification strategies, and culture conditions influence islet yield, purity, viability, and glucose-stimulated insulin secretion. Finally, we propose a practical evaluation strategy that combines morphology, viability, insulin secretion, stress-response assays, apoptosis markers, β-cell identity, and selected pathway readouts. Careful control of rodent islet preparation can make preclinical β-cell pharmacology more reproducible and informative for later validation in human-relevant models.

Keywords: bioactive substances, collagenase digestion, glucose-stimulated insulin secretion, pancreatic islets, rodent models, β-cell function

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Introduction

Diabetes mellitus remains difficult to prevent and treat, largely because deterioration in glucose control is closely related to the progressive loss of β-cell function and functional β-cell mass[1]. Understanding how β-cells respond to metabolic stress, injury, and candidate protective agents therefore requires experimental models that retain key features of the endocrine pancreas while allowing controlled *in vitro*

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testing.

Primary pancreatic islets provide such a model. They preserve greater multicellular and architectural complexity than immortalized β-cell lines, yet they are more experimentally accessible than whole-animal systems. After isolation, islets still contain mixed endocrine cell populations, preserve their three-dimensional organization, and maintain local cell-to-cell communication. These characteristics make them particularly useful for studying insulin secretion, cellular stress responses, and β-cell survival in a setting close to native tissue biology.

Isolated islets also possess several experimental advantages beyond their use as simple insulin-secretion preparations. Under appropriate culture conditions, intact islets can remain viable and functionally informative *ex vivo* for experimentally useful periods, allowing repeated interrogation of secretion, metabolism, and intracellular signaling. β-cells are particularly valuable for studying nutrient–signal coupling because changes in extracellular glucose rapidly alter cellular metabolic flux and the cytosolic adenosine triphosphate-to-adenosine diphosphate (ATP/ADP) ratio, which is tightly linked to membrane excitability, Ca^{2+} entry, and insulin exocytosis[2]. In addition, pancreatic islets are both sources and targets of multiple classical and nonclassical peptide signals, creating an integrated autocrine and paracrine network that regulates secretion, survival, and systemic nutrient metabolism[3]. These properties broaden the value of primary islets from a simple insulin-secretion assay to a model for integrated endocrine signaling and compound action.

Bioactive substances (BASs) are increasingly studied in this context. Bioactive food components and related agents may influence β-cell function and preservation[4]. Natural products have been reported to support β-cell survival, regeneration, and functional recovery under diabetic stress[5]. More specific examples include plant-derived coumarin scaffolds investigated for antidiabetic drug discovery[6] and *Ramulus Mori* alkaloids reported to enhance β-cell maturation and function through the cluster of differentiation 81 (CD81)/endoplasmic reticulum (ER) stress pathway[7].

The reliability of these findings, however, depends strongly on the quality of the islets used for testing. Islet size and the need to separate endocrine clusters from surrounding exocrine tissue can influence the material recovered for functional analysis[8]. Optimized purification protocols likewise demonstrate that isolation and purification conditions affect yield, purity, viability, and glucose responsiveness[9]. If these factors are not controlled, variation caused by the isolation procedure may be misinterpreted as a true pharmacological effect of the tested compound.

Species selection is another important source of variation. Rats and mice are both widely used for islet studies, but their pancreata differ in size, duct anatomy, tissue density, islet distribution, and sensitivity to enzymatic digestion. These differences affect cannulation, enzyme delivery, digestion timing, purification efficiency, and subsequent functional assays. For this reason, a protocol optimized in one species cannot simply be transferred to the other without validation.

Although many studies describe rodent islet isolation techniques, fewer discuss how preparation quality shapes the interpretation of candidate-compound experiments. In practice, the observed activity of a candidate compound reflects not only its biological properties but also the baseline condition of the islet preparation. This review therefore examines rat and mouse islet isolation, post-isolation quality control, functional testing, and translational limitations as connected parts of preclinical β-cell pharmacology.

This narrative review was informed by searches of PubMed/MEDLINE and Google Scholar conducted through 7 June, 2026. Search terms used alone and in combination included "pancreatic islet," "rodent," "rat," "mouse," "islet isolation," "collagenase digestion," "islet purification," "quality control," "glucose-stimulated insulin secretion," "bioactive compound," "β-cell function," "cytoprotection," "microfluidic," and "human islet." Relevant original studies, methodological reports, and reviews were selected to illustrate technical principles, quality-control considerations, and translational limitations. Human and large-animal studies were included only when they illustrated general methodological principles or translational issues not adequately addressed by rodent evidence. Reference lists of key articles were also screened. Because this was a narrative rather than a systematic review, formal duplicate screening, risk-of-bias assessment, and meta-analysis were not performed. Reporting of the literature-search approach was informed by the Scale for the Assessment of Narrative Review Articles (SANRA) framework[10].

Historical development and basic principles of pancreatic islet isolation

Pancreatic islet isolation has evolved from early

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mechanical and enzymatic approaches into increasingly controlled procedures designed to release endocrine clusters while preserving their structural and functional integrity. A major milestone was the collagenase-based rat-islet isolation method introduced by Lacy and Kostianovsky in 1967, followed by refinements in intraductal enzyme delivery and density-gradient purification that improved yield and reproducibility. For human and large-animal pancreata, development of the Ricordi automated digestion chamber in the late 1980s represented another major advance by combining controlled enzymatic and mechanical dissociation with continuous recovery of liberated islets. Modern rodent protocols retain the same underlying principle: sufficient digestion of the exocrine matrix must be achieved while limiting excessive enzymatic and mechanical injury to endocrine tissue[11–12].

Several practical considerations differ between rat and mouse preparations. Rat pancreata generally provide more tissue per donor and may offer a wider cannulation and digestion window, whereas mouse preparations often require finer ductal manipulation and closer control of enzyme exposure. These are typical tendencies rather than fixed species properties and may vary with strain, age, body size, enzyme lot, operator experience, and protocol[13–17].

Core technical procedures for rat and mouse islet isolation

Collagenase selection and enzyme preparation

Enzyme choice is one of the first variables that determines whether an isolation will produce usable islets. Collagenase preparations contain multiple proteolytic activities, and differences in collagenase classes, clostripain, or neutral-protease supplementation can alter tissue dissociation and islet recovery[18–21]. Quantitative assessment of collagenase blends further highlights the need to characterize enzyme activity before routine use[22]. Rat protocols that combine isolation with compound testing and standard murine isolation procedures demonstrate that concentration, buffer composition, and handling must be adapted to the species and experimental purpose[23–24]. Standardized purified enzyme blends should also be distinguished from crude collagenase preparations. Mouse studies show that enzyme-blend composition can materially influence islet yield[25]. Although human-islet studies report similar effects on yield and fragmentation, they are cited here only to illustrate a general enzyme-quality principle and should not be interpreted as rodent-specific effect estimates.

