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Mini-review

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Revisiting Regulators of Human β-cell Mass to Achieve β-cell–centric Approach Toward Type 2 Diabetes

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Abbreviations: BCA, fractional β-cell area; BCM, β-cell mass; BMI, body mass index; CP, chronic pancreatitis; DPP-4, dipeptidyl peptidase-4; GC, glucocorticoid; GLP-1RA, glucagon-like peptide-1 receptor agonist; IAPP, islet amyloid polypeptide; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; ROS, reactive oxygen species; SU, sulfonylurea; T1DM, type 1 diabetes; T2DM, type 2 diabetes.

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Abstract

Type 2 diabetes (T2DM) is characterized by insulin resistance and β-cell dysfunction. Because patients with T2DM have inadequate β-cell mass (BCM) and β-cell dysfunction worsens glycemic control and makes treatment difficult, therapeutic strategies to preserve and restore BCM are needed. In rodent models, obesity increases BCM about 3-fold, but the increase in BCM in humans is limited. Besides, obesity-induced changes in BCM may show racial differences between East Asians and Caucasians. Recently, the developmental origins of health and disease hypothesis, which states that the risk of developing noncommunicable diseases including T2DM is influenced by the fetal environment, has been proposed. It is known in rodents that animals with low birthweight have reduced BCM through epigenetic modifications, making them more susceptible to diabetes in the future. Similarly, in humans, we revealed that individuals born with low birthweight have lower BCM in adulthood. Because β-cell replication is more frequently observed in the 5 years after birth, and β cells are found to be more plastic in that period, a history of childhood obesity increases BCM. BCM in patients with T2DM is reduced by 20% to 65% compared with that in individuals without T2DM. However, since BCM starts to decrease from the stage of borderline diabetes, early intervention is essential for β-cell protection. In this review, we summarize the current knowledge on regulatory factors of human BCM in health and diabetes and propose the β-cell–centric concept of diabetes to enhance a more pathophysiology-based treatment approach for T2DM.

Key Words: beta-cell mass, beta-cell regulator, beta-cell workload, human pancreas, type 2 diabetes

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Diabetes can be broadly classified into type 1 diabetes (T1DM) caused by autoimmune disorder and type 2 diabetes (T2DM) with insulin resistance and β-cell dysfunction. Although it is widely known that T1DM is caused by a marked decrease in β-cell mass (BCM) because β cells are destroyed and insulin deficiency leads to marked hyperglycemia, studies on human pancreatic tissue have revealed that patients with T2DM also have inadequate BCM [1]. Judging from the pathological changes in T2DM, it is necessary to develop therapeutic and preventive approaches to protect β cells and maintain BCM, which is controlled within a narrow range of 0.6 to 1.2 g [2], rather than simply focusing on lowering blood glucose level [3-5]. Over the past 2 decades, it has been revealed that human BCM is regulated and changed by various conditions and situations even before the onset of T2DM, by analyzing pancreatic tissue obtained at autopsy and surgery. Furthermore, the mechanisms by which β cells increase or decrease have also been investigated. As β-cell fate has been investigated in detail, it has become clear that the regenerative capacity of β cells is somewhat limited in humans and that it is important not to overload β cells by reviewing diet and exercise habits and losing weight to protect them.

This review summarizes the mechanisms by which BCM is increased or decreased. In addition, we detail situations and conditions that affect BCM, including T2DM, and discuss treatment strategies fulfilling a β-cell–centric approach.

Regulation and Turnover of Human β-Cells

β-cell Apoptosis

It is known that the rate of decrease in BCM caused by the duration of T2DM is very small, 1.5% per year [6]. Therefore, although it is very difficult to accurately measure cell death, the decrease in BCM is caused by an imbalance between cell death and neogenesis/replication [6].

β-cell apoptosis, a type of programmed cell death, is one of the possible mechanisms of β-cell loss. Pancreatic tissue cultured with a high concentration of glucose showed increased apoptosis and expression of proapoptotic genes such as Bad, Bid, and Bik [7], indicating that glucotoxicity is a trigger for apoptosis [8]. One cause of glucose toxicity is chronic oxidative stress [9,10]. Glucose produces reactive oxygen species (ROS) through oxidative phosphorylation, and when the ROS concentration exceeds the physiological range as a result of hyperglycemia, it can cause tissue damage including β cells, which are especially susceptible to oxidative stress due to poor intrinsic antioxidant defenses [11]. Specifically, when β cells are attacked by ROS, mitochondrial dysfunction occurs and signaling that normally links glucose metabolism and insulin secretion is disrupted [12,13]. Furthermore, hyperglycemia induces the production and release of inflammatory cytokines such as IL-1β, which exerts β-cell toxicity [14]. When the process of glucose intolerance leads to overnutrition, lipids deposited in non-adipose tissue are not effectively oxidized, and their product, ceramide, increases nitric oxide production and causes apoptosis of pancreatic β cells [15]. In other words, β-cell loss may be secondary to ectopic lipid deposition and lipotoxicity.

The formation of islet amyloid, a hallmark of the pathological changes in T2DM, is caused by the aggregation of islet amyloid polypeptide (IAPP; also called amylin) secreted by β cells, along with insulin [16]. It has been shown that the aggregation process of IAPP causes cytotoxicity, resulting in apoptosis of β cells in in vitro studies [17]. In particular, IAPP oligomers, which are generated from IAPP monomers, are known to cause β-cell-specific toxicity and consequently diabetes [18,19]. A study of human pancreatic islets also showed that β-cell apoptosis was significantly associated with both an increase in islet amyloid and a decrease in fractional β-cell area (BCA), defined as the area occupied by β cells in a cross-section of the pancreatic parenchyma [16]. Excessive levels of glucose, free fatty acids, and IAPP trigger endoplasmic reticulum stress in β cells, which also induces β-cell failure [20].

Recently, dedifferentiation has been proposed as another possible mechanism of β-cell loss [21,22], but it has been indicated that its effects are limited in humans [23].

β-cell Neogenesis and Replication

There are 2 main processes of β-cell expansion; β-cell neogenesis by pancreatic progenitor cells and/or ductal precursor cells and replication from existing β cells [24]. β-cell growth occurs during the neonatal period in rodents and is primarily derived from the replication of preexisting β cells, and there is skepticism regarding the possibility that it originates from the development of new β cells (ie, neogenesis) [25]. An increased replication rate of β cells is also involved mainly in rodents in the process of a compensatory increase in BCM induced by partial pancreatectomy [26], short-term glucose infusion [27], or obesity [28]. In addition, a recent report suggests that endogenous repair of existing β cells, a process that normalizes β-cell function and structure, may act to prevent the progression of T2DM independently of these mechanisms in mild glucose intolerance, as long as it is promptly resolved [29].

In contrast, replication of β cells in humans is quite limited. At birth, β-cell replication is observed at a frequency of about 2% to 2.5% (ie, by double staining for insulin + Ki67), but by 5 years of age, it is observed at best at about 0.5%, and it becomes almost absent in adults [24,

30]. Furthermore, there are a few reports suggesting the involvement of increased β-cell replication in the process of a compensatory increase in BCM to increase insulin secretion in adulthood [31, 32]. In humans, shortening of telomeres limits replication and causes replicative aging, but this does not occur in rats, and replication is not impaired for several generations even when telomerase is ablated in mice [33]. These factors are considered to account for the differences in β-cell replication between humans and rodents.

β-cell replication peaks during the neonatal period, whereas neogenesis (insulin and CK-19 double-positive cells and/or pancreatic ductal CK-19-positive cells) peaks earlier, during the fetal period [24]. Although the rate of postnatal β-cell neogenesis is low, it has been reported that neogenesis is accelerated under the conditions of obesity and insulin resistance requiring increased insulin secretion in adulthood [1, 34]. Therefore, the process of β-cell compensation to maintain glucose homeostasis has been suggested to involve β-cell neogenesis rather than replication in adult humans. β-cell turnover has also been found to be a process of neogenesis rather than replication in adult nonhuman primates (vervet monkeys) [35].

Factors Influencing Human β-Cell Mass

Birthweight

In 1991, Hales et al first reported an inverse relationship between birthweight and incidence of T2DM [36]. Since then, there have been a series of reports that people born with low birthweight have a higher incidence of future T2DM [37-45]. The idea that the environment during the prenatal period influences the risk of noncommunicable diseases in the future is called the developmental origins of health and disease hypothesis [46,47]. Recent reports have revealed that epigenetic modification such as DNA methylation, histone modification, and microRNA interaction is involved in fetal undernutrition [48,49]. In rodent models with low birthweight caused by intrauterine malnutrition, it is known that microRNA interaction causes changes in the cell cycle and a decrease in BCM, leading to glucose intolerance [50]. In addition, genetic variants that predispose to T2DM might also reduce birthweight [51]. Especially, 2 loci that are associated with low birthweight (*CDKAL1* and *HHEX-IDE*) have been shown to significantly impair β-cell function [52,53]. Taken together, these results suggest that individuals born with low birthweight are at increased risk of future T2DM because of potentially low β-cell function and BCM even in humans. We have recently reported that nondiabetic adults born with low birthweight have lower BCA and islet size and that birthweight is a determinant of BCM not only in rodents but also in humans [54]. In the fetal period, BCM is known to increase due to an increase in islet size rather than islet density, which is defined by the number of islets in the pancreatic parenchyma [55], but this is expected to be insufficient in low birthweight infants. We also revealed that birthweight was correlated with β-cell neogenesis (ie, density of insulin-positive duct cells), rather than replication. Pancreatic duct cells can convert to β cells even in adults [56], and these results together suggest that being born with low birthweight reduces the ability to produce β cells throughout life. Individuals born with low birthweight subsequently have lower BCM, which might explain, in part, the relationship between low birthweight and higher risk of developing T2DM in the future.

