Supplemental material intended for publishing online only

Supplement 1

TABLE 1: References (1-17)

RECOMMENDATIONS
At the population level:

1. Given epidemiological, functional, histological, molecular and genetic findings (Table 1, Figures 1-4, main text), and the importance of earlier screening and diagnoses of BCOD/F in both T2D and T1D, the overall goal of beta cell content preservation appears appropriate. Further, diagnostic shortcomings of current definitions of dysglycemia result in inclusion of prediabetic "controls" limiting the potential of GWAS, organ donor consortia and case definition in T2D and T1D prevention trials, and controlled diabetes studies. We thus propose that both civilian and academic entities, in part, rotate to the quest for earlier dysglycemic diagnoses, in addition to addressing diabetic heterogeneity. Current population-based diabetes testing lack utility compared to procedures for other pandemics, be they screening, diagnostic, or staging to achieve earlier prevention and treatment strategies, e.g., those used for cancer and cardiovascular diseases. We suggest earlier identification of BCOD using methods similar to those identified in the main text and Figures 1 and 3 before and Figure 4 *after* initial screening.
    a) **Proposal for initial screening in adolescent and adult BCOD associated with T2D:** Improved screening could include more widespread use of annual laboratory (or even home-based for family members) fasting glucose levels, using a potential threshold of >85-90 mg/dL on repetitive testing (18-22). After upward glycemic trends exceeding the threshold are reached, or for those subjects with strong T2D historical risk factors, achieving the lower threshold estimates should trigger a referral to local tertiary diabetes care centers for detailed, state-of-the-art testing, as recommended by The Lancet Diabetes Commission in 2021(23). The goal is to achieve earlier diagnoses than currently recommended, since current guidelines do not address specific laboratory findings at these earlier disease phases. Further, approaches enlisting US general and specialist healthcare providers have proven inadequate for most levels of diabetes care, thus the need for tertiary care centers as also detailed in The Lancet Diabetes Commission and by us and others as well (20, 23-26). However, the Commission did not address earlier screening and diagnosis, which we now emphasize again (20).
    b) **Proposal for initial screening in children, adolescent and adult BCOD associated with T1D:** Assessment could be *continuously* rising fasting, point of care capillary blood glucose level trends, rather than a single test, as suggested in Figure 3 and main text. For example, >5% increase at a threshold >85mg/dL, rather than the more complicated, population-based venipunctures for laboratory tests, e.g., glucose, oGTT, C-peptide levels or autoantibodies. These latter tests would be tasked to tertiary care centers (27, 28). Simple, point of care glucose testing and historical assessment of T1D risk factors using simple

written protocols embedded in electronic medical records or the equivalent, could be performed during those ~18 visits for routine vaccinations, etc., during the first 18 years of life with built-in higher visit intensity during the first seven years of life in the US. Subsequently, at annual medical visits the same protocols could be used for screening adults. Those at risk could be referred to tertiary care centers for detailed, state-of-the-art testing to achieve earlier diagnoses than currently recommended (Figures 1, 3 and 4 main text) (27-29).

2. Enlisting the support and cooperation of governmental agencies, e.g., the US CDC, especially *normally functioning* local and regional public health departments, which could be tasked with local health care provider training and follow up of just the above screening procedures in those private and governmental facilities administering routine vaccines for children and adolescents. For adult screening, these agencies could provide awareness and training for all local medical practitioners for annual testing using the same methods as for the COVID-19 pandemic.
3. Industry, media and diabetes advocacy organizations and investigators could be tasked at local and regional levels to ensure enhanced public awareness for health care providers and the public, to *encourage* population-based screening, e.g., national, state and local messaging used with COVID-19.
4. Effective screening provides an enlarged window for implementation of lifestyle changes and medications provided by tertiary care centers, thereby limiting acute and chronic complications both in patients and their families.
5. Lifestyle interventions/medications should be more aggressively pursued by national diabetes advocacy organizations, to recognize diabetes as similar to other disease pandemics, thus, promoting public awareness and rationalization for early therapeutic attention to prevent the disorder.