Ductal perfusion: Species-specific anatomy and technique

Ductal perfusion is often the point at which the quality of an isolation is first determined. If collagenase does not enter the pancreas evenly, some areas remain poorly digested while others are exposed to excessive enzyme activity. Common bile duct perfusion is therefore widely used in rodent protocols, as it delivers enzyme through the biliopancreatic ductal system and limits the need for harsh mechanical disruption during digestion[26–27]. In practice, good perfusion requires clear identification of the biliary tract, control of leakage toward the intestine, and visible inflation of the pancreatic tissue[28].

Several procedural considerations differ between rat and mouse preparations. Ductal injection methods have been described for both rat and mouse pancreatic-islet isolation[29–30]. In addition to cannulation technique, collagenase storage is a preanalytical variable because crude collagenase can lose islet-isolating efficacy regardless of storage conditions[31]. In practice, rapid and uniform pancreatic expansion remains a useful indication that the enzyme has adequately entered the ductal network. Detailed species-specific considerations are summarized in ***Table 1***.

Controlled enzymatic digestion

After perfusion, the objective is to release the islets while preserving structural integrity. This stage is affected by enzyme strength, temperature, digestion time, and the amount of movement applied during tissue dispersion. Mild shaking or pipetting helps separate the exocrine tissue, but excessive agitation can expose islets to shear stress and reduce their subsequent functional response[32–34].

The endpoint should therefore be judged by the state of the tissue rather than by time alone. Under comparable conditions, rat pancreata may permit a longer digestion window, whereas mouse preparations may be more susceptible to overdigestion; the endpoint should nevertheless be determined empirically for each strain, age group, enzyme lot, operator, and protocol. Loosening of the gland, partial tissue breakup, and dispersion with gentle handling indicate that digestion is approaching completion. Intracellular insulin content can change during the isolation process, showing that visual tissue release alone does not fully define functional quality[35]. Rat-isolation studies further demonstrate that digestion

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Table 1 **Species-specific anatomical and methodological considerations for rat and mouse pancreatic islet isolation**

Domain	Rat pancreas	Mouse pancreas	Methodological implication
Gross anatomy	Typically provides more pancreatic tissue per donor; the splenic segment is relatively compact, whereas the duodenal segment is dispersed	Generally smaller, with a diffuse mesenteric organization	These are typical tendencies and may vary with strain, age, and body size
Duct access	Cannulation may be technically easier because of the larger anatomy	Cannulation is finer and may be more technically demanding	Operator experience and protocol influence success in both species
Perfusion approach	Ductal enzyme delivery with control of outflow	Fine cannulation with careful pressure control	Uniform pancreatic expansion is more important than applying a fixed volume
Typical perfusion range	Often higher, protocol dependent	Usually lower, protocol dependent	Volume should be adjusted to species, tissue size, and pressure tolerance
Digestion behavior	May permit a longer digestion window under comparable conditions	May be more susceptible to overdigestion under comparable conditions	Optimize the endpoint for each strain, age group, enzyme lot, and laboratory
Islet distribution and yield	Often provides more islet material per donor	Yield and size distribution vary substantially with strain and protocol	Select the species according to material requirements and the biological question
Islet size	Generally larger	Generally smaller	Size affects IEQ estimation, oxygen diffusion, purification, and GSIS normalization
Purification concern	Greater exocrine mass may increase contamination	Small islets may be harder to identify and collect	Purification should match downstream BAS endpoints
Use in BAS studies	May be advantageous for yield-demanding or dose-response assays	Frequently advantageous for genetically defined mechanistic studies	These are common use patterns rather than exclusive capabilities

Abbreviations: BAS, bioactive substance; GSIS, glucose-stimulated insulin secretion; IEQ, islet equivalent.

conditions materially influence yield and function and that digestion should be stopped promptly once adequate tissue dispersion has been achieved[36–37].

Purification: Density gradients and manual picking

After digestion, endocrine clusters must be separated from the larger volume of exocrine debris. Classic Ficoll gradients established density-based purification as a standard approach[38]. Comparative mouse-islet studies subsequently showed that Histopaque and related density-gradient approaches can yield viable and functional preparations[39–40]. The choice of gradient should therefore reflect the required purity, throughput, cost, and downstream assay rather than reliance on a single historical medium.

Manual picking under a stereomicroscope remains useful when maximal purity is required or when damaged and exocrine-contaminated clusters need to be excluded individually. However, it is labor-intensive and becomes impractical for large-scale preparations. Histopaque-based purification or other density-based approaches can therefore reduce operator burden, whereas manual picking remains valuable as a final clean-up step or for small experimental preparations[41–43].

Post-isolation culture and recovery

A short recovery period is usually needed before compound exposure so that acute effects of enzymatic digestion and mechanical handling can subside. Recovery culture does not remove all biological variability, because intrinsic functional and molecular abnormalities may persist in islets from diabetic donors[44]. Culture-support strategies, including small-intestinal-submucosa matrices, can improve islet survival and function[45], while local peptide signals such as islet amyloid polypeptide can influence β-cell proliferation[46]. High-throughput human-islet culture systems[47], contemporary rat isolation and transplantation protocols[48], and classic short-term and organ-culture studies[49] collectively show that culture format and duration should be matched to the intended functional assay. The complete species-adapted isolation workflow and the major go/no-go quality-control checkpoints are illustrated in ***Fig. 1***.

Quality control before candidate-compound testing

Based on the evidence reviewed here, we propose treating quality control as a pre-exposure entry checkpoint for candidate-compound experiments. Morphology, purity, viability, and function should be documented sufficiently to establish whether the preparation is interpretable[50].

