

# Intracellular free fatty acid upholds $\beta$ -cell glucose competence: The role of peroxisome proliferator-activated receptor $\delta$ and mitochondrial metabolism

## WHAT IS KNOWN REGARDING $\beta$ -CELL INTRACELLULAR FATTY ACID IN RELATION TO GLUCOSE-STIMULATED INSULIN SECRETION?

Elevation of extracellular glucose causes fusion of the  $\beta$  granules and the plasma membrane as a result of increased sub-membrane  $\text{Ca}^{2+}$  concentration. A raised adenosine triphosphate (ATP)-to-adenosine diphosphate (ADP) ratio is pivotal to this process, as it causes closure of the ATP-sensitive  $\text{K}^+$  (KATP) channel, membrane depolarization, opening of voltage-dependent calcium channels and finally  $\text{Ca}^{2+}$  influx from the cell exterior. Soon thereafter, glucose-stimulated insulin secretion (GSIS) is augmented as the releasable pool of  $\beta$  granules is replenished. Although the molecular basis of this augmentation has not been fully defined, free fatty acid (FFA) has been strongly implicated as having an indispensable role in this process. Quantities of FFA in the micromolar range, and therefore too minute to generate classic metabolic coupling factor(s), such as ATP for insulin exocytosis, enhance GSIS when added to the incubation in conjunction with a stimulatory concentration of glucose<sup>1</sup>. Furthermore, pre-exposure of  $\beta$ -cells to a similarly low concentration of FFA in the absence of a stimulatory concentration of glucose primes the cells so that the insulin release (IR) subsequently provoked by any stimulation is enhanced compared with that in  $\beta$ -cells not treated

with FFA<sup>1</sup>. Continued exposure of  $\beta$ -cells to a high concentration of glucose causes an anaplerotic output of citrate from the tricarboxylic acid (TCA) cycle, elevating cytosolic malonyl-CoA, which causes suppression of carnitine palmitoyltransferase 1 leading to decreased FFA entry to mitochondria<sup>2</sup>. The subsequent accumulation of cytosolic FFA might increase insulin secretion through fatty acylation of key protein(s) involved in exocytosis<sup>3</sup>. In contrast, increased cellular fatty acyl-CoA (FA-CoA) could also enhance IR through the glycerolipid/FFA cycle<sup>2</sup>. Knockdown of desnutrin/adipose triglyceride lipase (ATGL) in  $\beta$ -cells by short hairpin ribonucleic acid suppressed GSIS<sup>4</sup>. All of these data show that acute elevation and lowering of cellular FFA in  $\beta$ -cells, respectively, enhanced and suppressed GSIS. However, the downstream effector or effectors of FFA regulation of insulin secretion have remained elusive, especially under the conditions of sustained  $\beta$ -cell specific lowering of intracellular FFA.

## NEWER INSIGHTS OBTAINED BY $\beta$ -CELL SPECIFIC DESNUTRIN/ATGL KNOCKOUT

In an elegant recent study, Tang *et al.*<sup>5</sup> identified a novel downstream signaling pathway for intracellular FFA in pancreatic  $\beta$ -cells: activation of peroxisome proliferator-activated receptor  $\delta$  (PPAR $\delta$ ) followed by enhanced mitochondrial metabolism. This was accomplished by using  $\beta$ -cell specific desnutrin/ATGL knockout (desnutrin  $\beta$ KO) mice (Figure 1). The desnutrin  $\beta$ KO mouse showed postprandial and postglucose hyperglycemia with blunted

nutrient- or glucose-induced insulin secretion. Interestingly, the islets were enlarged and the insulin content increased by approximately 50%, while there was no evidence of increased insulin synthesis, so that islet insulin appeared to have increased as a result of decreased IR. Because desnutrin hydrolyzes triacylglycerol (TAG), TAG accumulated and the FFA content and glycerol output were reduced in the islets of the KO mouse. Extracellular application of oleate (concentration unspecified) in the presence of a stimulatory concentration of glucose failed to acutely elevate insulin exocytosis in the  $\beta$ -cells of the KO mouse. This indicated that a constitutive, normal level of intracellular FFA by desnutrin is required to uphold the  $\beta$ -cell machinery for IR competence in response not only to glucose, but also to fatty acid. IR directly triggered by high  $\text{K}^+$ -induced membrane depolarization in the presence or absence of the KATP channel opener, diazoxide, remained unaffected in the KO mouse. In other words, the FFA produced by desnutrin tonically maintains the nutrient-induced IR in the  $\beta$ -cells. Using mitochondrial membrane potential markers, Tang *et al.*<sup>5</sup> further showed that the loss of desnutrin/reduced TAG hydrolysis was associated with impaired mitochondrial metabolism, which was in this case as a result of defective activation of PPAR $\delta$ . Adenoviral transfection of desnutrin and pharmacological activation of PPAR $\delta$  mostly restored the altered phenotype of the desnutrin KO mouse, implying that the absence of desnutrin was in fact causal for impaired PPAR $\delta$  activation and the