Postnatal Growth

Referring to Scammon's growth curve, the general type of growth, such as organ formation, is complete at the age of 20, but steep growth is observed in the first 3 years of life and at puberty. Regarding the growth process of β cells, Meier et al studied in detail the process from birth to adulthood using pediatric specimens obtained at autopsy [30]. They reported that in early infancy, the pancreas consists of abundant small clusters of β cells and single β cells, but by 10 weeks of age, the small clusters of β cells have increased in size and typically occupy encapsulated islet-like structures. Thereafter, during the process of β-cell growth until the age of 20, islet size increases, although islet density gradually decreases [30,57]. In this process, BCA decreases from nearly 3% in infancy to about half that at 20 years, but the pancreatic volume increases 10s of times during this period. Therefore, BCM increases from at most 40 mg to approximately 1 g, with an increase in β-cell number from 5 million to 1 billion, as the size of individual β cells does not change during the growth process [30,57]. Interestingly, because both β-cell neogenesis and replication are highest in fetal life and decrease with age and β-cell apoptosis is rarely observed during growth, unlike the growth curve, β-cell growth is highest in infancy and decreases during adolescence and adulthood, with no secondary acceleration during puberty when secondary body growth occurs [24,30,58].

Aging

There are conflicting results regarding the changes in BCM with aging. We have previously reported that pancreatic mass reaches a plateau around the age of 20 years and then gradually decreases from about 60 years [59,60]. The most prominent change in pancreatic morphology with aging is atrophy of the exocrine pancreas and decrease in volume of the pancreatic parenchyma as fibrosis and fat infiltration

increase [32,60]. Hence, although BCA increases with age, BCM, expressed as the product of BCA and pancreatic parenchymal mass, does not change between the age of 20 and 100 years. Regarding β-cell turnover, although the frequency of β-cell replication was very low, subjects with advanced age did not show any change from that in the younger group [32].

However, Mizukami et al revealed that BCM peaked between the ages of 20 and 50, and then gradually decreased but was relatively well preserved [57]. These results are consistent with those of other previous studies, which found a weak negative correlation between BCM and age [6,61]. Although BCM decreases over the age of 70 years, it remains above 80% if it is not affected by glucose intolerance [6,57]. Based on previous reports, changes in BCM with aging are small, if any, which may be why it is difficult to draw consistent conclusions. Although it was consistently found that β-cell apoptosis was not enhanced with aging and, intriguingly, β-cell size increases with age [32,57], which may reflect cellular hypertrophy associated with cellular senescence, it will be necessary to verify whether BCM changes in the elderly, especially in those over 70 years old, by further increasing the sample size.

Obesity (in Childhood and Adulthood)

Obesity, especially visceral fat accumulation, results in insulin resistance due to decreased secretion of adiponectin released from adipocytes [62]. Insulin resistance results in a compensatory increase in the amount of insulin released from β cells. During the process of insulin resistance, β-cell replication was enhanced in a rodent model, resulting in a 3-fold increase in BCM [28]. However, studies on human pancreatic tissue showed a limited increase in BCM of 20-90% [1,6,32,63], although the definition of obesity differed slightly between these studies. Furthermore, in the process of increasing BCM, there was an increase in neogenesis rather than replication [1,32], which is different from the process in rodents. The fact that increased neogenesis during the obesity-induced increase in BCM in humans is an insulin resistance-induced change is supported by the fact that Mezza et al evaluated insulin sensitivity by conducting the hyperinsulinemic euglycemic clamp procedure and reported that β-cell neogenesis was increased during the increase in BCM in the insulin-resistant group [34].

Obese individuals with intrinsic β-cell dysfunction are more likely to develop T2DM; however, childhood obesity and adulthood obesity may have different effects on the future development of diabetes. Although having obesity in childhood, especially in those with low birthweight, increases the incidence of diabetes [64], another study reported that obesity in adolescence alone did not increase the risk of T2DM, unlike obesity in adulthood [65]. Furthermore, low body mass index (BMI) in adolescents increases the incidence of gestational diabetes [66,67]. In another population of Japanese women, adolescent BMI was inversely associated with the development of T2DM [68]. The inconsistent conclusions about preadulthood obesity and the future development of diabetes may be in part explained by the involvement of β-cell plasticity. In our previous study, we reported that BCA and islet size increased in the group with a history of childhood obesity compared with the group with obesity only in adulthood [54]. This may reflect the fact that β-cell neogenesis and replication are more active in childhood than in adulthood, and therefore β-cell compensatory responses are more likely to occur. Whether β-cell expansion in childhood obesity has a protective effect against the future development of T2DM needs to be further examined (Fig. 1).

Ethnicity

East Asians exhibit lower insulin secretion and higher insulin sensitivity compared with Africans and Europeans [69]. Previous reports from Japan, including ours using both autopsy and surgical cases, showed that obesity induced little increase in either β-cell neogenesis or BCM [54,57,70-73], which was different from the results of studies in Caucasian subjects [1,32,63]. Taking these results together, pathophysiological changes in BCM before the onset of T2DM may be different among races, and East Asians have lower ability of β-cell neogenesis and β-cell expansion in response to obesity compared with other ethnicities, which may be derived from the environmental or genetic background [2,74]. Although it is still unclear how these factors affect β-cell dynamics, we found in our recent study that individual β-cell size in obesity is unchanged regardless of race and that the increase in β-cell number in response to obesity varies by race [75]. The racial difference in β-cell neogenesis and β-cell hyperplasia rather than hypertrophy in obese individuals is supported by the idea that β-cell number is mainly regulated by β-cell neogenesis in human adults. Because Japanese people have a higher incidence of lifestyle-related diseases at a lower degree of obesity than Caucasians, a BMI of 25 or higher, which is lower than the World Health Organization classification, is defined as obesity in Japan [76]. One of the possible reasons for the differences in the degree of obesity that increases the incidence of the previously discussed diseases is the difference in changes of BCM and β-cell neogenesis induced by insulin resistance. East Asians, who are less likely to compensate for insulin resistance, are thought to be more prone to developing T2DM because BCM and β-cell number may not increase in that situation and the β-cell

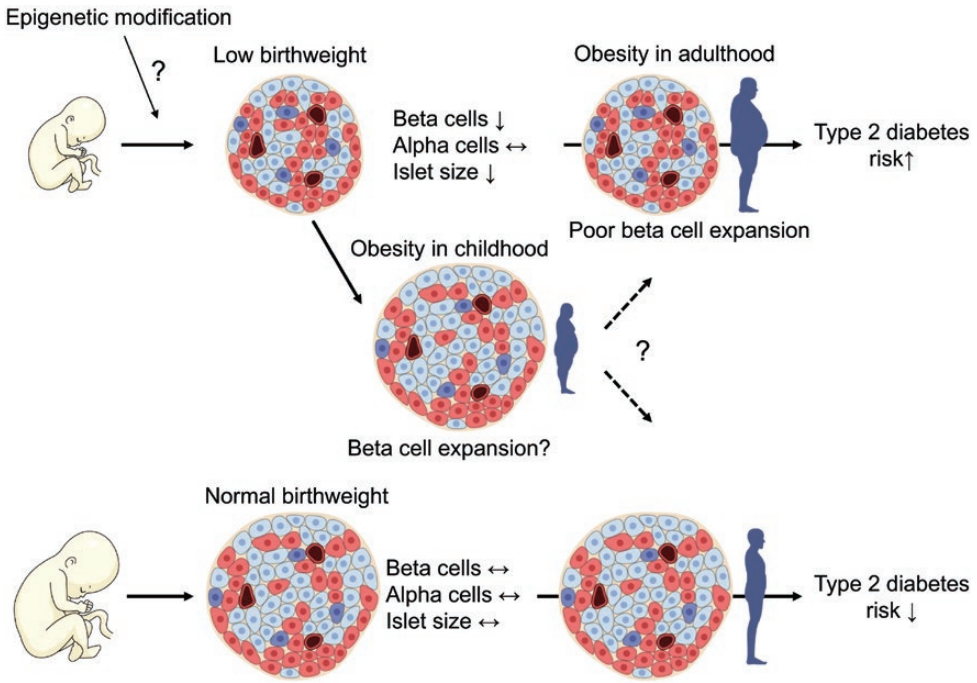

Figure 1. Mechanisms of effects of low birthweight and childhood obesity on islet morphology. Individuals born with low birthweight have lower β-cell mass (BCM) and islet size via epigenetic modification, although α-cell mass remains unchanged. Being born with low birthweight, which potentially results in low BCM and the relative predominance of α-cell mass, may contribute to the high incidence of type 2 diabetes mellitus (T2DM) in adults with low birthweight, as proposed in the developmental origins of health and disease hypothesis. A history of childhood obesity increases BCM and islet size, but it is not yet known whether this change brings a protective effect against future development ofT2DM.
Reproduced from Sasaki et al [54].

workload, which describes the stress on individual β cells to secrete insulin is likely greater than that in other ethnicities, making them more prone to β-cell dysfunction. For example, insulin secretion increases 2-fold in obese individuals to maintain normoglycemia. However, if BCM and β-cell number remain constant, workload of individual β cells (ie, β-cell workload) is assumed to increase 2-fold [5].