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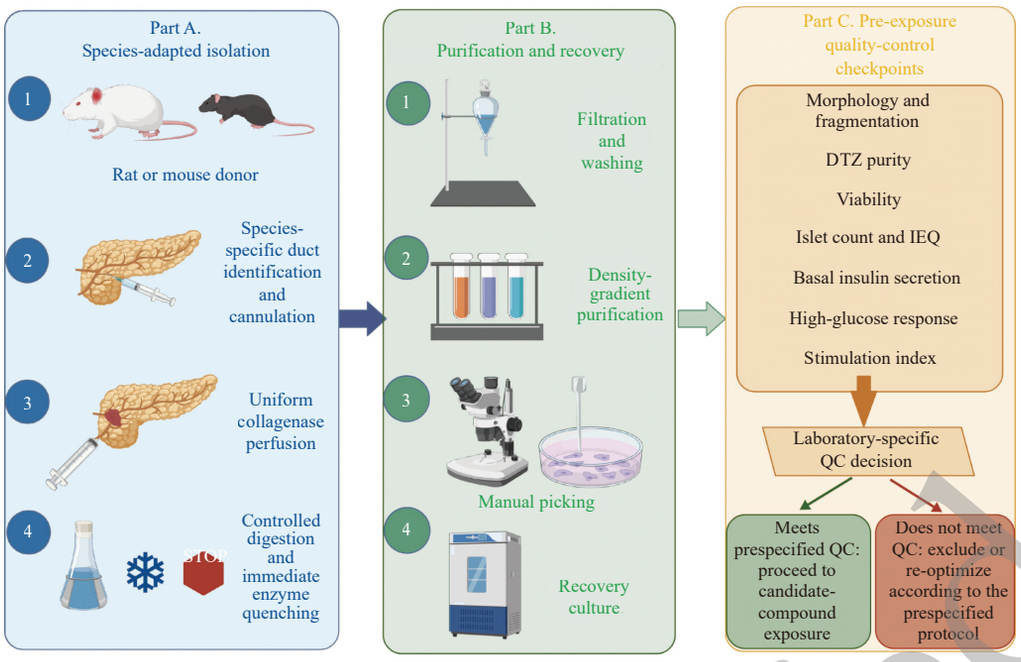


Fig. 1 **Species-adapted workflow and critical quality-control checkpoints for rodent pancreatic islet isolation.** The workflow includes species-specific duct cannulation, collagenase perfusion, controlled digestion, purification, manual picking, recovery culture, and pre-exposure quality assessment. Preparations meeting predefined laboratory-specific quality-control checkpoints proceed to candidate-compound testing; preparations that do not meet these checkpoints are excluded or reprocessed according to a prespecified protocol. Created by the authors.

Morphological integrity

Morphological inspection is usually the first indication of whether an islet preparation is suitable for further testing. Intact islets are typically compact, with clear borders, little fragmentation, and minimal necrotic debris. Islet size itself is biologically relevant because smaller islets may have functional and transplantation advantages, and systematic evidence links islet dimensions with transplantation outcomes[51–52]. Yield, quality, and purity also vary with perfusion, digestion, and purification conditions, as demonstrated in modified mouse-islet isolation protocols[53]. Therefore, morphological quality control should report islet count, size distribution, structural integrity, and fragmentation. Islet yield should be reported as both the actual islet count and islet equivalents (IEQ), with one IEQ representing the volume of a spherical islet 150 µm in diameter[54–55]. Because diameter binning and conversion to a 150-µm standard can introduce size-distribution-dependent error, actual islet count and IEQ should be reported together. Purity and functional performance are assessed separately in the following sections.

Purity assessment

Purity is a key factor in interpreting BAS experiments because contaminating exocrine tissue can affect functional and biochemical readouts. Dithizone staining is widely used to identify insulin-rich endocrine clusters visually[56–57]. However, staining alone cannot fully establish preparation identity or quality: experimentally generated insulin-secreting islet-like clusters may also be detected[58], and additives, scaffolds, or extracellular-matrix components can alter subsequent islet survival and function[59]. Purity should therefore be reported quantitatively, together with an explicit description of residual or attached exocrine tissue.

Viability and cell death

Viability needs to be checked because damaged islets can give misleading functional and biochemical results. Acridine-orange/propidium-iodide staining and related fluorescent approaches provide practical measures of membrane integrity[60–62]. Culture format can independently influence maintenance of islet viability and mass, as demonstrated in microwell systems[63]. Metabolic assessment, including oxygen consumption rate (OCR) measurements normalized to deoxyribonucleic acid (DNA), can add information beyond membrane staining[64], which is important because fluorescein-diacetate/propidium-iodide and other nucleic-acid stains have recognized limitations in isolated islets[65].

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Functional competence: Glucose-stimulated insulin secretion

Glucose-stimulated insulin secretion (GSIS) is a central functional readout because it tests whether β-cells respond to increased glucose with regulated insulin release. Pharmacological restoration of secretion has been demonstrated with dihydroberberine acting through glucokinase regulation[66]. Static GSIS has also shown predictive value in human-islet bioassays[67]. The stimulation index should be reported together with the absolute or normalized insulin output under low- and high-glucose conditions and, where appropriate, total islet insulin content. The denominator used for normalization should be prespecified.

Functional imaging provides a complementary means of characterizing intact-islet responses with temporal and single-cell resolution. Fluorescent dyes and genetically encoded biosensors can monitor glucose- or compound-induced changes in cytosolic Ca^{2+}, cyclic adenosine monophosphate (cAMP) signaling, and ATP/ADP dynamics. Ca^{2+} imaging can reveal the timing, coordination, and heterogeneity of β-cell activation across an intact islet, whereas cAMP biosensors can resolve receptor- and glucose-dependent second-messenger dynamics. Genetically encoded ATP/ADP sensors further allow metabolic changes to be related directly to electrical activity and secretion. These approaches are therefore increasingly useful for functional profiling and compound screening, particularly when static insulin measurements alone cannot resolve differences in response dynamics or intercellular coordination[68].

Pre-exposure quality-control checkpoints for candidate-compound experiments

Before candidate-compound exposure, each laboratory should apply predefined and locally validated quality-control checkpoints. Universal numerical release criteria have not been established for rodent islet pharmacology; thresholds should therefore be prespecified and justified for the assay and laboratory. For context, the National Institutes of Health (NIH) Clinical Islet Transplantation Consortium required a post-culture glucose-stimulated insulin-release stimulation index > 1 and viability ⩾ 70%; fractions containing > 50 000 islets at < 30% purity underwent supplementary purification[69].

Study-specific thresholds illustrate how such metabolic measures can be linked to functional potency. In a rat-islet transplantation study, glucose-stimulated respiratory activity greater than 1.5-fold consistently identified preparations capable of reversing diabetes, whereas preparations below this value rarely restored glycemic control[70]. ATP-based measurements may also improve metabolic assessment: ATP/DNA has been reported to correlate with islet-cell viability more reliably than ADP/ATP, although neither metric currently provides a universally validated cutoff for rodent BAS experiments[71]. Accordingly, studies should report absolute or normalized values, prespecify their laboratory-specific exclusion ranges, and distinguish validated thresholds from exploratory biomarkers.

Quality thresholds are also important for avoiding false-positive secretory effects. Regulated insulin secretion depends on coordinated β-cell electrical activity and stimulus–secretion coupling rather than nonspecific membrane leakage[72]. When appropriate, a positive survival control may be included; mesenchymal-stromal-cell secretory molecules provide one example of an intervention reported to improve the functional survival of human islets[73].