At the research level:

1. Defining earlier beta cell functional loss in the context of demographic and genetic features could help define cases for inclusion into various T2D and T1D modalities for prevention trials.
2. How to improve integration of autoantibody or other immune or *active* cell death biomarkers in subject testing data with beta cell functional assays during the slowly progressive, subclinical prediabetic phase indicated in Figure 1 and Figure 3 - a need recently suggested by others (27-33).
3. Dysglycemic adult data could be more comprehensively compared with beta cell content estimates using autopsy, surgical or organ donor specimens. For example, large consortia patient cohorts with strong dysglycemic definitions, could be included in registries for potential organ donors. This would be difficult for children, since they are on the steep part of the sigmoid curve of beta cell mass (Figure 4, and main text).
4. Careful definitions of dysglycemias in research studies validating control cohorts with and without dysglycemia.
5. Improved definition of relevant disease pathways, e.g., ER stress, for T2D and T1D prevention enabling primary drug intervention (34-36). Additionally, the potential of including intensive insulin treatment during immune or other multiple drug interventions as previously suggested (33, 37, 38). Exogenous insulin could suppress compensating beta cells from excessive proinsulin synthesis in those individuals without gene variants

suppressing beta cell regeneration, especially in children where beta cell proliferation could be relatively more normalized (Figure 4).

6. Development of unique and multiple medication use for treatment of subclinical pre-T1D (autoantibody positive, and/or dysglycemia that current guidelines indicate as normal), e.g., judicious use of gliptins or glutides, which are suggested to increase beta cell mass in humans (39), and more attention to environmental factors, especially viruses (40, 41), and detrimental high protein and saturated fat (palmitate) diets (42-44).

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Supplement 2*

Beta cell autoAB-NonDM	AREA(%) autoAB+NonDM		Beta cell autoAB-NonDM	MASS(mg) autoAB+NonDM		Beta cell autoAB-NonDM	AREA(%) autoAB+NonDM	
2.51	0.90		2350	450		1.60	1.10	
2.60	0.10		1950	200		1.10	0.25	
1.75	0.30		1600	50		1.20	0.40	
1.70	0.30		1620	50		0.90	0.42	
0.75	0.50		1620	500		0.85	0.50	
0.70	0.85		800	500		0.68	0.25	
1.55	0.60		750	500		0.65	0.30	
1.80	0.25		100	250		0.55	0.34	
1.90	0.25		500	250		0.49	0.60	
1.85	0.30		400	250		0.46	0.30	
1.80	1.00		400	250		0.45		
3.20	0.40		400	700		0.35	**X (SD) =**	
2.50	1.35		400	700		0.39	**0.45(.26)**	
2.30	0.70		550	650		0.52	**P<0.05**	
2.15	0.70		600			0.50		
1.55			600	**X (SD) =**		0.47		
1.50	**X (SD)=**		600	**378(223)**		0.49		
1.00	**0.56(.35)**		900	**P<0.0024**		0.51		
1.25	**P<0.0013**		1000			0.40		
1.15			850			0.49		
1.00			1300					
0.90			1400			**X (SD) =**		
0.80			1400			**0.65(.32)**		
0.50			1250					
0.30			650	**Ref 10**			**Ref 14**	
0.65			650	**Main**			**Main**	
0.65			600	**Text**			**Text**	
0.65			500					
0.65			300					
0.06			250					
0.06			**X (SD) =**					
0.06			**876(554)**					
0.70								
0.65								
0.70								

0.06								
0.70								
0.60								
X (SD) =	**1.19(.80)**							

*Data shown are estimates derived from charts reported in references 10 (age 20-80) and 14 (age 20-60) main text

