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Reversibility of Defects in Proinsulin Processing and Islet β-Cell Failure in Obesity-Related Type 2 Diabetes

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COMMENTARY

Islet β-cell failure is mostly progressive in type 2 diabetes, resulting in the need for serial escalations in glucose-lowering therapies for many patients with this condition (1–4). This failure is a consequence of impaired β-cell function and loss of β-cell mass, with varying contributions of each likely to relate to the heterogeneity in causative factors from patient to patient (1–5). It is a commonly held view that impaired proinsulin synthesis contributes to the β-cell dysfunction aspect of β-cell failure (1,2). This can be a consequence of the endoplasmic reticulum (ER) stress response that inhibits protein synthesis (including proinsulin) and/or β-cell dedifferentiation in which the expression of the essential elements required for a mature β-cell function, including the transcription of the insulin gene, are reduced or absent (1,2).

In this issue of *Diabetes*, however, Alarcon et al. (6) convincingly show that islet β-cell proinsulin synthesis is increased rather than decreased in two obese mouse models of type 2 diabetes. They do find severe depletion in the number of mature insulin granules in the β-cells of these obese diabetic mice, but the evidence is that this results from accelerated, dysfunctional proinsulin processing and trafficking rather than the consequence of deficient proinsulin synthesis (6). Furthermore, these defects can be rapidly reversed by a short period of β-cell rest, at least in vitro (6). These findings are important, as reversibility of this form of islet β-cell dysfunction, if better understood, may lead to improved approaches for the prevention of progressive β-cell failure in affected patients with type 2 diabetes.

Alarcon et al. (6) used the C57BL/6J *db/db* and the C57BLKS/J *db/db* obese diabetic mouse models (referred to hereon as $6J^{db/db}$ and $KS^{db/db}$), the difference between them being that $KS^{db/db}$, compared with $6J^{db/db}$ mice, are less able to compensate for insulin resistance by β-cell mass expansion. At 16 weeks of age, both *db/db* strains were shown to have diabetes with fasting hyperglycemia and hyperinsulinemia. Following an intraperitoneal glucose load, however, only the $6J^{db/db}$ mice were able to mount a glucose-stimulated insulin secretory response, indicating that $6J^{db/db}$ mice have less severe islet failure than $KS^{db/db}$ mice (6). The fasting hyperinsulinemia of both these obese mouse strains, however, is consistent with them having some capacity to synthesize and release insulin, even though this is poorly regulated.

Ultrastructural analysis of the islet β-cells of both *db/db* mouse strains showed marked reduction in the number of mature insulin granules, an increased number of immature granules, and the expansion of both the Golgi apparatus and the ER (6). Also seen were some very complex multivesicular bodies (MVBs), which are elaborated on further in the Supplementary Data online (see ref. 6) using elegant tomographic electron microscopy methods. The authors speculate that the appearance of these MVBs may be a consequence of increased requirements for membrane bilayers for β-cell granule formation (6).

The proinsulin synthesis rates, as measured by the incorporation of L-[3,4,5-^{3}H]leucine into proinsulin, were markedly increased within the islets of both *db/db* mice strains compared with their respective wild-type counterparts (6). This finding, together with the reduction in mature and an increase in immature insulin granules within the β-cells of these islets, is indicative of a failure of normal proinsulin processing, including insulin granule formation and trafficking. Importantly, a failure of proinsulin synthesis could not be demonstrated. At basal glucose levels, proinsulin biosynthesis was increased 4- and 11-fold in the islets of $6J^{db/db}$ and $KS^{db/db}$ mice, respectively, relative to the islets of their respective nonobese controls (6). Additionally, proinsulin biosynthesis was shown to be glucose responsive (measured at 17 mmol/L compared

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with 3 mmol/L glucose) in islets of both obese mouse strains (6).

The ER expansion in the $6J^{db/db}$ and $KS^{db/db}$ islets did not show evidence of increased protein kinase RNA-like ER kinase (PERK) phosphorylation and CCAAT-enhancer-binding protein homologous protein (CHOP) expression. These findings are consistent with an ER adaptive rather than stress response. In fact, the observed increased proinsulin biosynthesis in these obese type 2 diabetic mouse islets make the presence of ER stress very unlikely, as ER stress is known to be an inhibitor of protein synthesis.