Vehicle-treated islets from the same isolation should remain the immediate reference condition. The experimental unit should be defined according to the level at which treatment is independently allocated. Pooling is acceptable when a single donor yields too few islets for all paired treatment conditions or when a common starting population is needed for distribution across treatment groups; however, pooling removes donor-level information. A non-overlapping donor pool should therefore be treated as one biological source for donor-level inference, and multiple wells derived from the same isolation or pool should not be counted as independent donor replicates. Wells independently allocated to treatments can serve as experimental units for within-pool comparisons, but generalizable conclusions require replication across independent isolations or non-overlapping pools. Authors should report the number of donors per pool, independent isolations or pools, wells per condition, and the unit used for statistical inference[74–75]. In addition, GSIS results should be interpreted in the context of the triggering and amplifying pathways through which glucose regulates insulin secretion[76], particularly when compounds may act at different points in stimulus–secretion coupling.

A tiered quality-control and reporting framework for primary-islet BAS experiments is provided in ***Table 2***.

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Table 2 **Minimum and extended quality-control domains for candidate-compound testing in primary rodent isletsDomain Prespecified laboratory-specific requirements for morphology, purity, viability, and baseline GSIS Extended assessment for mechanistic claims Interpretation and reporting requirement**

Donor and isolation record	Species, strain, sex, age, metabolic state, enzyme source/lot, perfusion route, and digestion endpoint	Operator, isolation date, pancreas mass, temperature, agitation, and stop procedure	Allows preparation-related variation to be separated from the BAS effect
Morphology and size	Intact borders, fragmentation, debris, islet count, size distribution, and IEQ	Blinded image scoring or automated morphometry	A high yield does not compensate for structural damage; report count and IEQ together
Purity	DTZ-based quantitative estimate (%) and description of attached exocrine tissue	Objective imaging or endocrine/exocrine marker analysis	In the NIH Clinical Islet Transplantation Consortium protocol, fractions containing > 50 000 islets at < 30% purity underwent supplementary purification; this is provided for context and is not a universal rodent cutoff
Viability	Quantitative live/dead assessment (%) before exposure and after treatment	ATP/DNA, oxygen-consumption rate, mitochondrial potential, or other metabolic viability measure	The NIH Clinical Islet Transplantation Consortium used ⩾70% viability as a product criterion; rodent BAS studies should define laboratory-specific thresholds
Baseline function	Low- and high-glucose insulin secretion, stimulation index, and total insulin content	Dynamic perifusion, calcium imaging, or oxygen-consumption coupling	The NIH Clinical Islet Transplantation Consortium specified a post-culture GSIR stimulation index >1
Metabolic potency	ATP/DNA or ATP/ADP with absolute or normalized values reported	OCR/DNA, mitochondrial membrane potential, or glucose-stimulated respiratory activity	No universal rodent cutoff is established. Study-specific benchmarks should be stated explicitly; > 1.5-fold glucose-stimulated respiratory activity predicted functional transplantation outcome in one rat-islet study
BAS exposure	Source, chemical identity or extract characterization, purity, storage, vehicle, concentration range, and exposure time	Positive control and exposure verification when solubility or adsorption is a concern	Vehicle concentration and treatment schedule must be matched across groups
Stress model	Defined glucose, fatty-acid, cytokine, oxidative, or hypoxic condition and exposure duration	Recovery or washout phase and graded stress intensity	A cytoprotective claim requires a disease-relevant challenge rather than healthy islets alone
Cytoprotection and mechanistic support	At least one direct injury measure plus one functional readout	ATP/OCR, Ca^{2+} dynamics, β-cell identity, oxidative/inflammatory markers, and pathway-target validation	β-cell cytoprotection requires reduced injury together with preserved function. Pathway changes without functional rescue constitute mechanistic support only
Replication and statistics	Report donor animals per isolation or pool, number of independent isolations/non-overlapping pools, and treatment wells separately	Randomised allocation of aliquots to treatment, blinded readout, and multilevel analysis when appropriate	Pooling is acceptable when required for material yield or balanced treatment allocation, but it removes donor-level information. Wells from one pool do not create independent donor replication
Normalization and exclusion	Pre-specified denominator and pre-exposure exclusion rules	Sensitivity analysis using a second denominator such as DNA, protein, ATP, or insulin content	Report excluded preparations and justify the selected denominator
Pre-exposure QC checkpoint	Intact morphology, acceptable purity and viability, measurable GSIS	ATP/DNA, OCR, and dynamic perifusion	Preparations failing baseline GSIS or viability should not enter BAS testing

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; BAS, bioactive substance; DNA, deoxyribonucleic acid; DTZ, dithizone; GSIR, glucose-stimulated insulin-release; GSIS, glucose-stimulated insulin secretion; IEQ, islet equivalent; OCR, oxygen consumption rate; QC, quality control.

Evaluation of bioactive substance effects on β-cell phenotype and function

Candidate-compound screening should first determine whether a compound preserves physiologically appropriate β-cell survival and function, as emphasized in mechanistic reviews of flavonoids[77]. Because immature β-cells do not reproduce the full functional state of mature β-cells[78], secretion should be evaluated under both low- and high-glucose conditions rather than from basal release alone. Dynamic perifusion can then distinguish early release, sustained secretion, delayed responses, and recovery after stimulation[79].

Testing should also include stress conditions relevant to diabetes or transplantation. Glucose and lipid excess can produce glucolipotoxic β-cell dysfunction[80], while inflammatory injury is a

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recognized cause of dysfunction and apoptosis in transplanted islets[81]. Compound-specific studies illustrate the value of mechanistic injury models: linderane has been evaluated against streptozotocin-induced oxidative damage[82], and antioxidant-rich flavonoids from *Oreocnide integrifolia* have been studied for effects on glucose uptake, insulin secretion, and β-cell protection[83]. These designs are consistent with the broader role of oxidative stress in diabetic β-cell dysfunction[84].

β-cell identity is another important endpoint. A compound may improve short-term secretion yet fail to preserve the transcriptional and phenotypic features of mature β-cells. Markers of β-cell identity and dedifferentiation can therefore strengthen the interpretation of BAS effects[85]. Endocrine-cell composition and viability can also be examined using flow cytometry and laser-scanning cytometry when sufficient material and validated markers are available[86].