Pregnancy

As insulin resistance increases over the course of pregnancy, it is associated with the development of various perinatal complications such as gestational hypertension and gestational diabetes [77]. Although the mechanism of insulin resistance during pregnancy is not fully elucidated, it is known that hormones released from the placenta (ie, human placental lactogen, human placental growth hormone, and progesterone) and cytokines (ie, tumor necrosis factor α and interleukin 6) are involved in the development of this condition [78]. In response to increased insulin resistance, β-cell function and insulin secretion increase to maintain glucose homeostasis [79,80]. In rodent models, an approximately 2- to 5-fold increase in BCM in response to insulin demand is seen with increased β-cell replication [81-83]. Van et al first reported in humans a 2.4-fold increase in BCA, with an increase in islet size during gestation [84]. However, only 5 pregnant women were analyzed, and the sample size may have been insufficient. More recently, Butler et al have examined changes in BCM during gestation (at 10~40 weeks' gestation) using a larger sample of autopsy cases [31]. They reported a 1.4-fold increase in BCA during gestation, with an increase in islet number rather than size and that, unlike in rodent models, the increase was due to an increase in neogenesis but not replication. These results are consistent with those of previous studies, which suggested that β-cell neogenesis but not replication is responsible for changes in BCM in adults [1,32,34]. However, they emphasized that the increase in BCM is not sufficient for the amount of insulin secreted during pregnancy. The amount of insulin required during pregnancy increases 2-fold, which is greater than the increase in BCM. In terms of β-cell workload, people with gestational diabetes are likely to have β cells with insufficient reserve to adequately respond and thus may be more prone to develop T2DM in the future.

Drug Use

The effects of anti-diabetic drugs on β-cell turnover and BCM have been clarified to some extent in vitro and in

rodents in vivo. For example, glucagon-like peptide-1 receptor agonists (GLP-1RAs) [85,86] and dipeptidyl peptidase-4 (DPP-4) inhibitors [87] increased BCM, which may be induced by direct or indirect effects of GLP-1 to inhibit β-cell apoptosis and accelerate β-cell replication. However, no consistent conclusions have been obtained regarding the effects of incretin preparations on human β cells, exocrine function, and pancreatic morphology. Butler et al compared islet morphology in diabetic patients with and without incretin (ie, GLP-1RAs and DPP-4 inhibitors) treatment and reported a 6-fold increase in BCM even in humans with an increase in β-cell number rather than size in the group with incretin therapy, although β-cell neogenesis was not enhanced and β-cell function was not evaluated [88]. In addition, the effect on endocrine cells was accompanied by hyperplasia of α cells, which have the potential to develop into neuroendocrine tumors. Pancreatic weight was increased by 40%, indicating that the results of previous rodent studies that revealed that GLP-1 had a proliferative effect on the exocrine pancreatic system may also be applicable to humans [89]. However, this study has been challenged as the sample size of this study was small, and there were critiques of the inclusion criteria, such as type 2 diabetic patients not receiving incretin therapy may have had T1DM [90], as well as a number of confounding factors. It has also been claimed that the incretin treatment group included a large number of older patients whose pancreatic parenchymal area might be smaller (and more adipocytes within the pancreas) with aging, resulting in overestimation of BCA, a fraction of insulin positive area to pancreatic parenchymal area, and BCM [90]. Another study of the pancreas of a nonhuman primate (cynomolgus monkeys), which is anatomically and physiologically closely related to and relevant to humans, showed that high doses of liraglutide did not increase either β-, α-, δ-, or PP-cell mass, and there was no consistent dose-dependent increase in pancreatic weight with liraglutide and semaglutide administration [91]. Other reports regarding human pancreatic tissues concluded nonsignificant effects of incretin therapy on BCA, β-cell replication [92], and exocrine function [93,94], despite its improvement of β-cell responsiveness [95].

In the previous section, we described that obesity may increase BCM in response to insulin resistance in humans, although there are racial differences. Glucocorticoids (GCs) such as prednisolone and dexamethasone are anti-inflammatory drugs used mainly for autoimmune diseases, but they also induce insulin resistance [96] and augment insulin secretion [97]. In rodent models, insulin resistance induced by GCs increases BCM via β-cell replication [98,99]. However, in our previous study, GC administration did not increase BCM in Japanese adults either with or without diabetes [100]. It is possible that racial differences and the fact that adults have reduced neogenesis and replication of β cells may have contributed to the lack of increase in BCM. Although in humans, unlike rodents, GCs do not increase BCM, they increase insulin resistance and insulin secretion, which is expected to place a greater burden on individual β cells.

Pancreatic Diseases

The prevalence of diabetes in pancreatic cancer patients is very high, with approximately 50% having a diagnosis of diabetes [101]. Although pancreatic cancer worsened glycemic control, among pancreatic cancer patients with diabetes, about 80% were diagnosed with diabetes less than 24 months before the diagnosis of pancreatic cancer [102]. In other words, diabetes can be both a trigger and a consequence of pancreatic cancer. Diabetes does not develop until BCA is reduced by 65% [103], but some patients develop pancreatic diabetes because part of the pancreas is invaded by tumor. Furthermore, there are some data showing that blood glucose control improves after tumor resection if there are sufficient islets remaining in the pancreatic tissue [104]. Therefore, it has been suggested that diabetes associated with pancreatic cancer may be a paraneoplastic syndrome that develops when humoral factors released from the malignancy inhibit insulin secretion and action [102]. In our previous study, we found that patients with pancreatic cancer had lower BCA than patients with nonpancreatic cancer in both nondiabetic and diabetic cases [72]. The finding that pancreatic cancer alters the islet morphology in normal pancreatic tissue is supported by a study in which adrenomedullin released from the tumor affected endocrine cells [105]. Adrenomedullin, consisting of a 52 amino acid peptide, is upregulated in pancreatic cancer cell lines and has been shown to reduce insulin secretion from β cells [105]. However, since another study showed no difference in BCA between patients with pancreatic and nonpancreatic cancer in view of the negative effect of the tumor on normal pancreatic tissue [106], it is necessary to confirm the comparison by eliminating confounding factors of BCM.

Patients with chronic pancreatitis (CP) also have a high prevalence of diabetes and often require exogenous insulin administration. Exocrine dysfunction usually precedes the onset of endocrine dysfunction and diabetes [102], and most patients with CP-related diabetes have abnormalities in exocrine function [107]. Schrader et al showed that patients with CP had a 29% and 21% decrease in BCA and pancreatic volume, respectively [108]. However,

in CP, apoptosis was not enhanced in endocrine cells but was 10 times more frequent in acinar cells, suggesting that endocrine tissues are less susceptible to tissue destruction by inflammation than are exocrine tissues [108]. They further analyzed the pancreatic tissue of patients with CP in another study and found no difference in BCA between those who developed diabetes after undergoing pancreatic resection and those who did not; therefore, insulin resistance and β-cell dysfunction may be greatly involved in CP, which cannot be explained simply by change in BCM [109].

Table 1 summarizes current knowledge of changes in β-cell characteristics in various conditions.

Change in BCM in Glucose Intolerance

The question arises as to whether the timing of the decrease in BCM is at the initial stage of glucose intolerance or at the stage when T2DM is diagnosed. Although consistent results have not been obtained, a previous report showed that a 21% decrease in BCA indicated the development of impaired glucose tolerance (IGT) [103]. Other reports showed a decrease in BCA of 40% in subjects with impaired fasting glycemia [1] and 38% in IGT subjects [110] compared with the normal glucose tolerance (NGT) group, although the relative reduction was smaller than in the T2DM group. In studies that accurately assessed glucose tolerance by 75-g oral glucose tolerance test, including ours, BCA in impaired fasting glycemia and IGT subjects did not show a significant decrease compared with the NGT group; however, in all reports, BCA tended to decrease as glucose intolerance progressed [71,73,111,112]. In patients with IGT, β-cell replication was not enhanced, but neogenesis was activated [110], suggesting that some compensatory response to glucose intolerance may occur. At this stage, however, because BCM starts to decrease even though there is no increase in apoptosis [110], there is room for further investigation into what kind of turnover is occurring in islets in mild glucose intolerance. Taken together, these results indicate that BCM is likely to be reduced even before the onset of T2DM, and early intervention after the onset of glucose intolerance is necessary to develop a therapeutic strategy for β-cell protection.