Supplement 3

Dynamics of Beta Cell Loss and Regeneration

Beta cell neogenesis: Neogenesis occurs in human T2D and T1D as inferred from human neonatal genetic disorders of beta cell development as well as human embryonic, fetal, neonatal and childhood pancreatic histological studies, where beta cell development, replication and neogenesis have specific genetic signatures (1-6). Endocrine chromogranin positive, hormone negative (CPHN) cells in islets, but more prominently alone or in clusters of two to three cells in the exocrine pancreas are similar in children and adults with T1D and in lean and obese adults with T2D (1, 7-10). These findings appear to recapitulate those histological cell locations in mid to late human gestation and early infancy eventually becoming islet beta cells. Consistent with beta cell neogenesis beyond childhood is the increased presence of NKx6.1 in CPHN cells in adults with T1D, in the absence of increased replication marker Ki67 levels, although there is replication at a baseline, relatively low level (1, 8, 11). Further, these studies showed the presence and likely reoccurrence of progenitor-related ductal cells with endocrine cells containing insulin and the transcription factors PDX1, PAX6 and NEUROG3, also consistent with neogenesis in T2D, though less likely in the pancreatic ductal gland in T1D (12). Finally, the absence in T2D of increased Ki67, and the presence of adjacent one-three cell beta cell clusters, especially in or near ductal areas, are indicative of neogenesis, rather than replication, especially during times of compensatory insulin demands, e.g., human pregnancy and T2D (2, 10). Similar observations using IMC show the presence in T1D islets of strongly positive NKx6.1cells which contain high levels of intracellular C-peptide, i.e., beta cells, which these authors attributed to neogenesis (13). These results when linked to longitudinal human T1D IMC pseudo-time studies supports the potential for neogenesis to be, in part, responsible for residual low C-peptide levels in T1D (14).

Beta cell mass dynamics: A recent review has highlighted dynamic events resulting in beta cell loss vs. neogenesis and/or proliferation (11). The best human example is a dynamic favoring beta cell apoptosis in the two months before and after birth, which is subsequently reversed favoring beta cell proliferation resulting in enlarged beta cell mass (15). Given that beta cell mass depletion in T2D is less rapid or severe than in T1D, it follows that in subclinical, type 2 prediabetes, clinical prediabetes, early and late T2D, as well as T1D, the dynamic favors beta cell loss, given the relative lack of earlier diagnoses to abrogate that loss. After intensive dysglycemic treatment, that dynamic can change: first, there may be improved residual beta cell function as indicated in Figure 1C, green upward bold and dashed arrows, when beta cell mass is partially preserved; second, the dynamic potentially favors regeneration likely via neogenesis,

with a relative reduction of beta cell death associated with decreased compensatory proinsulin requirements, so that the relatively small increase in beta cells would be preserved, similar to pregnancy and T2D (2). Finally, T1D has >90% beta cell mass reductions in both children and adults, with detectable, yet very low non-fasting, circulating levels of C-peptide even after 15 years of disease (16). Thus, proinsulin synthesis and insulin secretion from functioning beta cells, due to compensatory insulin needs (17), neogenesis (9, 13, 14), and constituently low-level proliferation (11), alters the dynamic resulting in limited regeneration, i.e., patients remain on insulin treatment. These concepts appear clinically relevant in that HgbA1c values are lower (8.4%; [68 mmol/mol]) in meal stimulated C-peptide "responder" subjects (>600pg/ml;>0.2nmol/L) than HgbA1c values (9.2% [77mmol/mol]) in "non-responder" subjects (<600pg/ml) with recent onset (<3 years) T1D (18). Additionally, C-peptide concentrations, even above 0.1-0.2 nmol/L, are associated with improved glycemic control, e.g., lower glycemic variability and lower risk of hypoglycemia (19). Moreover, a comparatively higher C-peptide (defined as peak MMTT C-peptide >0.400 nmol/L) was associated with more "time in optimal range" and lower mean glucose levels. These data suggest that lower HgbA1c values were *caused* by residual C-peptide (insulin) levels. Given the beta cell loss/regeneration dynamic, and the reported coexistence of what appears to be beta cell neogenesis/proliferation, despite substantial beta cell content loss, it follows that intensive insulin treatment results in lower ambient hyperglycemia (lower HgbA1c) preventing residual and newly formed beta cell ER stress and cell death presumably resulting from less beta cell compensatory demands. It also follows that this dynamic provides an additional explanation for the clinical benefits of having residual fasting and stimulated C-peptide levels. Thus, measurable, but low C-peptide levels may not be the cause of improved clinical management, but also may result from it. Further clinical and organ donor studies can assess these potentially important dynamics.

