JULY 2020

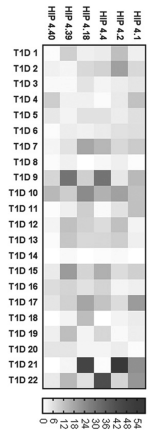
diabetes®

In This Issue of *Diabetes*

By Max Bingham, PhD

Hybrid Insulin Peptides Present in Type 1 Diabetes Might Explain Loss of Insulin

T cells that are reactive to hybrid insulin peptides (HIPs) can be detected at much higher rates in peripheral blood of individuals with type 1 diabetes, according to Arribas-Layton et al. (**p. 1492**). Specifically, they report a new class of HIPs associated with the HLA-DRB1*04:01 allele, which is part of the high-risk type 1 diabetes–associated DR4/DQ8 haplotype, and that they may activate pathogenic $CD4^+$ T cells, potentially explaining loss of insulin secretion from pancreatic β-cells. Their approach involved bioinformatics screening of a library of theoretical HIP sequences derived from insulin or other islet-derived protein fragments to identify potential peptides for further screening in vitro. After additional confirmatory steps, the authors then selected six HIPs to take further in the investigation. They found that T cells that recognize these HIPs are present in the peripheral blood of some individuals with type 1 diabetes, although there was considerable diversity present. For example, some had high frequencies of T cells for multiple HIPs, whereas others had very few HIP-reactive T cells. Age was reportedly the only characteristic that significantly correlated with the combined frequency of HIP-reactive cells. Further investigations showed that the $CD4^+$ T cells that recognize the HIPs also exhibited an effector memory phenotype and reacted to islet preparations. The authors also investigated the presence of HIP-reactive T cells in control subjects, and while they did detect the presence of some HIP-reactive $CD4^+$ T cells, these were present at significantly lower levels than in individuals with type 1 diabetes. Commenting more widely, author Eddie A. James told *Diabetes*: "Discovery of this new class of HIP-reactive T cells is an important confirmation that nongenetically encoded peptides play a role in breaking tolerance. It is our hope that ongoing studies will reveal how HIPs are formed in β-cells and why they elicit stronger T-cell responses in some individuals, inspiring strategies for restoring self-tolerance."



Heat map analysis of different HIP-reactive T-cell (horizonal axis) frequencies (shades of gray), indicating patterns of reactivity differing between subjects with type 1 diabetes (vertical axis).

Arribas-Layton et al. Hybrid insulin peptides are recognized by human T cells in the context of DRB*04:01. Diabetes 2020;69:1492–1502

NOX4-Generated H_2O_2 Is Required for Glucose-Stimulated Insulin Secretion

Elevated ATP in pancreatic β-cells does not fully explain glucose-stimulated insulin secretion (GSIS), according to Plecitá-Hlavatá et al. (**p. 1341**). Rather, parallel redox signaling in the form of H_2O_2 is required and it originates from the enzyme NADPH oxidase 4 (NOX4), according to the authors. They suggest that on top of elevated ATP, H_2O_2 is required to close the K_{ATP} channel and induce glucose-stimulated insulin exocytosis. Based on the findings, the authors propose that GSIS should now be viewed as having a dual signaling mechanism, calling into question current thinking on the mechanisms involved in impaired insulin secretion in diabetes and, for that matter, the thinking behind some of the strategies to treat type 2 diabetes. They found that when NOX4 was silenced in rat insulinoma (INS-1E) cells, GSIS rates fell away even when cells were preincubated with glucose. They also observed similar falls in rates when cells were incubated with various relevant pathway inhibitors and could even restore insulin secretion in silenced cells via chemical closure of the K_{ATP} channel. Moving to various NOX4 knockout mouse models and also pancreatic islets isolated from each model, they could largely recapitulate all the effects seen in the cell experiments, concluding that NOX4-mediated H_2O_2 is an essential comediator of GSIS in parallel with ATP and must be elevated for GSIS to occur. Further experiments then went on to explore the role of the K_{ATP} channel with patch clamp recordings, the calcium channel, the effects of sole stimulation with H_2O_2, and a possible role for mitochondrial sources of ATP and H_2O_2. As well as discussing the findings more widely, the authors go on to speculate about the possible consequences for diabetes treatments and disease progression. Commenting more widely, author Petr Ježek told us: "The next challenge will be to understand relationships between the repetitive redox signaling and maintenance of insulin gene expression and between these physiological pro-oxidative states and chronic oxidative state coming from early type 2 diabetes development."

