The role of an endogenous regulator of calcineurin in the regulation of glucose homeostasis

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We have studied the role in glucose homeostasis of a protein which acts as an endogenous inhibitor of the phosphatase calcineurin (CaN), which is important in the regulation of transcription and in protein phosphorylation associated with cell signalling. Treatment of post-operative transplant patients with immunosuppressants such as cyclosporin A and FK 506, which are CaN inhibitors, can induce diabetes (Weir & Fink, 1999). This occurs due to the inhibition of the CaN/NFAT transcription pathway which regulates pancreatic β-cell function including growth, proliferation and insulin secretion (Heit *et al.*, 2006). We are investigating the effect of increased expression of this protein and its role in the pathogenesis of diabetes. Transgenic mice were generated to overexpress this gene using human cDNA. Mouse pancreatic islets were isolated from dead mice by perfusion of the pancreas with *Collagenase P* following ligation of the bile duct at the entrance to the duodenum. mRNA expression levels of genes of interest were examined using quantitative real-time RT-PCR. Pancreatic islets were exposed *in vitro* to 16.7 mM glucose for 6 days and expression of our gene was found to increase 2.5 fold ($p < 0.05$). Using pancreatic islets from our transgenic mice, we are currently investigating the expression of genes regulated by CaN in β-cells such as those mutated in hereditary forms of monogenic type 2 diabetes (MODY) and other genes important in β-cell survival, proliferation and insulin production. Using an ACCU-CHEK® Performa glucometer we find that our transgenic mice develop age-dependent diabetes characterized by increased fasting blood glucose values of 5.8 ± 0.34 mmol/L (n = 9) at 60 days old compared to 4.2 ± 0.21 mmol/L (n = 9) in age and sex-matched wild-type mice ($p < 0.05$). Differences in fasting blood glucose values progressively increase with age, and body weight analysis confirms that these changes are not due to differences in body weight between the two genotypes. Glucose tolerance, measured by injecting 2 mg glucose/g body weight, is also reduced in our transgenic mice, with glucose values reaching peak levels of 27.5 ± 1.44 mmol/L (n = 5) after 60 minutes compared to 19 ± 1.27 mmol/L (n = 5) in wild-type mice ($p < 0.01$). This is not due to increased insulin resistance in our transgenic mice. These findings highlight a novel role of this gene in regulating glucose homeostasis, and its upregulation in hyperglycemia many be a causative link to the β-cell failure and hypoinsulinemia that occurs in the later stages of type 2 diabetes.

Heit J, Aapelqvist A, Gu X., Winslow M, Neilson J, Crabtree G & Kim SK. (2006) *Nature*, **443:** 345-9.
Weir M & Fink J. (1999) *American Journal of Kidney Diseases*, **34:** 1-13.