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Supplement 4

CELL DEATH MECHANISMS

Apoptotic cell death: This form of cell death can be subdivided into homeostatic and the more compensatory form, e.g., glucotoxic. The former is similar to beta cell removal during tissue remodeling or after replicative senescence. When the usual physiological UPR is overwhelmed by increasing proinsulin synthetic demands, e.g., insulin resistance or by other compensatory mechanisms, beta cell function can be compromised, and cell death could result (Figure 2). Indeed, in T2D apoptosis is a major cause of beta cell death. Following apoptotic cell death, anti-inflammatory macrophage and neutrophil phagocytes remove dead cells limiting leakage of beta cell contents. That these mechanisms are remarkably efficient is reflected by the relative lack of major immunological abnormalities in T2D, as compared to T1D. Nevertheless, even in older, obese and autoantibody-negative T2D, low-grade islet monocytic infiltrates have been observed, similar to adults with T1D, while transient, low affinity serum autoantibodies (potentially false positive antibodies) and circulating T-cells reactive to *in vitro* beta cell antigens are well described (1, 2). Such changes could result from phagocytic cell dysfunction consequent on hyperglycemia and altered protein glycation (3).

Immunogenic cell death: Autoimmune diseases involve activation of the immune response in dialogue with target tissue responses to "danger signals" (4). In other words, the beta cell target may be engaged in the adverse immune responses and is also a priori at-risk. Inflammation-induced mechanisms in autoimmune disease can converge on altered interferon expression in the target tissue. Hence, beta cells in T1D show abnormal expression of disease-risk genes (see above). Beta cell death can also result from extrinsic and direct immunogenic cell death, as exemplified by our assays of mononuclear- and nonT-mononuclear cell-mediated cytotoxicity, antibody-mediated cytotoxicity and/or antibody-dependent cell-mediated cytotoxicity (5-7), in addition to reactive oxygen species (8), complement activation (9) and NK cells (7). It is likely that these processes activate immunogenic target cell death mechanisms, (10, 11). Thus, there is a genetically controlled immune response to damage-associated molecular patterns including neoantigens leaking from damaged beta cells. Other resident antigens are recognized by cognate receptors on phagocytic, proinflammatory mononuclear and dendritic cells plus T-cells, that were not eliminated during thymic removal of potential autoreactive immune cells (11). These molecular processes restricted by specific HLA genes have been comprehensively reviewed (12). Altered interferon signaling by beta cells imply a response to viral infections which can directly kill beta cells (4). Such beta cell immune identity would be consistent with neonatal apoptosis (13), and increased body mass index (14, 15) promoting T1D in susceptible neonates and children. Further, immunocytotoxic assays could be developed as early diagnostic biomarkers of preT1D, to detect immune mechanism likely associated with beta cell specific *active* immunogenic cell death as recently suggested (10). Finally, even extensive immune beta cell death can be reversed in animal models of T1D by drug modulation of ER regulatory proteins, suggesting a prominent role for the apoptotic cell death mechanisms (16).

These broad observations of immunity in diabetes, in part, explain the overall heterogeneity found in the continuum of large immune effects in some clinical cohorts, less immune effects in others, and yet minimal or no effects in others. These clinical truisms are likely driven by the

highly variable gene loads related to immunity (Figure 2, blue arrows and Sensitization Chip) but importantly in the context of genes regulating proinflammatory phagocytosis, and lastly those much more prevalent gene variants regulating extrinsic beta cell function, e.g., BMI, insulin sensitivity, and intrinsic beta cell development, growth and maintenance (17).

Emerging forms of beta cell loss: There are a variety of additional mechanisms for potential cell loss involving both major forms of diabetes. While a role for them has not, as yet, been clearly described, they could have additive/synergistic roles in overall beta cell mass maintenance, and thus, a brief update follows.

Senescence. Proteins associated with senescence have roles in modulating beta cell mass, since T2D-associated common and low frequency gene variants involving cyclin proteins, e.g., cyclin D2, 3 and 4, are key regulators of neonatal beta cell proliferation, in part determining ultimate beta cell mass (18). For example, a low frequency cyclin D2 intronic variant is associated with half the risk of T2D (18). In the NODk paper described above, the authors examined human islets from controls and T2D and found that the latter's RNA expression data showed increased levels of the senescent markers *H2AFX* and *CDKN1A*, and others have found elevated P16Ink4a in T2D, a product of the *CDKN2A* locus (19, 20).

