

# The Role of PPAR-delta in the adaptation of $\beta$ -cells to high glucose levels

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**Introduction:** Pancreatic  $\beta$ -cells are programmed to adapt to elevated levels of glucose by increasing their insulin secretory function. The molecular mechanisms of this process are not fully understood. Peroxisome proliferator-activated receptor-delta (PPAR-delta), which has been shown to play essential roles in regulation of lipid and carbohydrate metabolism, has also been found to function as a lipid sensor in  $\beta$ -cells. Recently, we have found that high glucose levels activate PPAR-delta in vascular cells. Current reports have indicated that 4-hydroxyalkenals (the products of peroxidation of poly-unsaturated fatty acids), such as 4-hydroxynonenal (4-HNE), serve as endogenous ligands of PPAR-delta. Therefore, we asked whether the adaptation of  $\beta$ -cells to hyperglycemia is mediated by PPAR-delta and what could be the endogenous ligand of this nuclear receptor.

**Patients/ Methods:** The PPAR-delta agonist GW501515, its antagonist GSK0660 and 4-HNE were used in the INS-1E  $\beta$ -cell line and in isolated rat islets to study the role of PPAR-delta in modulating insulin secretion at varying glucose concentrations. Silencing of PPAR-delta in INS-1E cells and a luciferase reporter assay were employed to clarify the role of PPAR-delta activation in insulin secretion in  $\beta$ -cells.

**Results:** Increasing concentrations of glucose enhanced insulin secretory capacity of isolated rat islets and INS-1E cells. This effect was blocked in the presence of the selective PPAR-delta antagonist GSK0660. Both GW501516 and 4-HNE mimicked the effect of high glucose and increased insulin secretion, whereas GSK0660 blocked their effect. Using HPLC analysis we found that the capacity of INS-1E cells to generate 4-HNE was augmented significantly under high glucose conditions. The luciferase reporter assay in INS-1E cells revealed that high glucose levels, GW501516 and 4-HNE activated PPAR-delta, whereas the antagonist GSK0660 abolished this trans-activation effect. Silencing of PPAR-delta in INS-1E cells confirmed these results.

**Conclusions:** This study demonstrates that 4-HNE-dependent activation of PPAR-delta mediates the adaptive increase in insulin secretory capacity of  $\beta$ -cells exposed to high glucose levels.

## Dicer is essential for mice islet cells survival

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**Introduction:** microRNAs (miRNAs) are a subset of endogenous small RNA molecules that repress gene expression post-transcriptionally, by mediating mRNA degradation or translational repression. Increasing evidence suggests aberrant miRNA function in human diseases, but despite rapid progress in the miRNA research field, little is known about their role in glucose metabolism and diabetes.

**Patients/ Methods:** We generated a transgenic mouse model in which Dicer, a key enzyme in the biogenesis of miRNA, is ablated from all the endocrine cells of the pancreas using the Cre-Lox system for site-specific recombination. Consequently, these cells lose all miRNA in the cells of the islets of Langerhans at developmental stages (E14.5 the latest).

**Results:** As a result from the Dicer deletion, the endocrine cells of the pancreas die progressively, resulting in virtually complete loss of all insulin and glucagon. As insulin was undetectable in the serum of adult mutant mice (EIA kit), we conducted whole pancreas insulin content assay. Strikingly, insulin content which was not significantly different at birth was reduced by four orders of magnitude at 12 weeks of age. These exceptional results were supported by immunohistochemical examination of pancreata showing no detectable insulin or glucagon positive cells in adult mice. Not surprisingly, the extreme hypo-insulinemia was accompanied by severe hyperglycemia. Between birth and weaning the mutant mice experienced a marked deterioration in glycemic control. While at birth no differences in blood glucose levels were found between control and mutant mice, after weaning all the mutant pups displayed extreme hyperglycemia (>600mg/dl) which persisted through out their lives. Despite severe hypo-insulinemia, and in contrast to high-dose streptozotocin treated mice, the mutant mice did not display high blood ketone levels.

**Conclusions:** Challenging previously published reports, we demonstrated that deletion of Dicer in the endocrine pancreas at developmental stages (E14.5 the latest) does not prevent normal differentiation and function, but eventually results in programmed cell death, starting at the neonatal period. These findings are consistent with other developmental Dicer deletions models. Despite the complete loss of glycemic control, the mutant mice reach adult age. To the best of our knowledge, this is the first diabetes mouse model to survive untreated with constant severe hyperglycemia and virtually complete insulin depletion. This might be in part because mutant mice are spared from the lethal diabetic ketoacidosis (DKA), typical of other diabetes type 1 models. We suggest this might be due to the protective concomitant loss of glucagon, since it has been shown to play a critical role in the manifestation of DKA.