Truly remarkable was how fast the ultrastructural changes in the β-cells of both *db/db* obese mouse strains were reversed by culturing the islets overnight at 5.6 mmol/L glucose. With this short time frame of providing β-cell rest, reappearance of mature insulin granules, disappearance of immature granules, and regression of the Golgi and ER expansion were evident (6).

When in vitro insulin secretion was assessed using islet perifusion methodology, the major defect evident in the islets of the $6J^{db/db}$ mice was increased basal insulin release. The pattern of the first and second phases of glucose-stimulated insulin secretion, even in freshly isolated $6J^{db/db}$ islets, was surprisingly normal. The $KS^{db/db}$ mice islets again showed increased insulin release basally, but only when this was expressed as a percentage of insulin content. In the freshly isolated islets of $KS^{db/db}$ mice, there was also evidence of reduced first- and second-phase insulin secretion (6). With overnight rest, basal insulin secretion was essentially normalized in both mouse strains, and the abnormal glucose-stimulated secretion observed in the freshly isolated $KS^{db/db}$ islets was, for the most part, normalized.

Thus, the β-cells of these obese mice attempt to compensate for an increased insulin need, but they do not have the capacity to sustain normal maturation of insulin granules and their regulated release. Importantly, relief from continuous nutrient overload in vitro leads to rapid recovery of normal proinsulin processing and insulin granule dynamics (Fig. 1).

However, there are some unanswered questions: *1*) the relevance of these findings to β-cell failure in human obesity-related type 2 diabetes, *2*) if the phenomenon of dysfunctional proinsulin processing and insulin granule formation is simply a consequence of accelerated proinsulin synthesis to match secretory demand or a consequence of other "toxic" factors within the β-cell environment (e.g., fatty acids, cytokines, environmental toxins), *3*) the relative functional effectiveness of the proinsulin-enriched insulin released compared with normally released insulin, and *4*) the therapeutic approaches that could be used to improve the quality of proinsulin processing, insulin granule maturation, and physiological secretion (Fig. 1).

Interestingly, the fasting hyperinsulinemia related to human obesity, the major defect of the obese mice in the current study (6), was markedly reduced within only 6 days of bariatric surgery, consistent with short-term islet rest allowing rapid β-cell recovery (7). Also,

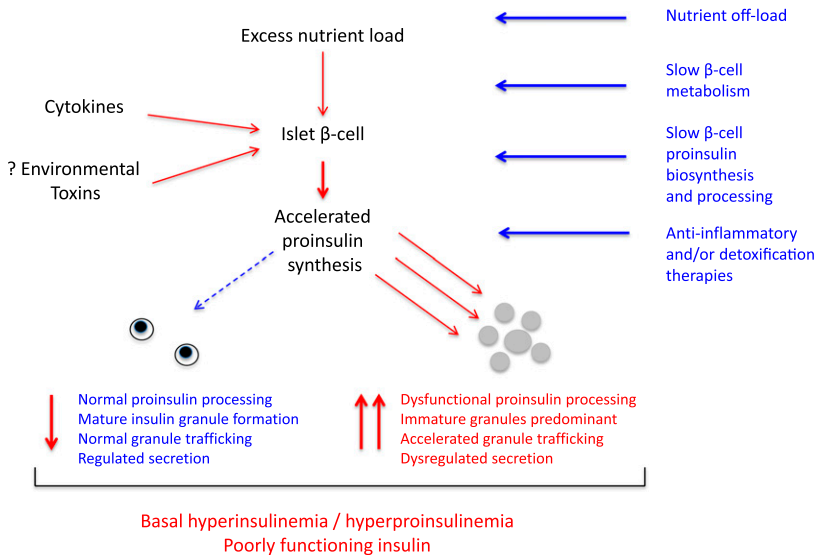

Figure 1—Islet β-cell dysfunction in obesity-related type 2 diabetes can be a consequence of accelerated proinsulin synthesis and a failure of normal proinsulin processing, insulin granule formation, granule maturation, and regulated insulin secretion. This leads to the abnormal basal release of poorly functioning insulin characterized by a high proinsulin/insulin ratio. Other factors such as cytokines and environmental toxins could contribute to the abnormal proinsulin processing. Islet β-cells with the greatest inherent predisposition to respond to excess nutrient or other stressors with insulin hypersecretion will be at greatest risk. This process is reversible if interventions are early enough. These may include therapies that off-load β-cell nutrient exposure, slow β-cell metabolism, slow proinsulin synthesis, and/or reverse inflammatory or other toxic processes within at-risk islet β-cells.