Experimental design considerations for candidate-compound testing

Candidate-compound testing in primary islets requires more than a single concentration and a single secretion endpoint. Candidate compounds may have narrow activity ranges, and early screening should therefore include a concentration range, an appropriate vehicle control, and a time-course design. Longer exposures should assess whether mature β-cell identity is maintained, including key regulators such as NKX6.1, which is required for preservation of the functional β-cell state[87].

Vehicle selection is another source of experimental variation and should be validated rather than assumed to be biologically inert. Dimethyl sulfoxide (DMSO), for example, is widely used to solubilize candidate compounds, but β-cell responses can change substantially as its concentration increases. In INS-1 cells, 0.5–2.5% DMSO enhanced glucagon-like peptide-1 (GLP-1)-stimulated insulin secretion and related transcriptional responses by approximately 2- to 2.5-fold in a concentration-dependent manner. More recent work in MIN6-K8 cells likewise showed that acute DMSO exposure can potentiate regulated insulin secretion[88–89]. These examples do not establish a universal safe DMSO concentration for primary islets; rather, they demonstrate that the lowest feasible solvent concentration should be used, the final vehicle concentration and exposure duration should be reported explicitly, and all compound-treated groups should be compared with an identically matched vehicle control.

Timing also changes interpretation. Short exposures may identify direct secretagogue activity, whereas longer exposures are often used to evaluate cytoprotection, insulin content, β-cell identity, or proliferative signaling. Wnt-dependent β-cell proliferation provides one example of a pathway-level proliferative endpoint[90]. Compounds proposed to protect β-cells should therefore be tested under a defined stress condition, such as cytokine exposure, high-glucose and/or lipotoxic stress, oxidative stress, or hypoxia, together with an appropriate stress-matched vehicle control.

Distinguishing secretory stimulation from β-cell protection

A practical interpretation should begin by determining whether a candidate compound alters insulin secretion and whether that effect remains glucose dependent. Insulin release should therefore be compared under basal (low-glucose) and stimulatory (high-glucose) conditions. A preferential increase under stimulatory glucose, together with a preserved or increased stimulation index, supports glucose-dependent secretagogue activity. Conversely, an increase predominantly under basal glucose or a reduced stimulation index raises concern for nonphysiological stimulation or nonspecific insulin release. Total insulin content can be measured where appropriate as an additional contextual or normalization variable for interpretation of GSIS[91].

The next question is whether the apparent secretory effect occurs without evidence of cell injury. Increased insulin in the extracellular medium accompanied by loss of membrane integrity, increased injury markers, or reduced viability may represent leakage from damaged cells rather than regulated secretion. For compounds proposed to be cytoprotective, testing should therefore include a defined stress model, such as cytokine exposure, glucolipotoxicity, oxidative stress, or hypoxia, together with a matched stress-plus-vehicle control. Evidence for cytoprotection is strengthened when the compound reduces injury while preserving GSIS, insulin content, or another functional or metabolic readout[92–93].

Pathway-level measurements should be interpreted as supportive mechanistic evidence after the functional phenotype has been classified. Changes in WNT/β-catenin, calcineurin/nuclear factor of activated T cells (NFAT), B-cell lymphoma 2 (BCL-2)-family signaling, oxidative-stress pathways, or inflammatory pathways may help explain an observed phenotype[94], but pathway modulation alone does not

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establish secretagogue activity or β-cell cytoprotection. Based on this sequence, we propose the decision framework illustrated in ***Fig. 2***.

Interpreting results across different classes of bioactive compounds

Different classes of bioactive compounds create distinct interpretive challenges in isolated-islet experiments. Natural products and partially purified phytochemical preparations may vary between batches because of differences in biological source, harvesting conditions, extraction procedures, chemical stability, and standardization methods[95]. Peptides and synthetic small molecules are generally more chemically defined, but they may still have narrow concentration ranges between biological activity and cytotoxicity[96]. Poorly soluble compounds may precipitate or form colloidal aggregates during incubation, while some hydrophobic molecules may be lost through nonspecific adsorption or absorption into polymeric culture materials, thereby changing the effective concentration reaching the islets[97–98]. The magnitude of this loss can be substantial in polymer-based assay systems. In one quantitative study of