As glucose intolerance progresses and T2DM is diagnosed, BCM is clearly reduced compared with that in NGT. In the last 2 decades, approximately 20 studies have compared BCM between patients with and without T2DM, and found a 20% to 65% decrease in BCM in patients with T2DM [2, 3]. In addition, even in patients with T2DM, BCM decreases further with prolonged disease duration and poor glycemic control [6,110,112,113]. β-cell neogenesis is enhanced as a compensatory response in patients with T2DM in the early postdiagnosis period [63,110] but gradually becomes equivalent to that in nondiabetic patients [1,72,110,112]. β-cell replication is similar in nondiabetic and diabetic patients [1,72,110,112]. However, several studies have reported that β-cell apoptosis is enhanced in T2DM [1,63,110], especially in those with a long duration of disease. In summary, the results of β-cell turnover suggest that the decrease in BCM in T2DM is not due to a decrease in β-cell neogenesis or replication but rather an increase in β-cell apoptosis as a possible mechanism.

There are approximately 250 000 to 1 million islets and each islet contains approximately 1000 β-cells [3], which means that there are 250 million to 1 billion β cells in total

Table 1. Current Knowledge of Changes in β-Cell Characteristics in Various Conditions in Humans

Situation	β-cell mass	β-cell size	β-cell number	β-cell neogenesis	β-cell replication	β-cell apoptosis
Low birthweight [54]	↓	→	NA	↓	→	NA
Postnatal growth [24,30]	↑	→	↑	↑	↑	→
Aging [6,24,32,57,61]	→or↓	↑	NA	→or↓	→or↓	→
Adulthood obesity						
Caucasian [1,32,34]	↑	→	↑	↑	→	→
Asian [54,70,72]	→	→	→	→	→	→
Childhood obesity [54]	↑	NA	NA	→	→	NA
Pregnancy [31,84]	↑	→or↑	↑	↑	→	→
Incretin treatment [88,90-92]	→or↑	→or↑	→or↑	→	→	NA
Glucocorticoid treatment [100]	→	NA	NA	↑?	→	→
Pancreatic cancer [72,106]	→or↓	NA	NA	NA	NA	NA
Chronic pancreatitis [108]	↓	→	NA	→	→	→
Borderline diabetes [1,103,110-112]	→or↓	NA	NA	→or↑	→	→
T2DM [1,72,75,110,112]	↓	↓	↓[a]	→	→	→or↑

Abbreviations: ↓, decrease; →, no change; ↑, increase; NA, not available.
[a]Relative reduction in β-cell number was greater than that in β-cell size in T2DM.

in humans [114]. Although it has been shown that both islet number and size decrease during the process of β-cell loss in T2DM [54,72], the question arises as to whether the β-cell size or number decreases in this process. To solve this problem, we examined the contribution of decrease in β-cell size and number in T2DM and clarified that the relative reduction of BCM in T2DM was 43%, of which β-cell size and number were significantly reduced by 10% and 37%, respectively, indicating that β-cell number is a major contributor to β-cell loss in T2DM (Fig. 2) [75]. However, it is noted that β-cell size was not accurately measured by staining for a membrane marker, and we did not measure all β cells, so we may have overlooked heterogeneity. Further research using 3D imaging techniques that can measure β-cell size more accurately is warranted to clarify the role of β-cell size in diabetes. Furthermore, the fact that HbA1c correlated with β-cell number rather than size confirms the importance of individual β cells in achieving glycemic control [75]. Based on the results of previous studies, we can say that treatment of T2DM requires protection from possibly induced β-cell apoptosis and dedifferentiation, which lead to a decrease in β-cell number and thus BCM.

Clinical Implications: Treatment and Prevention of T2DM

β-cell Workload Hypothesis

We have proposed the β-cell workload hypothesis of the process of β-cell loss (Fig. 3) [4]. Obese patients have a limited increase in BCM but need to secrete large amounts of insulin to stimulate glucose absorption in the face of increased insulin resistance. This is especially significant in East Asians who are obese and do not have an increase in BCM, which puts even more work on individual β cells. Also, pregnant women have a small increase in BCM but must release more insulin than the degree of increase in BCM to maintain glucose homeostasis. The burden on individual β cells would also be greater in subjects with a low birthweight because of the potentially low BCM.

This overload of β cells puts additional stress on the remaining cells if some of them are damaged or die by mechanisms such as apoptosis. Then, as more β cells die, the workload of the remaining cells increases, leading to a vicious cycle of further decline in both quality and quantity of β cells. Eventually, when functional BCM is unable to compensate for insulin resistance, hyperglycemia develops. Hyperglycemia further increases the workload of β cells, causing toxicity to β cells, known as glucotoxicity, leading to further β-cell loss. This concept is targeted in preventive strategies for T2DM, as current treatments for T2DM do not cure the disease to the original condition. However, the β-cell workload hypothesis allows an integrated explanation of the pathogenesis of T2DM without considering β-cell dysfunction and insulin resistance separately and indicates that it is important to reduce β-cell workload and achieve better glycemic control to break the vicious cycle of β-cell dysfunction in patients with T2DM [4].

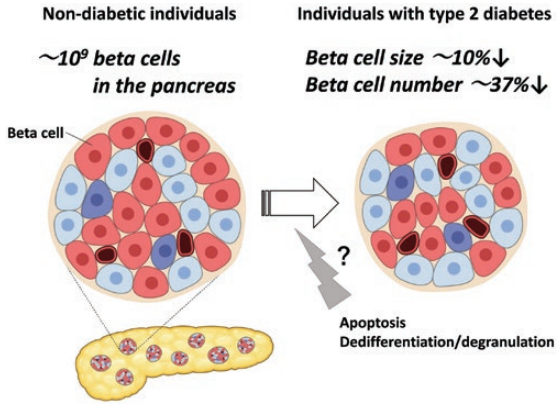

Figure 2. Contribution of β-cell size and number to the process by which β-cell mass (BCM) is reduced in type 2 diabetes mellitus (T2DM). There are approximately 250 million to 1 billion β-cells in humans. In the process of a 43% reduction in BCM via apoptosis and dedifferentiation, β-cell size and number were reduced by 10% and 37%, respectively, suggesting that reduced β-cell number is a major contributor to β-cell loss in T2DM.
Reproduced from Sasaki et al [75].

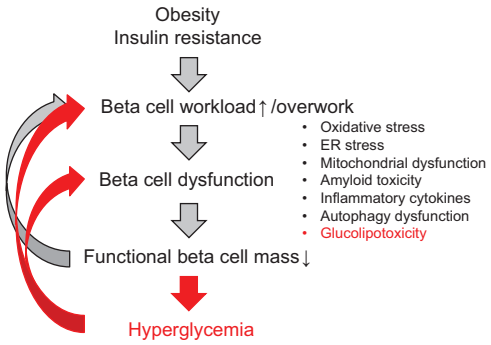

Figure 3. Proposed mechanisms of β-cell workload hypothesis. When the workload of β-cells increases as a consequence of obesity or insulin resistance, some of them disappear possibly through apoptosis, putting an additional burden on the remaining cells. If they are unable to cope with the stress, it leads to β-cell dysfunction and hyperglycemia develops. Hyperglycemia imposes additional burden on the remaining β-cells, causing glucotoxicity and a vicious cycle of β-cell depletion. This hypothesis emphasizes that earlier and simultaneous intervention aimed at improving both β-cell dysfunction (pathology) and insulin resistance (cause) is important to prevent the progression of the disease. Adapted from Inaishi and Saisho [2]. Reproduced from Saisho 2019 [4].

Treatment Strategy for T2DM

Adapting the β-cell–centric concept to clinical practice is expected to enhance a more pathophysiology-based treatment approach for T2DM that fosters early intensification of treatment and prevents clinical inertia. The β-cell–centric concept also emphasizes an early combination treatment strategy aiming at simultaneous intervention toward β-cell dysfunction and insulin resistance. A proposed treatment strategy for T2DM based on the β-cell–centric concept is shown in Figure 4. This treatment strategy, rather than the current glucose-centric stepwise intensification approach, will minimize exposure to excessive glucose during the disease course and prevent diabetic complications in the long term.