short-term pharmacologic β-cell rest in patients with type 2 diabetes is also associated with improved insulin secretory function (8,9). There is some evidence that secreted insulin from high-fat diet–induced obese C57BL/6 (HFDIO) mice islets is less functional in insulin bioassays (10), such that functional quality of secreted insulin deserves more attention. Furthermore, within the same study, treatment of the HFDIO mice with the anti-inflammatory interleukin-22 improved the degree of hyperinsulinemia, the pattern of glucose-stimulated insulin release, and the quality of the insulin released, suggesting that pharmacological interventions aimed at reducing islet β-cell inflammation may have the potential to reverse proinsulin processing defects (10). Insulin hypersecretion has been proposed as a causative factor for type 2 diabetes and obesity, as well as a risk factor for islet β-cell failure, such that therapies to prevent this may be protective (11–14).

In conclusion, the study by Alarcon et al. (6) shows that deficiencies in proinsulin processing and insulin granule formation and dynamics may be more important than deficiencies in proinsulin synthesis in the β-cell dysfunctional failure in obesity-related type 2 diabetes. Furthermore, the reversibility of these deficiencies should be a focus for the development of new approaches to prevention and treatment of this condition.

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References

1. Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. J Clin Invest 2006;116:1802–1812

2. Halban PA, Polonsky KS, Bowden DW, et al. β-Cell failure in type 2 diabetes: postulated mechanisms and prospects for prevention and treatment. Diabetes Care 2014;37:1751–1758

3. Weir GC, Bonner-Weir S. Five stages of evolving beta-cell dysfunction during progression to diabetes. Diabetes 2004;53(Suppl. 3):S16–S21

4. Leahy JL, Hirsch IB, Peterson KA, Schneider D. Targeting beta-cell function early in the course of therapy for type 2 diabetes mellitus. J Clin Endocrinol Metab 2010;95:4206–4216

5. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. The many faces of diabetes: a disease with increasing heterogeneity. Lancet 2014;383:1084–1094

6. Alarcon C, Boland BB, Uchizono Y, et al. Pancreatic β-cell adaptive plasticity in obesity increases insulin production but adversely affects secretory function. Diabetes 2016;65:438–450

7. Wickremesekera K, Miller G, Naotunne TD, Knowles G, Stubbs RS. Loss of insulin resistance after Roux-en-Y gastric bypass surgery: a time course study. Obes Surg 2005;15:474–481

8. Laedtke T, Kjems L, Pørksen N, et al. Overnight inhibition of insulin secretion restores pulsatility and proinsulin/insulin ratio in type 2 diabetes. Am J Physiol Endocrinol Metab 2000;279:E520–E528

9. Greenwood RH, Mahler RF, Hales CN. Improvement in insulin secretion in diabetes after diazoxide. Lancet 1976;1:444–447

10. Hasnain SZ, Borg DJ, Harcourt BE, et al. Glycemic control in diabetes is restored by therapeutic manipulation of cytokines that regulate beta cell stress. Nat Med 2014;20:1417–1426

11. Aston-Mourney K, Wong N, Kebede M, et al. Increased nicotinamide nucleotide transhydrogenase levels predispose to insulin hypersecretion in a mouse strain susceptible to diabetes. Diabetologia 2007;50:2476–2485

12. Lamontagne J, Jalbert-Arsenault E, Pepin E, et al. Pioglitazone acutely reduces energy metabolism and insulin secretion in rats. Diabetes 2013;62:2122–2129

13. Corkey BE. Diabetes: have we got it all wrong? Insulin hypersecretion and food additives: cause of obesity and diabetes? Diabetes Care 2012;35:2432–2437

14. Alemzadeh R, Langley G, Upchurch L, Smith P, Slonim AE. Beneficial effect of diazoxide in obese hyperinsulinemic adults. J Clin Endocrinol Metab 1998;83:1911–1915