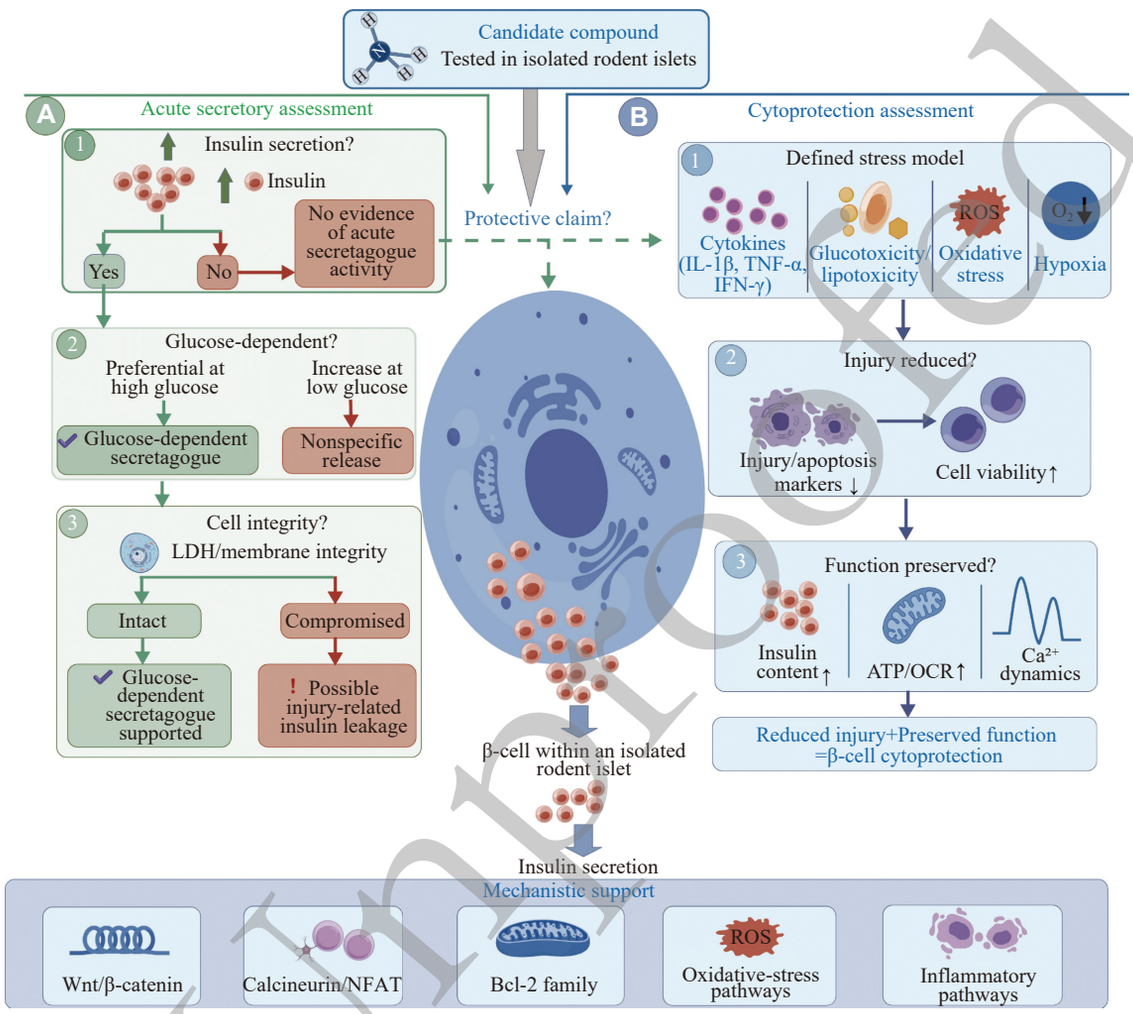


Fig. 2 **The proposed decision framework for distinguishing glucose-dependent secretagogue activity from β-cell cytoprotection in isolated rodent islets.** A: The decision framework for evaluating acute secretory activity. A preferential increase at stimulatory compared with basal glucose, together with a preserved or increased stimulation index (SI), supports glucose-dependent secretagogue activity; an increase at basal glucose or a reduced SI suggests nonphysiological or nonspecific release. Increased insulin release should be interpreted as regulated secretion only when lactate dehydrogenase (LDH) release or another membrane-integrity measure indicates preserved cell integrity; otherwise, injury-related insulin leakage should be considered. B: The decision framework for evaluating β-cell cytoprotection, irrespective of the Panel A classification. Under a prespecified stress model, cytoprotection is supported only when treatment both reduces cellular injury or apoptosis and preserves β-cell function relative to the stress-matched vehicle control. Functional readouts may include insulin secretion or content, ATP/OCR-based metabolic measurements, and Ca^{2+} dynamics. Changes in Wnt/β-catenin, calcineurin/NFAT, Bcl-2-family, oxidative-stress, or inflammatory pathways constitute supportive mechanistic evidence and are not sufficient by themselves to establish secretagogue activity or cytoprotection. Abbreviations: ATP, adenosine triphosphate; Bcl-2, B-cell lymphoma 2; IFN-γ, interferon-γ; IL-1β, interleukin-1β; NFAT, nuclear factor of activated T cells; OCR, oxygen consumption rate; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α. Created by the authors.

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poly(dimethylsiloxane) (PDMS) microfluidic channels, compounds with $\log_{10}P < 2.47$ showed minimal absorption (<10%), whereas compounds with $\log_{10}P > 2.62$ showed extensive absorption (>90%) under the tested conditions. Glass coating reduced absorption of the more hydrophobic compounds by approximately 4.5-fold. These values are platform-specific rather than universal cutoffs, but they illustrate how the nominal concentration added to an assay may differ markedly from the free concentration actually reaching the islets. Exposure verification or low-binding materials should therefore be considered when highly hydrophobic compounds are tested[99].

The source and preparation of each test substance should be reported in sufficient detail to permit interpretation and replication. For plant-derived extracts or complex natural products, relevant information includes botanical identification, extraction method, chemical characterization, purity or standardization, storage conditions, and treatment solvent. For purified compounds, the final concentration, vehicle composition, exposure duration, solubility, and stability under culture conditions are particularly important. Without this information, it can be difficult to determine whether a weak response reflects an inactive compound, inadequate delivery, chemical instability, or an inappropriate exposure range[100].

The experimental model should also correspond to the intended biological claim. A compound proposed as a secretory enhancer should demonstrate glucose-dependent activity without causing an inappropriate increase in basal insulin release. By contrast, a compound proposed as cytoprotective should be evaluated under a defined stress condition and should demonstrate both reduced cellular injury and preserved β-cell function relative to a stress-matched control. Cytokine exposure and glucotoxic culture provide distinct experimental contexts and activate different components of β-cell injury, so the selected challenge should be reported and mechanistically justified[101]. Results obtained under unstressed culture conditions should not automatically be interpreted as evidence of protection against diabetic injury. Replication and statistical inference should follow the experimental-unit definitions described in the section on pre-exposure quality-control checkpoints for candidate-compound experiments.

The available evidence illustrates why claims about compound activity need to be linked to the experimental context. Genistein has been reported to acutely stimulate insulin secretion through a cAMP-dependent protein kinase A (PKA) pathway, with functional evaluation extending beyond a purely descriptive increase in insulin release[102]. In contrast, epigallocatechin gallate has been examined for its ability to reduce cytokine-induced β-cell injury, representing a cytoprotective rather than primarily secretagogue-oriented experimental question[103]. These examples require different controls and readout panels: glucose-dependent stimulation should be assessed through basal and stimulated secretion, whereas cytoprotection should be evaluated using stress-matched viability, injury, and functional endpoints.

A single GSIS result is insufficient to establish β-cell cytoprotection. Functional rescue should be interpreted together with a direct measure of cellular injury, whereas pathway-level changes should be regarded as supportive mechanistic evidence rather than proof of efficacy. Dose–response assessment and orthogonal assays can also help distinguish a reproducible biological response from compound aggregation, nonspecific toxicity, or assay interference. Different experimental platforms should be matched to the question being addressed. Immortalized β-cell lines such as INS-1, MIN6, RIN, and related models are useful for reproducible molecular manipulation, reporter assays, gene perturbation, and pathway-focused experiments, but they may differ from primary β-cells in glucose sensitivity and differentiated phenotype[104]. Electrophysiological analysis is particularly informative in primary β-cells, intact islets, and pancreatic tissue slices, where native stimulus–secretion coupling and intercellular communication can be retained. Molecular docking, by contrast, is an *in silico* hypothesis-generating approach and should be discussed separately from cell-based experimental models. Docking-predicted compound–target interactions require biochemical, genetic, or pharmacological validation before they are used to explain a primary-islet phenotype[105]. Representative BAS studies, their experimental contexts, principal functional outcomes, and major interpretive limitations are summarized in ***Table 3***.