To maintain β-cell function, it is necessary to reduce β-cell workload and allow β cells to rest. In all stages of T2DM, lifestyle modification is the most important therapy for reducing the workload of β cells [3]; that is, adherence to diet and increased physical activity, resulting in weight loss, will improve insulin sensitivity and thus reduce β-cell workload. In fact, the Diabetes Prevention Program has reported that intensive lifestyle modification accompanied by weight loss prevents the progression of IGT to T2DM [115]. In addition, a study of insulin-naïve type 2 diabetic patients with disease duration of 10 years or less showed

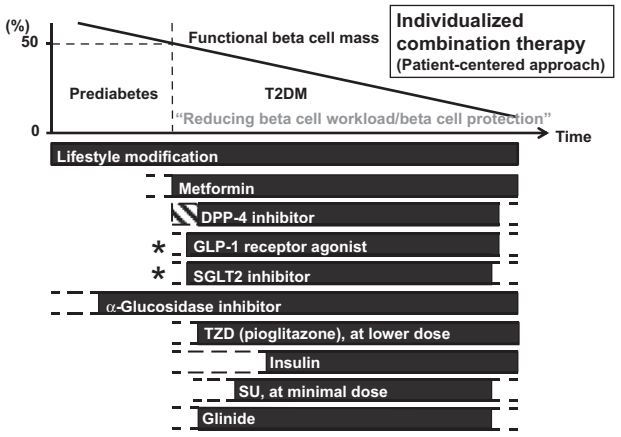

Figure 4. Proposed treatment strategy for type 2 diabetes mellitus (T2DM) based on β-cell centric concept. T2DM is a progressive loss of β-cell function against a background of insulin resistance. Therefore, treatment should focus on reducing the workload of β cells to maintain optimal glycemic control. Lifestyle modification and weight loss are the most basic and important treatments to reduce the burden on β cells. As drug therapy, dipeptidyl peptidase-4 (DPP-4) inhibitors are expected to improve β-cell function, and initial combination therapy with a DPP-4 inhibitor and metformin is expected to provide better glycemic durability compared with metformin alone. Glucagon-like peptide-1 receptor agonist and sodium glucose cotransporter 2 inhibitors (denoted by *) are expected to reduce cardiovascular events and should be considered for use in people with high cardiovascular risk, heart failure, or chronic kidney disease. The choice of therapy should be individualized with a patient-centered approach.
Adapted from Saisho [3,139,140]. Reproduced from Saisho 2020 [5].

that a lifestyle intervention of intensive exercise improved β-cell function with a decrease in low-grade inflammation and/or weight loss [116]. The Diabetes Remission Clinical Trial showed that almost half of the participants who were diagnosed with T2DM within the past 6 years achieved remission to a nondiabetic state after 12 months of intensive weight management in the context of routine primary care [117], and its post-hoc analysis has shown that remission with weight loss reversibly improves pancreatic morphology in T2DM [118]. Furthermore, accumulating evidence suggests that weight loss induced by metabolic surgery may lead to complete remission of T2DM [119,120].

The use of metformin and thiazolidinediones as pharmacotherapy is considered to improve insulin resistance and reduce β-cell burden. Metformin is effective in both obese and lean patients and should be used unless contraindications exist [121,122]. Pioglitazone has been shown to inhibit the progression of atherosclerosis [123], and thiazolidinediones reduce the workload of β cells, resulting in long-term glycemic durability [124]. Alpha-glucosidase inhibitors suppress postprandial hyperglycemia, thereby contributing to a decrease in the workload of β cells after meals. In addition, alpha-glucosidase inhibitors have been reported to inhibit progression to T2DM in patients with IGT [125,126], and their early introduction may be considered.

However, the indiscriminate use of insulin secretagogues increases the risk of hypoglycemia and the burden on β cells, so it may be better to be cautious or refrain from their use. Sulfonylureas (SUs) nonphysiologically increase insulin secretion from β cells, which increases the risk of hypoglycemia and weight gain, leading to management failure [124]. Glinides stimulate insulin secretion for a short time compared with SUs, so the increased workload of β cells is expected to be less than with SUs. However, nateglinide seems to be unable to inhibit the progression of IGT to T2DM [127], indicating that the use of drugs that compel β-cell workload is not effective in controlling glucose metabolism.

On the other hand, incretin-based drugs, DPP-4 inhibitors, and GLP-1RAs are expected to improve β-cell function because they stimulate insulin secretion in a physiological manner. Intriguingly, the Vildagliptin Efficacy in combination with metfoRmIn For earlY treatment of T2DM (VERIFY) study has shown that compared with metformin monotherapy, the initial combination of a DPP-4 inhibitor and metformin is reported to be more durable in terms of controlling blood glucose level for 5 years [128]. Since GLP-1RAs promote weight loss by inhibiting gastric emptying and food intake [129], they can simultaneously reduce the workload of β cells [4], which is the major advantage of this drug class compared to DPP-4 inhibitors. With the

recently developed GLP-1RAs, weekly injections and oral agents are now available [130], making them easier to use.

Furthermore, sodium glucose cotransporter 2 inhibitors have been shown to improve β-cell function by increasing urinary glucose excretion and reducing β-cell workload to induce weight loss [131]. Both GLP-1RAs and sodium glucose cotransporter 2 inhibitors have been shown to improve cardiovascular outcomes [132-135], and the use of these agents in combination with metformin should be considered for individuals at higher cardiovascular risk.

Intensive insulin therapy in the early stages should be considered because it has been shown to maintain β-cell function thereafter [136], but using insulin only aimed at lowering blood glucose level without eliminating excess caloric intake may exacerbate ectopic lipid deposition [137], resulting in increased insulin resistance and workload of β cells. Therefore, a recent American Diabetes Association/European Association for the Study of Diabetes consensus statement recommended that use of GLP-1RAs should be considered as the first injectable drug prior to insulin [138].

Because the concept of a cure does not exist for T2DM, it is difficult to motivate patients to continue treatment over the long term and to achieve good glycemic control on an ongoing basis [4]. Since maintaining patient motivation is an essential part of the management of T2DM, it is important for healthcare providers to encourage their patients [3].

Conclusion

This review focuses mainly on the current findings on β-cell regulators and changes in BCM in patients with glucose intolerance. At present, T2DM is not a curable disease but a disorder that requires long-term control and lifetime treatment. Therefore, it is important to shift from a glucose-centric to a β-cell–centric approach to protect β cells, rather than simply focusing on lowering blood glucose level.

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Data Availability: Data sharing is not applicable to this article as no data sets were generated or analyzed during the current study.

References

1. Butler AE, Janson J, Bonner-Weir S, Ritzel R, Rizza RA, Butler PC. Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes*. 2003;**52**(1):102-110.
2. Inaishi J, Saisho Y. Beta-cell mass in obesity and type 2 diabetes, and its relation to pancreas fat: a mini-review. *Nutrients*. 2020;**12**(12):3846.
3. Saisho Y. β-cell dysfunction: Its critical role in prevention and management of type 2 diabetes. *World J Diabetes*. 2015;**6**(1):109-124.
4. Saisho Y. Changing the concept of type 2 diabetes: beta cell workload hypothesis revisited. *Endocr Metab Immune Disord Drug Targets*. 2019;**19**(2):121-127.
5. Saisho Y. An emerging new concept for the management of type 2 diabetes with a paradigm shift from the glucose-centric to beta cell-centric concept of diabetes: an Asian perspective. *Expert Opin Pharmacother*. 2020;**21**(13):1565-1578.
6. Rahier J, Guiot Y, Goebbels RM, Sempoux C, Henquin JC. Pancreatic beta-cell mass in European subjects with type 2 diabetes. *Diabetes Obes Metab*. 2008;**10**(suppl 4):32-42.
7. Federici M, Hribal M, Perego L, et al. High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. *Diabetes*. 2001;**50**(6):1290-1301.
8. Poitout V, Robertson RP. Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocr Rev*. 2008;**29**(3):351-366.
9. Robertson RP. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *J Biol Chem*. 2004;**279**(41):42351-42354.
10. Robertson RP. Antioxidant drugs for treating beta-cell oxidative stress in type 2 diabetes: glucose-centric versus insulin-centric therapy. *Discov Med*. 2010;**9**(45):132-137.
11. Benáková Š, Holendová B, Plecitá-Hlavatá L. Redox homeostasis in pancreatic β-cells: from development to failure. *Antioxidants*. 2021;**10**(4):526.
12. Supale S, Li N, Brun T, Maechler P. Mitochondrial dysfunction in pancreatic β cells. *Trends Endocrinol Metab*. 2012;**23**(9):477-487.
13. Maechler P, Jornot L, Wollheim CB. Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic beta cells. *J Biol Chem*. 1999;**274**(39):27905-27913.
14. Dinarello CA, Donath MY, Mandrup-Poulsen T. Role of IL-1beta in type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes*. 2010;**17**(4):314-321.