Mitochondria. A comprehensive and forward-looking review has recently highlighted the roles of mitochondrial function and diabetes, e.g., the close physical association of mitochondrial and ER membranes, the importance of these associations regulating intra-organelle and intracellular calcium homeostasis, and revealing mitochondrial stress responses similarity to the ER UPR stress events, in part using some ER proteins to assist with mitochondrial stress reduction (21). In addition, this review describes other mechanisms involving the NRF2 role in mitophagy to remove damaged mitochondria due to toxic ROS. Further, in support of the role for mitochondria in T2D and T1D, are the gene variants *CLEC16A, PARK2* and *PINK1* (22-24).

Islet inflammation. Exocrine ductal proliferation in T2D is associated with changes in islet chemokines CXCL1, 4 and 10, which result in pancreatic interlobular ductal occlusion creating local subclinical pancreatitis and likely reduction of ductal related precursors important in beta cell neogenesis in both T2D and T1D (25, 26). Pyroptosis is an immunogenic form of programmed cell death involving inflammasomes and mitochondrial stress, and this topic in normal subjects has been recently reviewed (27).

Islet hemorrhages. Hypothesized to reduce beta cell mass, these local islet events could involve both major forms of diabetes, but recently have not been widely reported (28).

Summary. The mechanisms outlined above may not substantially impact overall pancreatic beta cell loss, since these studies focus on islets, whereas substantial numbers of beta cells are scattered, singly or in small cell clusters in the pancreatic parenchyma. For example, beta cell dedifferentiation can be elevated several-fold and yet only contribute 1-3% of total pancreatic beta cell loss as detailed earlier.

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Supplement 5

OBESITY

Currently, at diagnosis, the majority of adult diabetes cases are overweight or obese and the metabolic syndrome, a proxy for insulin insensitivity, is prevalent not only in T2D, but also in adult-onset T1D including cases not initially requiring insulin therapy (1). Genetic studies do support a link between childhood adiposity and T1D risk. Using inverse-variance weighted Mendelian randomization analysis, with genetic variants as variables to test for causal associations, childhood adiposity genetic variants were positively associated with T1D risk (2). As insulin secretion is compromised, pathways that maintain glucose homeostasis, e.g., glucose disposition should adapt to retain homeostasis. Allostasis, the ability to adapt to maintain overall glucose homeostasis, in this case by compensatory insulin secretion relative to insulin sensitivity, must be compromised once homeostasis is lost as dysglycemia ensues.

Failure of allostasis could explain why the T2D risk variants of *TCF7L2* are associated with accelerated conversion from single (IAA or IA-2A) to multiple autoantibodies in the context of growth and increased BMI in children thereby increasing T1D risk (3). In older children at T1D-risk, the same *TCF7L2* variants in single, autoantibody-positive (GAD65) children have lower conversion rates to multiple autoantibodies and show a less severe metabolic phenotype (3). Possible mechanisms for such an effect of BMI on T1D-risk through BCOD include beta cell secretory demand, increase in beta cell apoptosis and death provoking autoimmunity as well as obesity-induced low-grade inflammation in islets promoting local islet inflammation. These concepts are further advanced by additional genetic information relating to the common origins of T2D and T1D, which we have set out below as a hypothesis.

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Supplement 6

PERSPECTIVE SUMMARY

The main therapeutic point of this Perspective is to identify how we might improve preservation of beta cell mass to ensure maintenance of its function long term. This summary integrates each of the Perspective's main pillars. This view is derived from the observations of slowly progressive deterioration of fasting glucose levels and other glucose related biomarkers, followed by more rapid dysglycemic deterioration beginning ~2 years before clinical disease onset both in T2D and T1D (Figure 3, colored circles and squares) (1-3).

Normal beta cell function: The islet beta cell organ is remarkable in that it is small, fragile, operating at high capacity, yet critical to survival. Therefore, it must maintain itself against many insults with a robust ER stress response and UPR adaptation shown on Figure 2, thin blue arrows on left, Box 1 and Box 2 green arrows respectively. During these usual stress events, BCOD and thus dysglycemias do not occur, and attained beta cell mass can be maintained throughout life (Figure 3, Normal).