Pancreatic tissue slices as a complementary experimental configuration

Pancreatic tissue slices, developed and extensively characterized for islet physiology by Rupnik and colleagues, provide a complementary experimental configuration when preservation of the local pancreatic environment is important. In rodent preparations, the pancreas can be stabilized by intraductal injection of low-melting-point agarose and

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Table 3 **Representative bioactive substances evaluated in pancreatic islet or β-cell models**

Bioactive substance	Source	Stress condition	Main endpoints	Principal reported findings	Proposed mechanism	Major limitation	Ref.
Ramulus Mori alkaloids	Plant-derived alkaloids	ER stress/ maturation model	β-cell maturation, insulin secretion	Enhanced β-cell maturation and function	CD81/ER-stress pathway	Human-islet validation limited	[7]
Momordica dioica saponin	Plant saponin	STZ/high-glucose-induced β-cell stress	Viability, islet protection, insulin secretion	Reported islet-protective activity	Antioxidant protection *via* reduced lipid peroxidation and NO-associated oxidative stress	Single experimental setting	[23]
Dihydroberberine	Berberine derivative	Dysfunctional insulin secretion	GSIS, glucokinase activity	Normalized insulin secretion	Glucokinase regulation	Species and dose translation	[66]
Linderane	Plant-derived sesquiterpene	STZ-induced oxidative injury	ROS, viability, β-cell injury	Reduced oxidative β-cell damage	Antioxidant/cytoprotective action	STZ does not represent all T2DM stresses	[82]
Oreocnide integrifolia flavonoids	Flavonoid-rich preparation	STZ injury	Glucose uptake, insulin secretion, survival	Enhanced secretion and β-cell protection	Antioxidant-associated effects	Extract composition and standardization	[83]
Epigallocatechin gallate	Green-tea catechin	Cytokine-induced injury	Cell injury, inflammatory/ oxidative markers	Suppressed cytokine-induced β-cell injury	Cytoprotective/anti-inflammatory action	Primary-islet confirmation required	[103]
Genistein	Isoflavone	Acute unstressed stimulation	Insulin secretion, cAMP/PKA	Acutely stimulated insulin secretion	cAMP-dependent PKA pathway	Acute secretagogue effect	[102]

Abbreviations: cAMP, cyclic adenosine monophosphate; CD81, cluster of differentiation 81; ER, endoplasmic reticulum; GSIS, glucose-stimulated insulin secretion; NO, nitric oxide; PKA, protein kinase A; ROS, reactive oxygen species; STZ, streptozotocin; T2DM, type 2 diabetes mellitus.

subsequently sectioned with a vibratome, allowing endocrine and exocrine compartments to remain within their native tissue context. Unlike complete enzymatic isolation of islets, tissue slices preserve local architecture and cell–cell relationships, and support electrophysiological recordings, hormone-secretion measurements, and live-cell imaging, including Ca^{2+} dynamics. This configuration is therefore particularly useful for studies in which intact tissue organization, intercellular signaling, or preparation-induced changes are central to interpretation. Pancreatic slices nevertheless complement rather than replace isolated islets, because conventional islets remain more practical for higher-throughput compound exposure and standardized secretion assays[106–107].

Discussion

Rodent islet pharmacology cannot be separated from the biological and technical characteristics of the model. Standard murine isolation and transplantation procedures illustrate the precision required during preparation[108]. Cross-species analyses describe important structural differences in both the pancreas and islet architecture between the humans and mice[109–110]. In rats, isolation yield and quality are affected by donor and procedural confounders and by ductal-distension, digestion, and separation strategies[111–112]. Mouse preparations may use alternative vascular perfusion or high-efficiency isolation protocols[113–114]. Baseline interpretation can be further altered by β-cell senescence[115] and by islet loss during culture before downstream use[116].

Current limitations of rodent islet isolation models

Primary rat and mouse islets are useful because they preserve many features of native endocrine tissue, but the isolation process itself can change the material being studied. Enzyme digestion, mechanical dispersion, purification, recovery culture, and final quality selection all impose stress on the tissue. Changes introduced at any of these stages may shift the baseline structural and functional state of the preparation, creating preparation-related effects that can be mistaken for pharmacological responses. In BAS experiments, the measured response therefore reflects not only the compound being tested, but also the physiological condition of the islets at the start of treatment.

One recurring problem is variation between collagenase preparations. Recombinant collagenase can support successful islet isolation, but its activity profile must be controlled[117]. The digestion phase is

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also influenced by tissue and procedural factors that determine how effectively islets are released without excessive damage[118]. Too much proteolytic activity may damage surface proteins and matrix contacts, whereas insufficient digestion can leave islets trapped in exocrine tissue and require harsher mechanical handling.

Practical rodent-islet guides recommend local optimization of enzyme lots, perfusion, digestion, purification, and baseline assessment[119–120]. Although derived from clinical human-islet isolation, experience with collagenase and neutral protease produced under good manufacturing practice (GMP) conditions illustrates the general value of standardized enzyme manufacture, activity control, and lot documentation; it does not define a rodent-specific enzyme standard[121].

Digestion time is partly shaped by operator judgment, donor characteristics, perfusion quality, and enzyme activity. Functional consequences should therefore be assessed with assays capable of measuring metabolic fluxes in primary islets[122] and interpreted in the context of the islet as an integrated signaling hub[123]. Standardized tissue models for testing dissociation-enzyme combinations provide another way to refine digestion conditions before full-scale isolation[124]. Small pilot isolations and consistent donor and handling conditions can help narrow the acceptable digestion window.

Purity assessment can substantially influence interpretation. Although the cited human and porcine studies do not establish rodent-specific effect sizes, observations of visual overestimation of purity and post-purification viability loss illustrate general risks that should be assessed directly in rodent workflows rather than assumed[125–126].

Species differences in the amount and distribution of pancreatic collagen help explain why a single isolation protocol cannot be transferred unchanged across animals. After isolation, metabolic viability can be complemented by the ATP/DNA ratio, which has been reported to outperform the ADP/ATP ratio as an indicator of islet-cell viability[127]. Normalization should therefore be chosen according to the biological question and reported transparently.

Translational relevance: From rodent models to human islet isolation and transplantation

Rodent islets provide a highly controllable platform for mechanistic studies and early compound screening, but translation to human islets involves an additional set of pre-analytical variables that are largely absent from routine rodent isolation. Human donor pancreata undergo organ procurement, hypothermic preservation and transport before enzymatic digestion, and the duration and conditions of cold ischemia can substantially influence subsequent islet yield and functional quality. Human islet isolation itself is also more complex, typically involving intraductal enzyme perfusion, controlled enzymatic–mechanical dissociation in a Ricordi-type chamber, density-gradient purification, and post-isolation culture before experimental use or transplantation. Therefore, variation observed among human islet preparations may reflect procurement and isolation history in addition to intrinsic donor or species biology.