15. Kusminski CM, Shetty S, Orci L, Unger RH, Scherer PE. Diabetes and apoptosis: lipotoxicity. *Apoptosis*. 2009;**14**(12):1484-1495.
16. Jurgens CA, Toukatly MN, Fligner CL, et al. β-cell loss and β-cell apoptosis in human type 2 diabetes are related to islet amyloid deposition. *Am J Pathol*. 2011;**178**(6):2632-2640.
17. Janson J, Ashley RH, Harrison D, McIntyre S, Butler PC. The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles. *Diabetes*. 1999;**48**(3):491-498.
18. Jeong HR, An SS. Causative factors for formation of toxic islet amyloid polypeptide oligomer in type 2 diabetes mellitus. *Clin Interv Aging*. 2015;**10**:1873-1879.
19. Mukherjee A, Morales-Scheihing D, Butler PC, Soto C. Type 2 diabetes as a protein misfolding disease. *Trends Mol Med*. 2015;**21**(7):439-449.
20. Eizirik DL, Cardozo AK, Cnop M. The role for endoplasmic reticulum stress in diabetes mellitus. *Endocr Rev*. 2008;**29**(1):42-61.
21. Hunter CS, Stein RW. Evidence for loss in identity, de-differentiation, and trans-differentiation of islet β-cells in type 2 diabetes. *Front Genet*. 2017;**8**:35.
22. Marselli L, Suleiman M, Masini M, et al. Are we overestimating the loss of beta cells in type 2 diabetes? *Diabetologia*. 2014;**57**(2):362-365.
23. Butler AE, Dhawan S, Hoang J, et al. β-cell deficit in obese type 2 diabetes, a minor role of β-cell dedifferentiation and degranulation. *J Clin Endocrinol Metab*. 2016;**101**(2):523-532.
24. Gregg BE, Moore PC, Demozay D, et al. Formation of a human β-cell population within pancreatic islets is set early in life. *J Clin Endocrinol Metab*. 2012;**97**(9):3197-3206.
25. Dor Y, Brown J, Martinez OI, Melton DA. Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature*. 2004;**429**(6987):41-46.
26. Bonner-Weir S, Trent DF, Weir GC. Partial pancreatectomy in the rat and subsequent defect in glucose-induced insulin release. *J Clin Invest*. 1983;**71**(6):1544-1553.
27. Bonner-Weir S, Deery D, Leahy JL, Weir GC. Compensatory growth of pancreatic beta-cells in adult rats after short-term glucose infusion. *Diabetes*. 1989;**38**(1):49-53.
28. Tschen SI, Dhawan S, Gurlo T, Bhushan A. Age-dependent decline in beta-cell proliferation restricts the capacity of beta-cell regeneration in mice. *Diabetes*. 2009;**58**(6):1312-1320.
29. Abebe T, Mahadevan J, Bogachus L, et al. Nrf2/antioxidant pathway mediates β cell self-repair after damage by high-fat diet-induced oxidative stress. *JCI Insight*. 2017;**2**(24):e92854.
30. Meier JJ, Butler AE, Saisho Y, et al. Beta-cell replication is the primary mechanism subserving the postnatal expansion of beta-cell mass in humans. *Diabetes*. 2008;**57**(6):1584-1594.
31. Butler AE, Cao-Minh L, Galasso R, et al. Adaptive changes in pancreatic beta cell fractional area and beta cell turnover in human pregnancy. *Diabetologia*. 2010;**53**(10):2167-2176.
32. Saisho Y, Butler AE, Manesso E, Elashoff D, Rizza RA, Butler PC. β-cell mass and turnover in humans: effects of obesity and aging. *Diabetes Care*. 2013;**36**(1):111-117.
33. Bonner-Weir S, Li WC, Ouziel-Yahalom L, Guo L, Weir GC, Sharma A. Beta-cell growth and regeneration: replication is only part of the story. *Diabetes*. 2010;**59**(10):2340-2348.
34. Mezza T, Muscogiuri G, Sorice GP, et al. Insulin resistance alters islet morphology in nondiabetic humans. *Diabetes*. 2014;**63**(3):994-1007.
35. Saisho Y, Manesso E, Butler AE, et al. Ongoing beta-cell turnover in adult nonhuman primates is not adaptively increased in streptozotocin-induced diabetes. *Diabetes*. 2011;**60**(3):848-856.
36. Hales CN, Barker DJ, Clark PM, et al. Fetal and infant growth and impaired glucose tolerance at age 64. *BMJ*. 1991;**303**(6809):1019-1022.
37. Barker DJ, Hales CN, Fall CH, Osmond C, Phipps K, Clark PM. Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia*. 1993;**36**(1):62-67.
38. Lithell HO, McKeigue PM, Berglund L, Mohsen R, Lithell UB, Leon DA. Relation of size at birth to non-insulin dependent diabetes and insulin concentrations in men aged 50-60 years. *BMJ*. 1996;**312**(7028):406-410.
39. Curhan GC, Willett WC, Rimm EB, Spiegelman D, Ascherio AL, Stampfer MJ. Birth weight and adult hypertension, diabetes mellitus, and obesity in US men. *Circulation*. 1996;**94**(12):3246-3250.
40. Yarbrough DE, Barrett-Connor E, Kritz-Silverstein D, Wingard DL. Birth weight, adult weight, and girth as predictors of the metabolic syndrome in postmenopausal women: the Rancho Bernardo Study. *Diabetes Care*. 1998;**21**(10):1652-1658.
41. Carlsson S, Persson PG, Alvarsson M, et al. Low birth weight, family history of diabetes, and glucose intolerance in Swedish middle-aged men. *Diabetes Care*. 1999;**22**(7):1043-1047.
42. Forsén T, Eriksson J, Tuomilehto J, Reunanen A, Osmond C, Barker D. The fetal and childhood growth of persons who develop type 2 diabetes. *Ann Intern Med*. 2000;**133**(3):176-182.
43. Birgisdottir BE, Gunnarsdottir I, Thorsdottir I, Gudnason V, Benediktsson R. Size at birth and glucose intolerance in a relatively genetically homogeneous, high-birth weight population. *Am J Clin Nutr*. 2002;**76**(2):399-403.
44. Eriksson JG, Forsen TJ, Osmond C, Barker DJ. Pathways of infant and childhood growth that lead to type 2 diabetes. *Diabetes Care*. 2003;**26**(11):3006-3010.
45. Wadsworth M, Butterworth S, Marmot M, Ecob R, Hardy R. Early growth and type 2 diabetes: evidence from the 1946 British birth cohort. *Diabetologia*. 2005;**48**(12):2505-2510.
46. Gluckman PD, Hanson MA. Living with the past: evolution, development, and patterns of disease. *Science*. 2004;**305**(5691):1733-1736.
47. de Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. *Aust N Z J Obstet Gynaecol*. 2006;**46**(1):4-14.
48. Fernandez-Twinn DS, Hjort L, Novakovic B, Ozanne SE, Saffery R. Intrauterine programming of obesity and type 2 diabetes. *Diabetologia*. 2019;**62**(10):1789-1801.
49. Zhu Z, Cao F, Li X. Epigenetic programming and fetal metabolic programming. *Front Endocrinol (Lausanne)*. 2019;**10**:764.
50. Su Y, Jiang X, Li Y, et al. Maternal low protein isocaloric diet suppresses pancreatic β-cell proliferation in mouse offspring via miR-15b. *Endocrinology*. 2016;**157**(12):4782-4793.
51. Meier JJ. Linking the genetics of type 2 diabetes with low birth weight: a role for prenatal islet maldevelopment? *Diabetes*. 2009;**58**(6):1255-1256.
52. Pascoe L, Frayling TM, Weedon MN, et al; RISC Consortium. Beta cell glucose sensitivity is decreased by 39% in non-diabetic individuals carrying multiple diabetes-risk

