Supplementary Materials

Insulin physiology and metabolic control: current concepts and perspectives

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Supplementary Table 1.

Source	Representative search strategy	Filters/notes
PubMed/MEDLINE	("insulin" [MeSH Terms] OR insulin [Title/Abstract]) AND (biosynthesis OR secretion OR "beta-cell" OR clearance OR CEACAM1 OR "insulin-degrading enzyme" OR IDE OR "insulin signaling" OR "insulin resistance" OR adipose OR microbiota OR senescence OR "precision medicine")	1 Jan 2016–31 Jul 2026; no language restriction; seminal pre-2016 studies retained when conceptually necessary
Scopus/Web of Science	Equivalent syntax adapted to TITLE-ABS-KEY (Scopus) and TS= (WoS), using the same core terms	Relevance reassessed on retrieval; domain-based targeted searches used when required
ScienceDirect, SAGE, Taylor & Francis	Insulin AND (biosynthesis OR secretion OR clearance OR "insulin resistance" OR CEACAM1 OR IDE OR microbiota OR senescence)	Supplementary publisher-level searches to broaden full-text retrieval

CLINICAL TRANSLATION NOTE — INSULIN BIOSYNTHESIS AND PROCESSING: THE MOLECULAR BLUEPRINT

- The proinsulin/insulin ratio is measurable in routine laboratories but absent from standard screening panels; its elevation precedes HbA1c changes, identifies an actionable window before irreversible epigenetic silencing of PDX-1 and MAFA, and — because reversibility diminishes with disease duration — should inform the timing

and intensity of therapeutic escalation alongside stimulated C-peptide, not HbA1c trajectory alone.

- Differentiation protocols for stem cell-derived β-cells optimized in mice may not replicate human β-cell maturation; C-peptide secretion from transplanted cells remains the most accessible in vivo endpoint for assessing engraftment quality.

- Chemical chaperones (4-PBA, α-lipoic acid) and PPARβ/δ agonists target the proteostatic arm of the BQI circuit upstream of transcription factor loss — a mechanistically distinct intervention from glycemic control, currently without a dedicated clinical trial in early T2D.

CLINICAL TRANSLATION NOTE — INSULIN SECRETION: CLASSICAL MECHANISMS AND NOVEL INSIGHTS

- In hyperinsulinemic hypoglycemia, the full biochemical panel — insulin, C-peptide, proinsulin, and β-hydroxybutyrate — is required; omitting proinsulin risks misclassification and unnecessary pancreatectomy.

- Any child presenting diabetes before six months of age should undergo urgent genetic testing before initiating lifelong insulin therapy: KCNJ11/ABCC8 mutations respond to sulfonylureas and often allow insulin discontinuation.

- The PEP-driven pyruvate kinase model of KATP closure predicts secretory defects not correctable by mitochondria-targeted interventions, which may partly explain impaired GSIS in patients with intact mitochondrial function.

- Loss of the incretin effect in T2D reflects impaired β-cell GLP-1R responsiveness, not reduced GLP-1 secretion: DPP-4 inhibitors may be insufficient in advanced β-cell desensitization, where pharmacological GLP-1Ra concentrations are needed.

- Tirzepatide's GIPR dependence in human islets — absent in mice — means preclinical data for dual/triple incretin agonists cannot predict human β-cell responses without dedicated human islet validation.

CLINICAL TRANSLATION NOTE — THE PORTAL TRANSIT AND HEPATIC INSULIN CLEARANCE

- HICI (C-peptide/insulin molar ratio <1 during glucose challenge) adds mechanistic staging information to the OGTT at no additional cost. Combined with hepatic steatosis imaging (MRI-PDFF), it provides a practical composite index for patients in whom impaired hepatic clearance is clinically suspected.

- Persistent hyperglycemia despite escalating subcutaneous insulin doses, accompanied by progressive weight gain, should prompt reconsideration of the delivery route rather than automatic dose escalation. In this setting, the problem is often the non portal (systemic) pattern of insulin exposure, which favors peripheral insulin action and weight gain. Strategies that restore a more hepato preferential insulin profile, such as intraperitoneal or oral insulin delivery, address this mechanism directly and may improve glycemic control without further increasing the subcutaneous dose.

- MASLD and T2D share impaired CEACAM1-mediated clearance as a common upstream metabolic anomaly not captured by any approved diagnostic test or therapeutic target; no drug currently acts on CEACAM1 directly, making it an open and largely unexplored pharmacological opportunity.