# Expression and regulation of CRF receptor type 2 in the developing and mature mouse skeletal muscle

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**Introduction:** The corticotropin-releasing factor (CRF) and its family of ligands, including urocortin (Ucn)1, Ucn2 and Ucn3 and their cognate receptors, are involved in the maintenance and the adaptive responses necessary for energy homeostasis regulation. Studies focusing on the role of Ucn2 and Ucn3 signaling through their specific receptor, the CRF receptor type 2 (CRFR2), demonstrated diverse effects of CRFR2 signaling on energy homeostasis. Skeletal muscle (SM) tissue has been demonstrated to express high levels of the beta ( $\beta$ ) alternative splice form of the CRFR2 gene (CRFR2 $\beta$ ). CRFR2-null mice have enhanced glucose tolerance, increased insulin sensitivity and are protected from high-fat diet (HFD) induced insulin resistance. SM CRFR2 $\beta$  functions to inhibit interactions between insulin signaling pathway components by inhibiting insulin-induced Akt and ERK1/2 phosphorylation. However, little is known regarding SM CRFR2 regulation. To this end, we studied the regulation of CRFR2 expression during SM differentiation and examined the effect of different stressors on CRFR2 expression in the adult mouse

**Patients/ Methods:** We used RNase protection assays, RT-PCR and DNA sequencing in order to study CRFRs expression in SM tissue. The regulation of the CRFRs during the skeletal myogenic differentiation was determined using in vitro differentiation of the C2C12 cells. Luciferase reporter assays were used to study the activity of the CRFRs promoters during differentiation and to learn the involvement of putative muscle-specific transcription factors in CRFR2 promoter regulation. RNA extracted from SM of mice exposed to chronic variable stress (CVS) protocol or kept on HFD was used for determining CRFR2 and RBP4 expression

**Results:** We demonstrate a differential regulation of CRFR1 and CRFR2 mRNA expression, promoter activity and receptor functionality during the C2C12 myogenic differentiation. While C2C12 myoblasts exclusively expresses CRFR1, the C2C12 myotubes solely express CRFR2. In addition, using a site-specific mutagenesis we demonstrate the importance of the MEF2 consensus sequence, located at the 3' proximal region of CRFR2 promoter, to CRFR2 transcription. We further show that HFD and CVS challenges significantly increase the expression level of SM CRFR2 and RBP4, an adipokine whose serum levels are elevated in insulin resistant states

**Conclusions:** Our results are first to demonstrate the differential regulation of CRF receptors during SM differentiation and further contribute to our understanding of CRFR2 molecular and physiological regulatory mechanisms. Combining this new set of the data with our previously published findings we suggest that RBP4 and Ucn2 endogenously expressed by SM may synergistically act in an autocrine fashion to inhibit insulin signaling. A better understanding of SM CRFR2 pathways, its physiological roles and its regulation may pave the way for future management of type-2 diabetes and obesity

# Differential expression of novel adiponectin receptor-1 transcripts in skeletal muscle of normoglycemic and type 2 diabetic patients

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**Introduction:** Adiponectin receptor 1 (AdipoR1) expression in human skeletal muscle has been suggested to play an important role in insulin resistance and diabetes. We aimed at evaluating the potential existence of novel AdipoR1 splice variants in human muscle and their regulation under physiological and pathophysiological states.

**Patients/ Methods:** The presence of 5'UTR mRNA transcripts of AdipoR1, predicted from bioinformatics data, was evaluated in fetal and adult human tissues. The expression and function of the identified transcripts was assessed in cultured human skeletal muscle cells and in muscle biopsies obtained from normoglycemic and type 2 diabetic patients (n=49).

**Results:** Screening of potential AdipoR1 5'UTR splice variants revealed a novel highly abundant skeletal muscle transcript (R1T3), in addition to the previously described transcript (R1T1). Unlike R1T1, R1T3 expression significantly increased during fetal development and during myoblast-myotube differentiation. R1T3 silencing was associated with a profound reduction in AdipoR1 receptor expression in human muscle cells. Type 2 diabetes resulted with a 4-fold and a 2.2-fold reduction in R1T3 and R1T1 expression, respectively, in human muscles as compared with normoglycemic subjects, paralleled with decreased expression of the differentiation marker myogenin. R1T1 and R1T3 levels, as well as R1T3/R1T1 ratio, were found to be directly correlated with the degree of whole body insulin sensitivity.

**Conclusions:** The finding of a novel muscle-specific 5'UTR splice variant suggests that AdipoR1 receptor expression in human skeletal muscle, as opposed to other tissues, may be subjected to posttranscriptional regulation during development and differentiation, a process that may be attenuated in insulin resistance and type 2 diabetes.

# **p38 mitogen-activated protein kinase dependent transactivation of ErbB receptor family – A novel common mechanism for stress-induced IRS-1 serine phosphorylation and insulin resistance**

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**Introduction:** Stress stimuli such as TNF have been shown to induce IRS-1 serine phosphorylation and insulin resistance, by transactivation of the ErbB receptors. We aimed at elucidating the potential role of p38MAPK in mediating stress-induced ErbB receptors activation.

