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Glucosamine-mTORC1 signalling contributes to beta cell dysfunction during glucotoxicity

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Background and aims: Hyperglycemia accelerates β-cell dysfunction in diabetes by activating mTORC1 and promoting ER stress, though the upstream signals remain unclear. We studied glucosamine (GlcN), a glucose-derived amino sugar, in β-cell glucotoxicity.
Materials and methods: We treated diabetic Akita mice with dapagliflozin or insulin and incubated human islets at 5.5 mM glucose (LG) or 22.2 mM glucose (HG) followed by metabolic labeling with $^{13}C_6$-glucose, metabolomics and metabolic fluxes analyses by LCMS. We studied the effects of glucosamine on β-cell function (GSIS and Seahorse), mTORC1 activity (Western blotting and immunostaining for pS6 and 4EBP1), ER stress (BiP immunostaining), and RNA-seq. We generated inducible β-cell-specific *Raptor* KO Akita mice to test the effects of inhibiting mTORC1 on diabetes, β-cell function and stress in vivo.
Results: In diabetic mice, GlcN levels in tissues and plasma were elevated 2-3 fold and strongly correlated with blood glucose (r = 0.86-0.98, n = 6). Treatment with dapagliflozin or insulin significantly reduced GlcN levels. In human subjects with impaired fasting glucose or T2D, the glucosamine metabolite GlcNAc-1P was significantly increased (log2 fold change = 0.47 ± 0.11 and 0.48 ± 0.13, respectively, $p < 0.01$, n = 20) and correlated with fasting glucose, HbA1c, and reduced β-cell function (HOMA-B). In human islets, exposure to HG increased GlcN levels ~6-fold. $^{13}C_6$-glucose tracing showed that glucosamine is de novo generated by glucose amination. GlcN treatment activated mTORC1, evident by increased lysosomal localization of mTOR (fold increase of Pearson's coefficient = 1.14 ± 0.075 in GlcN treatment vs control, $p < 0.05$, n = 10) and phosphorylation of its downstream targets 4EBP1 and S6. Prolonged islets incubation at HG upregulated the expression of key enzymes of the hexosamine pathway, including *Gfat1* (1.65 ± 0.07, $p < 0.05$), *Pgm3* (1.73 ± 0.14, $p < 0.05$), and *Oga* (1.42 ± 0.12, $p = 0.075$). GlcN treatment significantly increased mitochondrial respiration (basal OCR= 0.1407 vs. 0.2335, Δ = 0.092 ± 0.025, $p < 0.05$, n = 3) and GSIS (Δ = 6.634 ± 1.736, n = 14-16), indicating increased β-cell work; this was preventable by low-dose (10 nM) rapamycin, suggesting that the GlcN effects on β-cell work are mediated via mTORC1. RNA-seq on islets treated with GlcN revealed enrichment of genes involved in oxidative stress, ER stress, and mTORC1 signaling. Genetic mTORC1 inhibition in Akita β-cells (β*Raptor* AkitaKO) improved glucose tolerance, increased insulin content and decreased the expression of BiP. Treatment of diabetic Akita mice with the SGLT2 inhibitor dapagliflozin, followed by drug washout, showed that dapagliflozin inhibited mTORC1 activity, along with improvement of diabetes (IPGTT), decreased ER stress (BiP) and islet inflammation.
Conclusion: Glucosamine is elevated in diabetes and induces sustained β-cell mTORC1 activation, promoting ER stress and dysfunction. Targeting the GlcN-mTORC1 axis may preserve β-cell function and slow diabetes progression.
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Pyruvate dehydrogenase kinase 4 inhibition or deletion in alpha cells enhances glucagon secretion at low glucose in mouse and human islets and improves glucose tolerance in diet-induced obese male mice

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Background and aims: The mechanisms of control of glucagon secretion are still largely unknown. In particular, it is unclear why glucose inhibits glucagon secretion while fatty acids stimulate it. These divergent effects may result from an intrinsic property of α-cells, potentially linked to their high expression of pyruvate dehydrogenase kinase 4 (PDK4). PDK1-4 phosphorylate and inactivate pyruvate dehydrogenase, which converts pyruvate into acetyl-CoA. This study aims to investigate the role of PDK4 in α-cells in the control of glucagon secretion and glucose homeostasis.
Materials and methods: The experiments were performed on islets or mice with (*αPdk4KO*) or without (*αPdk4WT=control*) deletion of *Pdk4* specifically in α-cells and on human islets (IIDP). Islets were incubated for 1h in a medium with 5mM arginine, various glucose (G) concentrations (1 mM (G1) or 10 mM (G10), and, when indicated, with dichloroacetate (DCA, at 0.5 and 1mM), a PDK inhibitor. Insulin tolerance tests (ITT) and intraperitoneal glucose tolerance tests (IPGTT) were performed on mice fasted for 4h. For ITT, due to sex-specific differences in insulin sensitivity, females received 0.7 U/kg and males 0.85 U/kg insulin (Actrapid). For IPGTT, 2g/kg glucose was injected intraperitoneally into mice fed a control diet (CTL) or a high fat diet (HFD; 60% of calories from fat for 16 weeks).
Results: As expected, glucagon secretion was stimulated at low glucose (G1) compared to high glucose (G10). Interestingly, G1 stimulated more potently glucagon secretion in *αPdk4KO* than in *αPdk4WT* islets (by 70%; p<0.05). The addition of DCA (0.5mM for *αPdk4WT* islets, 1mM for human islets) increased glucagon secretion in response to G1 (by 24%, p<0.05 in mouse islet and by 65%, p<0.01 in human islets). No differences in glucagon and in insulin secretion were observed at G10 between islets expressing or not *Pdk4*. In CTL diet mice, glycemia after 4h of fasting was similar in *αPdk4WT* and *αPdk4KO* males or females. However, ITT revealed a reduced insulin-induced drop in glycemia in *αPdk4KO* mice compared to *αPdk4WT* mice of both sexes (glycemia of 46.2mg/dl in *αPdk4WT* vs 76.8mg/dl in *αPdk4KO* females, p<0.05; and of 60.5mg/dl in *αPdk4WT* vs 90mg/dl in *αPdk4*KO males; p<0.05 at T=90 min). Glucagon levels 30 min after insulin injection were significantly higher in *αPdk4KO* than in *αPdk4WT* female mice (by 53%; p<0.01). IPGTT revealed no significant differences in glucose tolerance between *αPdk4WT* and *αPdk4KO* mice within the same sex. In HFD mice, glycemia after 4h of fasting or after insulin injection during ITT were similar in *αPdk4WT* and *αPdk4KO* males or females. IPGTT confirmed glucose intolerance in HFD mice compared to CTL diet mice. Notably, this glucose intolerance was less severe in *αPdk4KO* males (glycemia of 327mg/dl in *αPdk4WT* vs 239mg/dl in *αPdk4KO*, *p<0.01* after T=120min). However, no significant difference in glucose tolerance was seen between *αPdk4WT* and *αPdk4KO* female mice.
Conclusion: These results demonstrate that deletion or inhibition of PDK4 in α-cells improves glucagon secretion in response to low glucose (G1) *in vitro* in mouse and human islets. This echoes the reduced *in vivo* hypoglycemic excursion in response to insulin in CTL diet mice. In addition, α-cell PDK4 deletion improves glucose tolerance in diet-induced obese male mice.
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