- IDE animal model contradictions indicate its primary role is in hepatic insulin signaling, not systemic elimination: IDE modulators should be framed as hepatic insulin sensitizers, not clearance-enhancing agents.

CLINICAL TRANSLATION NOTE — PERIPHERAL INSULIN SIGNALING: THE MOLECULAR ARCHITECTURE OF INSULIN ACTION

- A normal fasting blood glucose level, along with elevated fasting insulin levels and decreased adiponectin, indicates that insulin resistance in adipose tissue is the primary pathological process—a stage in which standard glycemic biomarkers provide no information, but in which metabolic risk is already accumulating; measuring fasting insulin and adiponectin in high-risk individuals may provide an earlier mechanical classification than HbA1c or HOMA-IR alone.

- Treating fasting hyperglycemia with secretagogues or insulin alone, without addressing the concurrent lipogenic drive, risks worsening steatosis and MASLD progression; SGLT2i or GLP-1Ra address both the gluconeogenic and lipogenic arms simultaneously.

- Exercise restores GLUT4 translocation via AMPK/Rac1 mechanisms that bypass the defective PI3K/AKT axis: it is pharmacologically non-redundant with drug therapy in muscle-predominant insulin resistance, not merely adjunctive.

- Once the dominant resistance phenotype is identified — adipose-driven (high BMI and WHR, low adiponectin) versus hepatic-lipid-predominant (elevated liver fat on imaging, low HICI, normal BMI) — therapeutic selection follows: GLP-1Ra and thiazolidinediones for the former; SGLT2i and clearance-restoring strategies for the latter.

- Central insulin resistance is mechanistically plausible but clinically unvalidated: intranasal insulin and CNS-directed approaches should not influence therapeutic decisions outside dedicated trials.

CLINICAL TRANSLATION NOTE — ADIPOBIOLOGY AND INSULIN RESISTANCE: THE ENDOCRINE DIMENSION

- Adiponectin is a validated biomarker of insulin resistance and cardiometabolic risk but is absent from routine panels; low adiponectin in a normoglycemic individual with central adiposity identifies a high-risk state amenable to early lifestyle or pharmacological intervention.

- Two patients with identical BMI may carry fundamentally different metabolic risk based on subcutaneous adipose expandability; waist-to-hip ratio and triglyceride/HDL ratio provide accessible surrogates where imaging is unavailable.

- Broad IL-6 pathway blockade (IL-6R antagonists) may paradoxically impair muscle insulin sensitivity; metabolic status should be monitored in patients with comorbid inflammatory disease receiving these agents.

- Recombinant leptin has clinical utility only in absolute deficiency states (congenital deficiency, lipodystrophy); serum leptin should be measured before any leptin-based intervention to distinguish deficiency from resistance.

CLINICAL TRANSLATION NOTE — GUT MICROBIOTA AND THE INSULIN LIFE CYCLE

- Dietary fiber (≥25–30 g/day, diverse sources) is the microbiome intervention with the strongest human evidence and a defined mechanism (SCFA → GLP-1/PYY

from L-cells); it should be prescribed as a pharmacologically grounded recommendation, not general dietary advice.

- Incomplete glycemic remission after Roux-en-Y gastric bypass may partly reflect insufficient microbiome remodeling; dietary optimization or adjunctive probiotics represent a mechanistically rational complement, particularly in patients with poor post-surgical bile acid reconstitution.

- LPS-binding protein and soluble CD14 are measurable in clinical laboratories and can identify a metabolic endotoxemia phenotype — unexplained insulin resistance disproportionate to adiposity — that may respond preferentially to gut barrier-restoring interventions.

- FMT for T2D is investigational; insulin sensitivity improvements are not maintained consistently beyond 12 weeks and engraftment is highly donor-dependent — patients should be informed of these limitations explicitly.

CLINICAL TRANSLATION NOTE — TEMPORAL DYNAMICS OF β-CELL FAILURE: DEDIFFERENTIATION, CLEARANCE ADAPTATION, AND DISEASE STAGING

- A rising proinsulin/insulin ratio with preserved fasting glucose identifies a pharmacologically actionable dedifferentiation stage that precedes HbA1c elevation; waiting for HbA1c ≥6.5% risks treating at a stage of partially irreversible epigenetic remodeling.