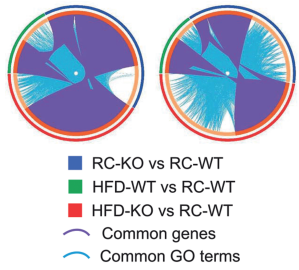
Plecitá-Hlavatá et al. Glucose-stimulated insulin secretion fundamentally requires H_2O_2 signaling by NADPH oxidase 4. Diabetes 2020;69:1341–1354

JULY 2020

diabetes®

β-Cell Failure Linked to Sustained Intracellular Ca^{2+} Increases and Overnutrition

The multiple factors involved in β-cell failure are explored by Osipovich et al. (**p. 1476**), with the authors suggesting that a sustained increase in intracellular Ca^{2+} augments overnutrition to cause β-cell failure. They identify many genes and pathways in β-cells that are affected by overnutrition, concluding that their failure is likely not due to the disruption of any single cellular process. Rather, the process appears to be rather more complex and additive in which Ca^{2+} signaling likely plays a role. To come to the conclusions, the authors used the *Abcc8* knockout mouse model (and controls) to look at the additive effects of sustained intracellular Ca^{2+} concentration (termed excitotoxicity in the article) and a high-fat diet on pancreatic islet function and gene expression of purified β-cells. They found that sustained intracellular Ca^{2+} concentrations in the knockout mice led to β-cells being more susceptible to impaired glucose metabolism due to a high-fat diet. Moreover, the effects were mitigated by verapamil, which is Ca^{2+} channel blocker, suggesting that a lowering of intracellular Ca^{2+} concentrations may attenuate β-cell failure. Meanwhile, individual effects of overnutrition and sustained intracellular Ca^{2+} concentrations and the combination of the two stresses all resulted in changes in β-cell transcription of which some overlapped. According to the authors, these included increases in activity of genes associated with mitochondrial energy metabolism, fatty acid β-oxidation, and mitochondrial biogenesis, among many others. Commenting further, author Mark A. Magnuson told us: "It has long been known that Ca^{2+} plays an essential role in insulin secretion. Our studies now demonstrate that an increase in intracellular Ca^{2+}, likely brought on by metabolic stress, is a critical determinant of β-cell failure. It will be important to determine how changes in intracellular Ca^{2+} affect gene expression in β-cells and to use systems biology approaches to understand how β-cells fail in type 2 diabetes."

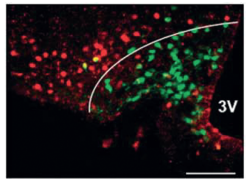


Cord diagrams showing genes (purple curves) and GO terms/pathways (blue curves) shared among lists of upregulated genes (URGs) and downregulated genes (DRGs) from three comparisons. Excitotoxicity (blue, RC-KO vs. RC-WT comparison), overnutrition (green, HFD-WT vs. RC-WT comparison), excitotoxicity+overnutrition (green, HFD-KO vs. RC-WT comparison).

Osipovich et al. Excitotoxicity and overnutrition additively impair metabolic function and identity of pancreatic β-cells. Diabetes 2020;69:1476–1491

α-Klotho Involvement in Central Regulation of Metabolism: Mechanisms and Therapeutic Options

Landry et al. (**p. 1368**) explore the role of central α-klotho in metabolic disease states, demonstrating that the antiaging protein likely does have a hormonal role as an antagonist of NPY/AgRP neurons. Specifically, the protein also appears to centrally regulate whole-body metabolism via a signaling axis that also involves fibroblast growth factor receptor (FGFR) and PI3 kinase. As well as providing novel insights into the pathophysiology of metabolic disease, they also suggest that it might help identify novel regulators that could (eventually) lead to therapeutic tools for metabolic diseases. Using mouse models of type 1 or 2 diabetes, they found that central administration of α-klotho suppressed food intake, improved glucose and insulin profiles, and reduced body weight compared with controls. Central inhibition of α-klotho meanwhile impaired glucose tolerance, and the authors suggest that this, along with other indicators, means there is a distinct glucoregulatory role for α-klotho that is independent of body weight and food intake. They also found that in ex vivo electrophysiology and immunohistochemical analyses, α-klotho appears to suppress NPY/AgRP neuron activity, at least in part by increasing receptor-mediated inhibitory signals. Moving to hypothalamic GT1-7 cell experiments, they found that treatment with α-klotho resulted in phosphorylation of various signaling and transcription factors and blunted gene expression of AgRP. Looking further into mechanisms in the mouse models, they found that inhibition of FGFR1 abolished the therapeutic effects of α-klotho in terms of body weight and food intake. Separately, inhibition of PI3 kinase abolished the ability of α-klotho to reduce food intake and improve glucose metabolism. From this, they conclude there is a novel axis involving α-klotho/FGFR and PI3 kinase involved in central regulation of metabolism.



Representative image of colocalization of cFOS (red) with NPY/AgRP neurons (green).

Landry et al. Central α-klotho suppresses NPY/AgRP neuron activity and regulates metabolism in mice. Diabetes 2020;69:1368–1381

https://doi.org/10.2337/db20-ti07