alleles compared with those with no risk alleles. *Diabetologia*. 2008;**51**(11):1989-1992.
53. Groenewoud MJ, Dekker JM, Fritsche A, et al. Variants of CDKAL1 and IGF2BP2 affect first-phase insulin secretion during hyperglycaemic clamps. *Diabetologia*. 2008;**51**(9):1659-1663.
54. Sasaki H, Saisho Y, Inaishi J, et al. Associations of birthweight and history of childhood obesity with beta cell mass in Japanese adults. *Diabetologia*. 2020;**63**(6):1199-1210.
55. Meier JJ, Köhler CU, Alkhatib B, et al. Beta-cell development and turnover during prenatal life in humans. *Eur J Endocrinol*. 2010;**162**(3):559-568.
56. Zare M, Rastegar S, Ebrahimi E, Roohipoor A, Shirali S. Role of pancreatic duct cell in beta cell neogenesis: a mini review study. *Diabetes Metab Syndr*. 2017;**11**(suppl 1):S1-S4.
57. Mizukami H, Takahashi K, Inaba W, et al. Age-associated changes of islet endocrine cells and the effects of body mass index in Japanese. *J Diabetes Investig*. 2014;**5**(1):38-47.
58. Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B. Beta-cell proliferation and apoptosis in the developing normal human pancreas and in hyperinsulinism of infancy. *Diabetes*. 2000;**49**(8):1325-1333.
59. Saisho Y, Butler AE, Meier JJ, et al. Pancreas volumes in humans from birth to age one hundred taking into account sex, obesity, and presence of type-2 diabetes. *Clin Anat*. 2007;**20**(8):933-942.
60. Saisho Y. Pancreas volume and fat deposition in diabetes and normal physiology: consideration of the interplay between endocrine and exocrine pancreas. *Rev Diabet Stud*. 2016;**13**(2-3):132-147.
61. Henquin JC, Rahier J. Pancreatic alpha cell mass in European subjects with type 2 diabetes. *Diabetologia*. 2011;**54**(7):1720-1725.
62. Engin A. Adiponectin-resistance in obesity. *Adv Exp Med Biol*. 2017;**960**:415-441.
63. Hanley SC, Austin E, Assouline-Thomas B, et al. {beta}-Cell mass dynamics and islet cell plasticity in human type 2 diabetes. *Endocrinology*. 2010;**151**(4):1462-1472.
64. Bhargava SK, Sachdev HS, Fall CH, et al. Relation of serial changes in childhood body-mass index to impaired glucose tolerance in young adulthood. *N Engl J Med*. 2004;**350**(9):865-875.
65. Tirosh A, Shai I, Afek A, et al. Adolescent BMI trajectory and risk of diabetes versus coronary disease. *N Engl J Med*. 2011;**364**(14):1315-1325.
66. Yeung EH, Hu FB, Solomon CG, et al. Life-course weight characteristics and the risk of gestational diabetes. *Diabetologia*. 2010;**53**(4):668-678.
67. Yachi Y, Tanaka Y, Nishibata I, et al. Low BMI at age 20 years predicts gestational diabetes independent of BMI in early pregnancy in Japan: tanaka women's clinic study. *Diabet Med*. 2013;**30**(1):70-73.
68. Katanoda K, Noda M, Goto A, Mizunuma H, Lee JS, Hayashi K. Being underweight in adolescence is independently associated with adult-onset diabetes among women: the Japan Nurses' Health Study. *J Diabetes Investig*. 2019;**10**(3):827-836.
69. Kodama K, Tojjar D, Yamada S, Toda K, Patel CJ, Butte AJ. Ethnic differences in the relationship between insulin sensitivity and insulin response: a systematic review and meta-analysis. *Diabetes Care*. 2013;**36**(6):1789-1796.
70. Kou K, Saisho Y, Satoh S, Yamada T, Itoh H. Change in β-cell mass in Japanese nondiabetic obese individuals. *J Clin Endocrinol Metab*. 2013;**98**(9):3724-3730.
71. Fujita Y, Kozawa J, Iwahashi H, et al. Increment of serum C-peptide measured by glucagon test closely correlates with human relative beta-cell area. *Endocr J*. 2015;**62**(4):329-337.
72. Inaishi J, Saisho Y, Sato S, et al. Effects of obesity and diabetes on α- and β-cell mass in surgically resected human pancreas. *J Clin Endocrinol Metab*. 2016;**101**(7):2874-2882.
73. Fujita Y, Kozawa J, Iwahashi H, et al. Human pancreatic α- to β-cell area ratio increases after type 2 diabetes onset. *J Diabetes Investig*. 2018;**9**(6):1270-1282.
74. Inaishi J, Saisho Y. Ethnic similarities and differences in the relationship between beta cell mass and diabetes. *J Clin Med*. 2017;**6**(12):113.
75. Sasaki H, Saisho Y, Inaishi J, et al. Reduced beta cell number rather than size is a major contributor to beta cell loss in type 2 diabetes. *Diabetologia*. 2021;**64**(8):1816-1821.
76. Kanazawa M, Yoshiike N, Osaka T, Numba Y, Zimmet P, Inoue S. Criteria and classification of obesity in Japan and Asia-Oceania. *World Rev Nutr Diet*. 2005;**94**:1-12.
77. Mastrogiannis DS, Spiliopoulos M, Mulla W, Homko CJ. Insulin resistance: the possible link between gestational diabetes mellitus and hypertensive disorders of pregnancy. *Curr Diab Rep*. 2009;**9**(4):296-302.
78. Kampmann U, Knorr S, Fuglsang J, Ovesen P. Determinants of maternal insulin resistance during pregnancy: an updated overview. *J Diabetes Res*. 2019;**2019**:5320156.
79. Catalano PM, Tyzbir ED, Roman NM, Amini SB, Sims EA. Longitudinal changes in insulin release and insulin resistance in nonobese pregnant women. *Am J Obstet Gynecol*. 1991;**165**(6 Pt 1):1667-1672.
80. Homko C, Sivan E, Chen X, Reece EA, Boden G. Insulin secretion during and after pregnancy in patients with gestational diabetes mellitus. *J Clin Endocrinol Metab*. 2001;**86**(2):568-573.
81. Parsons JA, Brelje TC, Sorenson RL. Adaptation of islets of Langerhans to pregnancy: increased islet cell proliferation and insulin secretion correlates with the onset of placental lactogen secretion. *Endocrinology*. 1992;**130**(3):1459-1466.
82. Scaglia L, Smith FE, Bonner-Weir S. Apoptosis contributes to the involution of beta cell mass in the post partum rat pancreas. *Endocrinology*. 1995;**136**(12):5461-5468.
83. Karnik SK, Chen H, McLean GW, et al. Menin controls growth of pancreatic beta-cells in pregnant mice and promotes gestational diabetes mellitus. *Science*. 2007;**318**(5851):806-809.
84. Van Assche FA, Aerts L, De Prins F. A morphological study of the endocrine pancreas in human pregnancy. *Br J Obstet Gynaecol*. 1978;**85**(11):818-820.
85. Xu G, Stoffers DA, Habener JF, Bonner-Weir S. Exendin-4 stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rats. *Diabetes*. 1999;**48**(12):2270-2276.
86. Farilla L, Bulotta A, Hirshberg B, et al. Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology*. 2003;**144**(12):5149-5158.
87. Pospisilik JA, Martin J, Doty T, et al. Dipeptidyl peptidase IV inhibitor treatment stimulates beta-cell survival and islet neogenesis in streptozotocin-induced diabetic rats. *Diabetes*. 2003;**52**(3):741-750.