- The same fasting insulin value has different implications depending on C-peptide: adding C-peptide to insulin measurement at negligible cost distinguishes hypersecretion from reduced clearance as the source of hyperinsulinemia.

- Functional islet imaging capable of detecting hub β-cell network disorganization could serve as a pre-glycemic staging biomarker; research investment in this direction is mechanistically justified by the hierarchical role of hub cells in coordinating pulsatile secretion.

- ALDH1A3 inhibition and stem cell-derived islet replacement are not interchangeable: the former requires residual β-cell identity markers and is relevant

only in early-to-intermediate dedifferentiation; the latter is the appropriate strategy in advanced epigenetic silencing with near-total loss of GSIS.

CLINICAL TRANSLATION NOTE — FROM PATHOLOGY TO TREATMENT: THE INSULIN LIFE CYCLE AS A THERAPEUTIC MAP

- The six variables needed for Ahlqvist et al. cluster assignment (GADA, age at diagnosis, BMI, HbA1c, HOMA-B, HOMA-IR) are available from routine encounters; applying this framework informally today — distinguishing a SIDD patient (low HOMA-B, low BMI) from a SIRD patient (high HOMA-IR, high BMI) — avoids defaulting to the same drug for mechanistically different phenotypes.

- Stimulated C-peptide below 0.2 nmol/L indicates near-total secretory failure where GLP-1Ra and DPP-4 inhibitors have limited efficacy; above 0.6 nmol/L with elevated proinsulin/insulin ratio it identifies the patient most likely to respond to incretin-based identity-rescue strategies.

- The Cardoso et al. SGLT2i-versus-GLP-1Ra selection algorithm requires only age, renal function, BMI, and baseline HbA1c — variables already in the clinical record; the primary barrier to adoption is the absence of validated decision-support tools in electronic health record systems, not data availability.

- SGLT2i cardiorenal benefits are at least partly insulin-independent; they should not be withheld in patients with limited glycemic efficacy due to advanced renal impairment — the cardiorenal indication and the glycemic expectation should be communicated separately.

CLINICAL TRANSLATION NOTE — AREAS OF CONTROVERSY AND OUTSTANDING QUESTIONS

- Selective hepatic insulin resistance: measure HICI alongside HOMA-IR in patients with MASLD and fasting hyperglycemia to determine whether clearance impairment or signaling defect is dominant before selecting a therapeutic strategy.

- Dedifferentiation versus apoptosis: a rising proinsulin/insulin ratio with preserved C-peptide suggests dedifferentiation (→ escalate identity-rescue interventions); a falling C-peptide with normalized ratio suggests net mass loss (→ shift toward cell replacement planning).

- Gut microbiota and central insulin resistance — two unresolved causal questions, two different clinical responses: dietary fiber and prebiotics are low-risk and mechanistically justified regardless of causal direction and can be recommended now; FMT and CNS-directed strategies (intranasal insulin) carry procedural risk or remain unvalidated in humans and should be restricted to clinical trials — though central insulin resistance remains relevant for understanding appetite dysregulation and weight resistance disproportionate to glycemic status.

- Rodent-to-human translational gap: biological plausibility from murine data is necessary but not sufficient for clinical adoption; require human trial evidence — ideally with mechanistic endpoints — before recommending any intervention based solely on preclinical data.

CLINICAL TRANSLATION NOTE — METABOLIC AGING, CELLULAR SENESCENCE, AND GEROSCIENCE: A FRAMEWORK FOR FUTURE PRECISION STRATEGIES IN T2D

- A high PRS for secretion loci (TCF7L2, KCNJ11) versus resistance loci (IRS1, PPARγ, FTO) identifies biologically distinct prevention targets; translating PRS into actionable intervention thresholds is one of the most tractable unmet needs in precision diabetology.

- Enriching T2D prevention trials with two complementary aging biomarker layers would maximize their mechanistic yield without new drug development: epigenetic clocks to stratify participants by biological aging rate at enrollment, and SASP biomarkers (IL-6, IL-1β, GDF15, p16INK4a in cell-free DNA) as secondary endpoints to measure target engagement of senomorphic agents such as metformin and rapamycin already in clinical use.

- Broad or indefinite senolytic therapy carries unquantified risks from disruption of physiological senescence roles; until dose, duration, and cell-type specificity are established in powered human trials, senotherapeutics should be communicated to patients as a research strategy, not a preventive intervention.