Subclinical dysfunction (BCOD): Many sensitization risks, including common developmental, growth and environmental factors challenge the beta cell (Figure 2, arrows on left) creating an increased demand on proinsulin synthesis (Figure 2, Box 1). If these demands exceed the ER's adaptative capacity, beta cells could be forced into the terminal UPR and apoptosis (Figure 2, Box 2 red arrows). Yet most beta cells can adapt. These low levels of reversible BCOD and dysglycemia are not recognized using current clinical guidelines, and actual human beta cell content has not been histologically estimated during these earliest phases of potential BCOF (Figures 1A, yellow circles and Figure 3, yellow circles and squares, Subclinical Prediabetes). Since the total beta cell population (organ) is composed of individual cells known to be heterogeneous in nature, these events likely involve subpopulations of beta cells. Unrecognized earlier, clinical stages of BCOD and dysglycemia could be leveraged for earlier identification in both T2D and T1D, e.g., using the disposition index derived from an oGTT as reported above.

Prediabetic BCOD/F: BCOD *can* deteriorate further as shown on Figure 1A, orange circles and Figure 3, orange circles and squares, and additional ER stress creates more proinsulin demands upon the remaining beta cells. For example, clinical dysglycemias associated with IFG and IGT using current guidelines can be identified in subjects at risk of T2D or T1D. Even at the stage of IFG and IGT, BCOD and dysglycemia are potentially reversible by treatments aimed at diabetes-related pathways (Figure 2, arrows on left) and beta cells (Figure 2, Box 2 Environmental Factors), e.g., diet, exercise and/or tablet medications (Figure 1C, green arrows). Thus, the ER machine's normal function can be sufficiently restored, even with up to 50% beta cell content reduction (Figure 1B, orange circles and Figure 3, black square in Prediabetes area).

Diabetic BCOD/F: If severe ER demands continue untreated then beta cell dysfunction and mass further deteriorate suggesting an initial slow progression related to primary causes, e.g., gene variants and/or environmental factors, etc., but a critical beta cell mass threshold has been reached (Figure 3, black square just above the solid red line in T1D/T2D column). While compensatory demands continue in remaining healthy beta cells and perhaps aided by neogenesis

and low-level proliferation creating new beta cells, even in this earliest stage of frank T2D and T1D, reversibility can be achieved as evidenced by diabetes remissions in a sizable minority of both T2D and T1D (Figure 1C, blue arrows). Rescuing severe BCOD usually comes in the form of diet, exercise, tablets or temporary insulin and/or immunotherapy (Supplement 1, Recommendations). However, when beta cell mass is reduced below the critical mass threshold, BCOD becomes irreversible and severe BCOF requires long-term insulin treatment in both T2D and T1D (Figure 3, black square below solid red line).

Each of the dysglycemic phases we describe can be further modulated by genetic and non-genetic factors downstream from the ER, e.g., the proinsulin pathway through trafficking to and from the Golgi to secretory granules, the conversion from proinsulin to insulin and exocytosis. Given the extreme numbers of genetic and epigenetic variants, many of which are transcription factors potentially having thousands of DNA targets, some of which are locally clustered, and then linking these elements to the cumulative behavior of low impact, common gene variants at many critical check points, it's no wonder that clinical heterogeneity is represented by more of a blend, than distinct cohorts. Only at the two extremes are clinical decisions more facile. One goal is to determine the role of these elements in the earlier phases of the beta cell loss/regeneration dynamic, which appears a daunting task. The BCOD/F paradigm provides a framework for understanding how the myriad pathways and ER machine elements could be crucial to advancing clinically apparent and reversible dysglycemias, up to and including diabetes in both forms of the disorder, and suggesting earlier screening, diagnosis, prevention and treatment strategies. The paradigm also suggests a change in both the research and care management of the diabetes pandemic. The Lancet Diabetes Commission has initiated this project with regard to clinical care, since current treatment elements are relatively advanced by the standards of improved outcomes for chronic complications and deaths in smaller, medically advanced countries. Similar refocusing approaches appear relevant for the investigative arm of diabetes towards earlier diagnosis and prevention (Supplement 1, Recommendations).

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