88. Butler AE, Campbell-Thompson M, Gurlo T, Dawson DW, Atkinson M, Butler PC. Marked expansion of exocrine and endocrine pancreas with incretin therapy in humans with increased exocrine pancreas dysplasia and the potential for glucagon-producing neuroendocrine tumors. *Diabetes*. 2013;**62**(7):2595-2604.
89. Matveyenko AV, Dry S, Cox HI, et al. Beneficial endocrine but adverse exocrine effects of sitagliptin in the human islet amyloid polypeptide transgenic rat model of type 2 diabetes: interactions with metformin. *Diabetes*. 2009;**58**(7):1604-1615.
90. Bonner-Weir S, In't Veld PA, Weir GC. Reanalysis of study of pancreatic effects of incretin therapy: methodological deficiencies. *Diabetes Obes Metab*. 2014;**16**(7):661-666.
91. Gotfredsen CF, Mølck AM, Thorup I, et al. The human GLP-1 analogs liraglutide and semaglutide: absence of histopathological effects on the pancreas in nonhuman primates. *Diabetes*. 2014;**63**(7):2486-2497.
92. Ueberberg S, Jütte H, Uhl W, et al. Histological changes in endocrine and exocrine pancreatic tissue from patients exposed to incretin-based therapies. *Diabetes Obes Metab*. 2016;**18**(12):1253-1262.
93. Smits MM, Tonneijck L, Muskiet MH, et al. Pancreatic effects of liraglutide or sitagliptin in overweight patients with type 2 diabetes: a 12-week randomized, placebo-controlled trial. *Diabetes Care*. 2017;**40**(3):301-308.
94. Saisho Y. Incretin-based therapy and pancreatitis: accumulating evidence and unresolved questions. *Ann Transl Med*. 2018;**6**(7):131.
95. Tanaka K, Saisho Y, Manesso E, et al; KIND-LM Study Investigators. Effects of liraglutide monotherapy on beta cell function and pancreatic enzymes compared with metformin in japanese overweight/obese patients with type 2 diabetes mellitus: a subpopulation analysis of the KIND-LM randomized trial. *Clin Drug Investig*. 2015;**35**(10):675-684.
96. Tappy L, Randin D, Vollenweider P, et al. Mechanisms of dexamethasone-induced insulin resistance in healthy humans. *J Clin Endocrinol Metab*. 1994;**79**(4):1063-1069.
97. van Raalte DH, Nofrate V, Bunck MC, et al. Acute and 2-week exposure to prednisolone impair different aspects of beta-cell function in healthy men. *Eur J Endocrinol*. 2010;**162**(4):729-735.
98. Rafacho A, Cestari TM, Taboga SR, Boschero AC, Bosqueiro JR. High doses of dexamethasone induce increased beta-cell proliferation in pancreatic rat islets. *Am J Physiol Endocrinol Metab*. 2009;**296**(4):E681-E689.
99. Rafacho A, Abrantes JL, Ribeiro DL, et al. Morphofunctional alterations in endocrine pancreas of short- and long-term dexamethasone-treated rats. *Horm Metab Res*. 2011;**43**(4):275-281.
100. Sato S, Saisho Y, Inaishi J, et al. Effects of glucocorticoid treatment on β- and α-cell mass in japanese adults with and without diabetes. *Diabetes*. 2015;**64**(8):2915-2927.
101. Pannala R, Leirness JB, Bamlet WR, Basu A, Petersen GM, Chari ST. Prevalence and clinical profile of pancreatic cancer-associated diabetes mellitus. *Gastroenterology*. 2008;**134**(4):981-987.
102. Andersen DK, Korc M, Petersen GM, et al. Diabetes, pancreatogenic diabetes, and pancreatic cancer. *Diabetes*. 2017;**66**(5):1103-1110.
103. Meier JJ, Breuer TG, Bonadonna RC, et al. Pancreatic diabetes manifests when beta cell area declines by approximately 65% in humans. *Diabetologia*. 2012;**55**(5):1346-1354.
104. Permert J, Ihse I, Jorfeldt L, von Schenck H, Arnquist HJ, Larsson J. Improved glucose metabolism after subtotal pancreatectomy for pancreatic cancer. *Br J Surg*. 1993;**80**(8):1047-1050.
105. Aggarwal G, Ramachandran V, Javeed N, et al. Adrenomedullin is up-regulated in patients with pancreatic cancer and causes insulin resistance in β cells and mice. *Gastroenterology*. 2012;**143**(6):1510-1517.e1.
106. Yoon KH, Ko SH, Cho JH, et al. Selective beta-cell loss and alpha-cell expansion in patients with type 2 diabetes mellitus in Korea. *J Clin Endocrinol Metab*. 2003;**88**(5):2300-2308.
107. Rickels MR, Bellin M, Toledo FG, et al; PancreasFest Recommendation Conference Participants. Detection, evaluation and treatment of diabetes mellitus in chronic pancreatitis: recommendations from PancreasFest 2012. *Pancreatology*. 2013;**13**(4):336-342.
108. Schrader H, Menge BA, Schneider S, et al. Reduced pancreatic volume and beta-cell area in patients with chronic pancreatitis. *Gastroenterology*. 2009;**136**(2):513-522.
109. Schrader H, Menge BA, Zeidler C, et al. Determinants of glucose control in patients with chronic pancreatitis. *Diabetologia*. 2010;**53**(6):1062-1069.
110. Yoneda S, Uno S, Iwahashi H, et al. Predominance of β-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. *J Clin Endocrinol Metab*. 2013;**98**(5):2053-2061.
111. Meier JJ, Menge BA, Breuer TG, et al. Functional assessment of pancreatic beta-cell area in humans. *Diabetes*. 2009;**58**(7):1595-1603.
112. Inaishi J, Saisho Y, Hirakawa Y, et al. Association of glucose tolerance status with pancreatic β- and α-cell mass in community-based autopsy samples of Japanese individuals: the Hisayama study. *J Diabetes Investig*. 2020;**11**(5):1197-1206.
113. Mizukami H, Takahashi K, Inaba W, et al. Involvement of oxidative stress-induced DNA damage, endoplasmic reticulum stress, and autophagy deficits in the decline of β-cell mass in Japanese type 2 diabetic patients. *Diabetes Care*. 2014;**37**(7):1966-1974.
114. Weir GC, Gaglia J, Bonner-Weir S. Inadequate beta-cell mass is essential for the pathogenesis of type 2 diabetes. *Lancet Diabetes Endocrinol*. 2020;**8**(3):249-256.
115. Knowler WC, Barrett-Connor E, Fowler SE, et al; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*. 2002;**346**(6):393-403.
116. Johansen MY, Karstoft K, MacDonald CS, et al. Effects of an intensive lifestyle intervention on the underlying mechanisms of improved glycaemic control in individuals with type 2 diabetes: a secondary analysis of a randomised clinical trial. *Diabetologia*. 2020;**63**(11):2410-2422.
117. Lean ME, Leslie WS, Barnes AC, et al. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet*. 2018;**391**(10120):541-551.
118. Al-Mrabeh A, Hollingsworth KG, Shaw JAM, et al. 2-year remission of type 2 diabetes and pancreas morphology: a post-hoc

analysis of the DiRECT open-label, cluster-randomised trial. *Lancet Diabetes Endocrinol.* 2020;**8**(12):939-948.
119. Tsilingiris D, Koliaki C, Kokkinos A. Remission of type 2 diabetes mellitus after bariatric surgery: fact or fiction? *Int J Environ Res Public Health*. 2019;**16**(17):3171.
120. Yoshino M, Kayser BD, Yoshino J, et al. Effects of diet versus gastric bypass on metabolic function in diabetes. *N Engl J Med*. 2020;**383**(8):721-732.
121. DeFronzo RA, Barzilai N, Simonson DC. Mechanism of metformin action in obese and lean noninsulin-dependent diabetic subjects. *J Clin Endocrinol Metab*. 1991;**73**(6):1294-1301.
122. Kim CH, Han KA, Oh HJ, et al. Safety, tolerability, and efficacy of metformin extended-release oral antidiabetic therapy in patients with type 2 diabetes: an observational trial in Asia. *J Diabetes*. 2012;**4**(4):395-406.
123. Dormandy JA, Charbonnel B, Eckland DJ, et al; PROactive Investigators. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet.* 2005;**366**(9493):1279-1289.
124. Kahn SE, Haffner SM, Heise MA, et al.; ADOPT Study Group. Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N Engl J Med.* 2006;**355**(23):2427-2443.
125. Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A, Laakso M; STOP-NIDDM Trail Research Group. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. *Lancet.* 2002;**359**(9323):2072-2077.
126. Kawamori R, Tajima N, Iwamoto Y, Kashiwagi A, Shimamoto K, Kaku K; Voglibose Ph-3 Study Group. Voglibose for prevention of type 2 diabetes mellitus: a randomised, double-blind trial in Japanese individuals with impaired glucose tolerance. *Lancet.* 2009;**373**(9675):1607-1614.
127. Holman RR, Haffner SM, McMurray JJ, et al. Effect of nateglinide on the incidence of diabetes and cardiovascular events. *N Engl J Med*. 2010;**362**(16):1463-1476.
128. Matthews DR, Paldánius PM, Proot P, Chiang Y, Stumvoll M, Del Prato S; VERIFY Study Group. Glycaemic durability of an early combination therapy with vildagliptin and metformin versus sequential metformin monotherapy in newly diagnosed type 2 diabetes (VERIFY): a 5-year, multicentre, randomised, double-blind trial. *Lancet.* 2019;**394**(10208):1519-1529.
129. Drucker DJ. Mechanisms of action and therapeutic application of glucagon-like peptide-1. *Cell Metab.* 2018;**27**(4):740-756.
130. Drucker DJ. Advances in oral peptide therapeutics. *Nat Rev Drug Discov.* 2020;**19**(4):277-289.
131. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J Clin Invest.* 2014;**124**(2):499-508.
132. Marso SP, Daniels GH, Brown-Frandsen K, et al; LEADER Steering Committee; LEADER Trial Investigators. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N Engl J Med.* 2016;**375**(4):311-322.
133. Marso SP, Bain SC, Consoli A, et al; SUSTAIN-6 Investigators. Semaglutide and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med*. 2016;**375**(19):1834-1844.
134. Zinman B, Wanner C, Lachin JM, et al; EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med*. 2015;**373**(22):2117-2128.
135. Neal B, Perkovic V, Mahaffey KW, et al; CANVAS Program Collaborative Group. Canagliflozin and cardiovascular and renal events in type 2 diabetes. *N Engl J Med.* 2017;**377**(7):644-657.
136. Weng J, Li Y, Xu W, et al. Effect of intensive insulin therapy on beta-cell function and glycaemic control in patients with newly diagnosed type 2 diabetes: a multicentre randomised parallel-group trial. *Lancet*. 2008;**371**(9626):1753-1760.
137. Unger RH. Reinventing type 2 diabetes: pathogenesis, treatment, and prevention. *JAMA*. 2008;**299**(10):1185-1187.
138. Buse JB, Wexler DJ, Tsapas A, et al. 2019 update to: Management of hyperglycaemia in type 2 diabetes, 2018: a consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia.* 2020;**63**(2):221-228.
139. Saisho Y. Importance of beta cell function for the treatment of type 2 diabetes. *J Clin Med*. 2014;**3**(3):923-943.
140. Saisho Y. Obesity, type 2 diabetes and β-cell failure: an Asian perspective. *J Mol Genet Med* 2014;**2014**(**suppl 1**):8.