This distinction is particularly relevant because human donor pancreata may remain under hypothermic preservation for several hours before digestion, whereas rodent pancreata are generally processed shortly after animal euthanasia under more tightly controlled conditions. Cold-ischemia-related injury can therefore become an important source of preparation-to-preparation variation in human islets[128–129]. Human and rodent islets show quantitative differences in endocrine-cell organization and vascular architecture, but these differences should not be interpreted as implying fundamentally different stimulus–secretion physiology. High-quality preparations from both species retain the core sequence linking glucose metabolism to changes in membrane excitability, Ca^{2+} signaling, and regulated insulin exocytosis[130]. Bile acids represent an additional potential translational consideration because G protein-coupled bile acid receptor 1 (TGR5/GPBAR1) is expressed in both mouse and human pancreatic islets, and bile-acid/TGR5 signaling can modulate β-cell Ca^{2+} signaling and insulin secretion[131]. Whether procurement-related biliary exposure materially alters the quality or function of isolated human islets remains poorly characterized.

An additional consideration is that enzymatic isolation removes islets from their native tissue and extracellular-matrix context[132]. Thus, comparisons between isolated human and rodent islets should distinguish intrinsic species differences from phenotypes introduced by procurement, digestion, devascularization, and subsequent culture.

These considerations do not imply that well-prepared human and rodent islets are functionally incomparable. Rather, they define how the two models should be used within a translational sequence. Rat and mouse islets are well suited for controlled mechanistic studies and early compound screening, whereas key findings should subsequently be tested in

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well-characterized human preparations whose procurement, preservation, isolation, culture, and quality history are documented. For transplantation-oriented applications, these pre-analytical variables form part of the biological context of the human islet product and should be considered when interpreting subsequent functional outcomes.

Standardization of compound evaluation in primary islets

Standardization refers primarily to consistent documentation, predefined laboratory-specific quality-control checkpoints, assay-specific reporting, and within-laboratory reproducibility; it does not require adoption of a single universal isolation protocol across laboratories. Each laboratory should validate its own workflow while reporting the variables needed for cross-study interpretation. The relevant preparation-quality domains are summarized in ***Table 2***.

Metabolic viability requires endpoints beyond simple membrane staining. Hyperspectral autofluorescence imaging offers a nontraditional approach to pancreatic-islet viability assessment[133]. Nucleotide ratios can also provide metabolic information: the ADP/ATP ratio has been evaluated as a predictive quality assay[134], while foundational studies describe the broader energy metabolism of islets of Langerhans[135]. No single measure is sufficient, so membrane integrity, metabolic activity, and functional secretion should be interpreted together.

Disease-relevant stress conditions are important when BASs are evaluated as potential therapeutic agents. In diabetes and after transplantation, β-cells may encounter inflammatory mediators, nutrient excess, oxidative stress, hypoxia, and loss of matrix support. Reviews of islet transplantation identify these biological and procedural limitations as major barriers to successful outcomes[136]. BAS studies are therefore more convincing when secretion is accompanied by viability, stress-response, and pathway-level readouts.

Complete reporting is another component of reliable BAS interpretation. Calls for improved reporting of human-islet characteristics emphasize the need to describe donor information, preparation quality, culture conditions, and relevant functional data[137]. For rodent BAS studies, the same principle should be extended to enzyme source and lot, perfusion and digestion conditions, purification method, recovery time, normalization strategy, and exclusion criteria.

Standardized access and distribution systems can also improve reproducibility. A web-based automated cell-distribution system demonstrated the feasibility of coordinated allocation and tracking[138], and the Integrated Islet Distribution Program subsequently emphasized improved human-islet phenotyping and reporting[139]. These models show how transparent sample characterization can strengthen comparisons across laboratories.

Transparent reporting also helps identify why promising compounds fail to reproduce. A BAS that performs well in highly purified, healthy islets may show weaker effects in preparations with greater exocrine contamination or lower metabolic reserve. Conversely, a compound may appear beneficial in a stressed preparation because the control islets were already compromised. Presenting quality-control data alongside functional results therefore strengthens the biological interpretation and helps distinguish reproducible compound activity from preparation-specific artifacts.

Future directions

Emerging microfluidic and islet-on-a-chip platforms can support dynamic glucose stimulation, continuous insulin or C-peptide measurement, imaging, and repeated sampling while reducing the number of islets required. These platforms may improve concentration-response testing and integration of functional and metabolic readouts, provided that device materials, flow conditions, compound adsorption, and starting-islet quality are reported explicitly[140–144].

Single-cell transcriptomic and related molecular approaches can add cellular-resolution information about islet heterogeneity and β-cell state. These methods should complement rather than replace baseline quality control, viability assessment, and functional testing[145–147].

A practical translational sequence therefore begins with well-characterized rodent islets, adds dynamic or molecular measurements when they address a specific question, and confirms key findings in human islets or other human-relevant models[148–150]. Advanced platforms improve measurement depth, but they cannot compensate for poorly prepared or inadequately documented starting material.

Conclusions

Rodent pancreatic islets remain valuable for evaluating compounds that influence β-cell secretion, stress responses, and survival, but the interpretability of these experiments depends on the condition of the starting preparation. Species, enzyme delivery,

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digestion endpoint, purification, recovery culture, and pre-exposure quality control can alter morphology, viability, insulin content, and glucose responsiveness; these variables should therefore be documented and distinguished from compound-related effects.

Based on the evidence reviewed here, we propose integrating predefined laboratory-specific quality-control checkpoints with endpoint-specific functional assessment. Glucose-stimulated insulin secretion should be interpreted alongside total insulin content and a prespecified normalization strategy, whereas evidence for cytoprotection should include a direct injury measure together with preserved β-cell function. Biological and technical replication should be reported separately, and statistical inference should reflect the true experimental unit.

Overall, reliable evaluation of bioactive compounds in rodent islets requires standardized reporting, within-laboratory reproducibility, biologically appropriate replication, functional testing, and transparent interpretation. Rodent islets are valuable for mechanistic and screening studies but should not be treated as direct surrogates for human islets. Confirmation in human islets or other human-relevant models remains essential before therapeutic relevance is inferred.

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Author contributions

Tianjiao Li: Conceptualization, investigation, visualization, project administration, and writing—original draft. **Irina Georgievna Danilova:** Conceptualization, supervision, project administration, and writing—review and editing. **Ilia Andreevich Rakhov:** Investigation, methodology, and writing—review and editing. **Maria Valerievna Ulitko:** Investigation, methodology, and writing—review and editing. **Jun Luo:** Supervision, resources, and writing—review and editing. **Peipei Pan:** Resources, project administration, and writing—review and editing. All authors read and approved the final manuscript.

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