**Patients/ Methods:** Fao, HepG2 cells and ob/ob or C57/BL6 mice were used.

**Results:** High-fat diet fed mice and ob/ob mice exhibited elevated hepatic p38MAPK activation associated with glucose intolerance and hyperinsulinemia. Liver expression of dominant-negative p38MAPK $\alpha$  (DN-p38MAPK $\alpha$ ) in ob/ob mice reduced fasting insulin levels and improved glucose tolerance, whereas overexpressing a constitutively-active p38MAPK activator, MKK3, in C57/BL6 mice induced glucose intolerance and hyperinsulinemia. Moreover, C57/BL6 mice overexpressing wild-type p38MAPK $\alpha$  exhibited reduced insulin-stimulated IRS-1 tyrosine phosphorylation. Fao or HepG2 cells exposed to TNF, anisomycin or sphingomyelinase demonstrated rapid transactivation of the ErbB receptors leading to PI3-kinase/Akt activation, and IRS-1 serine phosphorylation. p38MAPK inhibition either by SB203580, by siRNA or by DN-p38MAPK $\alpha$  decreased ErbB receptors transactivation and IRS-1 serine phosphorylation and restored insulin stimulated IRS-1 tyrosine phosphorylation. When incubating cells with specific ErbB receptors antagonists or utilizing cells lacking ErbB receptors, anisomycin- and TNF-induced IRS-1 serine phosphorylation was attenuated, despite intact p38MAPK activation. The stress-induced p38MAPK activation leading to ErbB receptors transactivation was associated with intracellular reactive-oxygen species (ROS) generation and was completely prevented by treatment with antioxidants.

**Conclusions:** Hepatic p38MAPK is activated following various stress stimuli in a ROS dependent manner. This event is upstream to ErbB receptors transactivation and essential for stress-induced IRS-1 serine phosphorylation and insulin resistance.

# Nitric oxide synthase protects the pancreatic beta-cell from glucolipotoxicity-induced endoplasmic reticulum stress and apoptosis

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**Introduction:** Cytokines stimulate nitric oxide production leading to endoplasmic reticulum (ER) stress and apoptosis. Treatment of pancreatic beta-cells with glucose and free fatty acids induces nitric oxide synthase (NOS) and ER stress. However, the role of NO in glucolipotoxicity-induced ER stress in beta-cells is not clear.

**Patients/ Methods:** We studied the effect of high glucose and palmitate on the expression of NOS isoforms in INS-1E beta-cells, and rat and P. obesus islets. The effects of nNOS knockdown and NOS inhibition by NG-nitro-L-arginine methyl ester (L-NAME) on beta-cell function, ER stress and apoptosis under conditions of glucolipotoxicity were investigated.

**Results:** Overnight incubation of rat and P. obesus islets at 22.2 mmol/l glucose with 0.5 mmol/l palmitate induced the expression of nNOS, but not iNOS, contrasting the robust stimulation of iNOS by cytokines. NOS inhibition by L-NAME did not prevent the attenuation of glucose-stimulated insulin secretion and proinsulin biosynthesis or the depletion of islet insulin content observed under conditions of glucolipotoxicity. Moreover, treatment of beta-cells with palmitate and L-NAME together resulted in marked activation of the IRE1 $\alpha$  and PERK pathways of the unfolded protein response (UPR), leading to apoptosis. Similarly, nNOS knockdown increased CHOP expression, JNK phosphorylation and caspase 3 cleavage in beta-cells exposed to high glucose and palmitate. Treatment of INS-1E cells with the JNK inhibitor SP600125 decreased beta-cell apoptosis induced by palmitate and L-NAME.

**Conclusions:** In beta-cells subjected to glucolipotoxic conditions, genetic and pharmacological inhibition of nNOS exacerbates ER stress and activates JNK. Therefore, induction of nNOS is an adaptive response to glucolipotoxicity, protecting beta-cells from ER stress and apoptosis.

## **The NFkB paradox: suppression of GLUT4 gene expression with concomitant enhancement of GLUT4 translocation**

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**Introduction:** The link between inflammation, insulin resistance and type 2 diabetes mellitus (DM2) is now well-established. While NFkB activity is enhanced in DM2, its role in regulating glucose transporter 4 (GLUT4) gene expression and function is yet unclear.

**Results:** GLUT4 regulation at transcriptional level was studied in vitro in differentiated L6 culture using co-transfection assays. Transient co-transfection GLUT4 promoter (GLUT4-P), with NFkB subunits (p65, p50 or both), dose-dependently suppressed GLUT4-P activity to 10%, 40% and 20% of basal levels, respectively. Glut4 function was assessed in Glut4-Myc L6 myoblasts. Compared to mock transfection in the basal state p65 transfection increased Glut4 translocation to the plasma membrane by 2 fold similar to insulin effect. Adding insulin to these cells further enhanced the translocation by 50%. While Glut4 translocation was unaffected by p50 transfection, equimolar amounts of both subunits increased translocation up to 300%, compared to basal, while insulin had no further effect. Over expression of NFkB subunits also increased 14-3-3 gene expression, while silencing significantly reduced 14-3-3 cellular levels. Further, p65 transfection enhanced 14-3-3 and AS160 protein – protein interaction a critical step for Glut4 translocation.

**Conclusions:** Thus, while NFkB represses GLUT4 promoter activity, it also upregulates Glut4 translocation machinery by enhancing 14-3-3 and AS160 interaction. These NFkB modes of action are potential targets for type 2 diabetes therapy.