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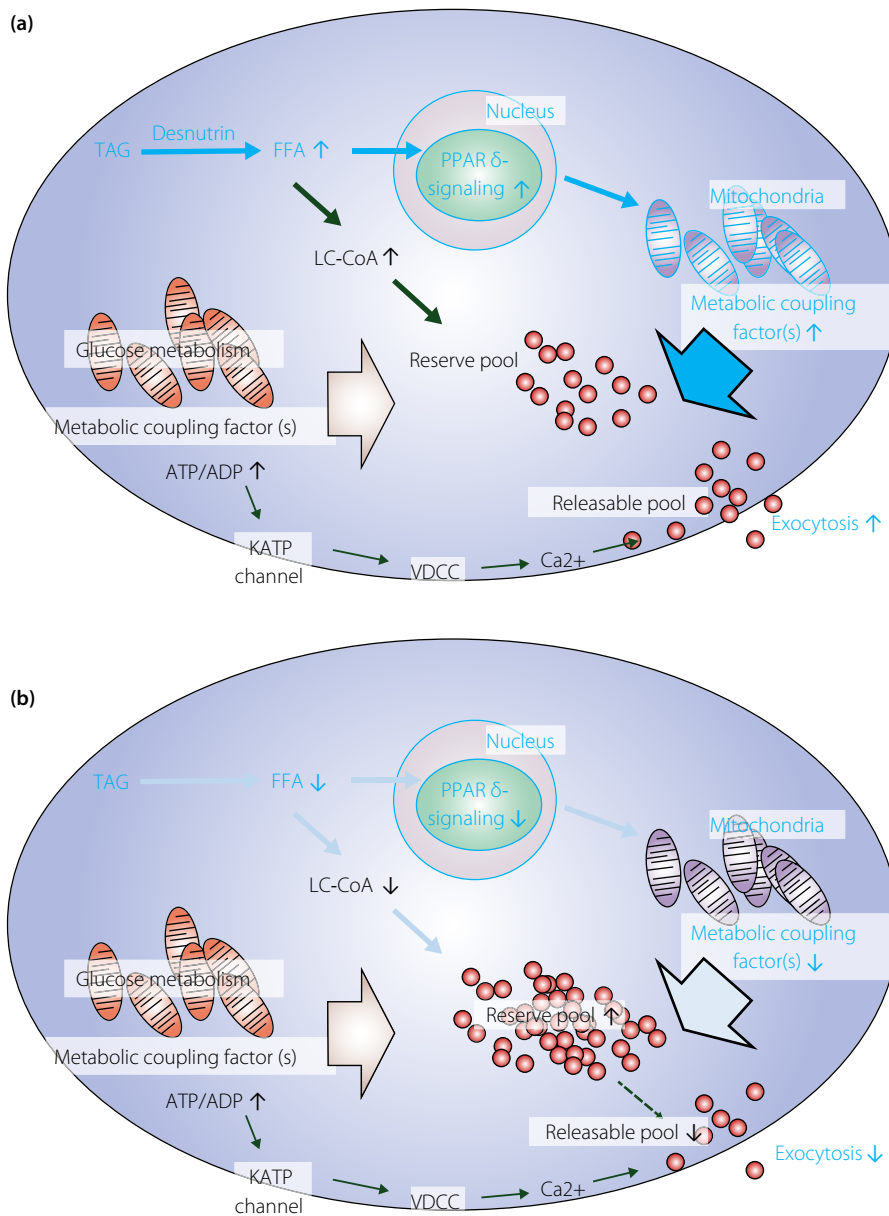
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**Figure 1** | A proposed newer view on the free fatty acid (FFA) signaling in (a) pancreatic  $\beta$ -cells, on the basis of the findings in (b) the  $\beta$ -cell specific desnutrin knockout mouse. The novel pathway shown by Tang *et al.*<sup>5</sup> is shown in blue. Downward or upward arrows indicate increase or decrease, respectively, in the substrate or signaling. (b) Faintness in blue implies attenuated signaling. The scheme is focused on the topic of the work of Tang *et al.*<sup>5</sup>, and is not intended to cover all aspects of  $\beta$ -cell metabolism-secretion coupling. ADP, adenosine diphosphate; ATP, adenosine triphosphate; KATP channel, adenosine triphosphate-sensitive  $K^+$  channel; LC-CoA, long-chain acyl-CoA; PPAR $\delta$ , peroxisome proliferator-activated receptor- $\delta$ ; TAG, triacylglycerol; VDCC, voltage-dependent calcium channel.

series of phenotypic, cellular and subcellular abnormalities in the  $\beta$ -cell specific desnutrin KO mouse/islets. Feeding a normal mouse on a high-fat diet for 8 weeks caused 50% suppression of desnutrin protein and messenger ribonucleic acid in the islet cells.

**CRITICAL APPRAISAL**

The  $\beta$ -cell specific desnutrin KO mouse shows a very unique phenotype from the viewpoint of islet physiology and pathophysiology. The desnutrin  $\beta$ KO mouse has large islets with increased insulin content in the absence of attenuated

insulin sensitivity *in vivo*. Having large islets with increased insulin content is a typical phenotype for animals fed a high-fat diet, and decreased whole-body insulin sensitivity with increased insulin synthesis is the rule under this condition. Nevertheless, in the desnutrin  $\beta$ KO

mouse, there was no enhancement of insulin synthesis as indexed by expression of the messenger ribonucleic acid. Despite increased insulin islet content, basal insulin secretion was not at all elevated either *in vivo* or *in vitro*. Although GSIS by the islets was depressed, the IR triggered by a depolarizing concentration of  $K^+$  was normal, with no increase. Taken together, these findings imply that, in the KO mouse: (i) the islet insulin content increases mostly, if not entirely, as a consequence of reduced insulin secretion; that is, the accumulation of insulin in the  $\beta$ -cells caused by impaired secretion; and (ii) the distribution of  $\beta$  granules clearly shifts to the reserve, or poorly releasable, pool. In other words, there is a diminution in the size of the readily releasable pool of  $\beta$  granules.

It should be noted that TAG hydrolysis by desnutrin was not the only source of FFA in the cells. In addition to TAG hydrolysis by desnutrin, an influx of FFA from outside the cells, a *de novo* accumulation of FFA in part as a result of the inhibition of carnitine palmitoyl-transferase-1 (CPT-1) by malonyl-CoA and hydrolysis of diacyl glycerol by hormone sensitive lipase all contributed to maintaining cellular FFA in the  $\beta$ -cells. Accordingly, the level of islet free FFA in the desnutrin KO mouse was modestly (approximately 40%), but not radically, suppressed. Therefore, it can be inferred that the deranged *in vivo* and *in vitro* phenotype of the KO mouse was a result of attenuation, not total obliteration, of FFA signaling. Thus, it can be assumed that a certain level of intracellular FFA is a required positive effector for the maintenance of the glucose competence of the IR machinery. There is a similarity between the data for the  $\beta$ -cell specific desnutrin KO mouse and the  $\beta$ -cells of fasted rats<sup>6</sup>. In the latter case, nutrient-induced, but not depolarization-induced, IR is preferentially suppressed with selective impairment of the mitochondrial metabolism. However, there is a crucial difference between the two conditions in that islet insulin content increased in the former, but decreased in the latter.

The data from the IR experiments was presented 'per islet'; that is, it was not adjusted for increased islet insulin content. The values for basal insulin output in the presence of substimulatory concentrations of glucose, so called constitutive release, might have been significantly lower if they had been expressed 'per islet insulin content'. Also, the degree of suppression in GSIS would have been much more pronounced if the data had been expressed as 'per islet insulin content'. Similarly, IR directly triggered by high  $K^+$  could have been more appropriately interpreted as 'decreased' if the increased insulin content of the islets had been taken into account. The authors concluded that the treatment with PPAR $\delta$  agonist of the KO mouse for 2 weeks caused 'normalization' of GSIS in the islets of the KO mouse. However, knowing that insulin content is grossly increased in the islets of the KO mouse, we are afraid that this interpretation might have been too simplistic. In other words, it appears that treatment with the PPAR $\delta$  agonist only partially restored the relative decrease of the releasable pool of  $\beta$  granules. This finding strongly indicates the existence of PPAR $\delta$ -independent, insulinotropic signaling of FFA in the  $\beta$ -cells. If the imbalance in the distribution of  $\beta$  granules had been completely corrected by the PPAR $\delta$  agonist, GSIS in the agonist-treated islets should have been significantly greater than in the control islets. Nevertheless, the novel findings in the present study, especially the PPAR $\delta$  mediation of the effects of FFA, constitute a valuable addition to previously accumulated knowledge relating to the role of FFA in  $\beta$ -cells. Because the effect of PPAR $\delta$  would have been manifested through changes in gene transcription and protein synthesis within a timeframe of several hours, this mechanism provides an elegant explanation for the chronic, sustained effects of FFA in  $\beta$ -cells. If the data from the heterozygous desnutrin KO mouse and from those fed a high-fat diet with a halving of desnutrin had been somewhere in between homozygotes and the control mice, desnutrin might have been appropriately

described as a 'regulator' of IR. Although activation of PPAR $\delta$  and the mitochondrial metabolism, especially raised ATP/ADP, were implicated as downstream effectors of FFA, pharmacological activation of PPAR $\delta$  in flox mouse did not cause upregulation of IR.

## CONCLUSIONS

Free fatty acid signaling in islet  $\beta$ -cells is complicated. Tang *et al.*<sup>5</sup> have identified PPAR $\delta$ -mediated activation of mitochondrial metabolism as a novel downstream effector of FFA in a comprehensive study of the  $\beta$ -cell specific desnutrin KO mouse. Specific pharmacological modulation of this signaling branch, if possible, would provide an innovative treatment for type 2 diabetes.

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