

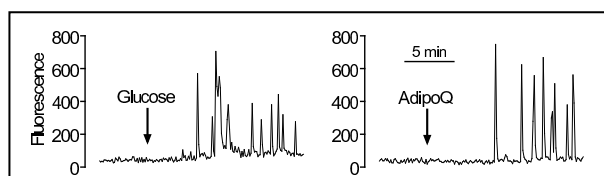
Adiponectin causes insulin secretion with increased cytoplasmic calcium and inhibition of AMP Kinase in MIN6 cells

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Type 2 diabetes is a growing concern and, according to World Health Organisation (WHO) figures, is poised to increase by 114% in the next 20 years. It is characterised by an initial reduction in glucose uptake by skeletal muscle and adipose tissue due to insulin resistance. This is compounded, later in the disease, by a reduction in insulin secretion by pancreatic β cells in response to increasing blood glucose. Type 2 diabetes is tightly linked to obesity, an increase in adipose tissue. Adipose tissue is an active endocrine organ, secreting many adipokines with important physiological actions. Adiponectin is a recently discovered adipokine whose levels, paradoxically, are decreased in obesity despite the increase in adipocyte mass (Pajvani & Scherer, 2003). Adiponectin suppresses triglyceride accumulation, increases fatty acid oxidation and activates AMP kinase (AMPK) in skeletal muscle, improving insulin signalling. Adiponectin also suppresses glucose production and activates AMPK in liver. Hence, adiponectin is an insulin sensitizer in skeletal muscle (Berg *et al.*, 2001; Yamauchi *et al.*, 2003). Adiponectin receptors have been reported in pancreatic β cells (Kharroubi *et al.*, 2003). AMPK indicates cellular energy status and has been implicated in the process of insulin secretion. Elegant studies in clonal β cell lines and primary islets demonstrate that increasing glucose concentration inhibits AMPK activity, accompanied by enhanced insulin secretion (da Silva Xavier *et al.*, 2003).

We hypothesised that adiponectin modifies insulin secretion and AMPK activity via its receptors on pancreatic β cells. We investigated the effects of adiponectin on pancreatic β -cell function by assaying insulin secretion using ELISA, quantifying AMPK phosphorylation using western blotting and imaging intracellular free calcium concentration ($[Ca^{2+}]_i$), using the Ca^{2+} sensitive fluorophore, Fluo-3, using MIN6 cells, a murine pancreatic β -cell line. We investigated the effect of adiponectin on AMPK and insulin secretion using AICAR, a known AMPK activator, and KN-62 an inhibitor of this enzyme.

Adiponectin (2 μ g/ml) suppressed AMPK activity and caused an increase in insulin secretion. Acute (30min) application of adiponectin caused a prompt increase in $[Ca^{2+}]_i$ (Figure). The increases in $[Ca^{2+}]_i$ and insulin production were prevented by nifedipine, a blocker of L-type Ca^{2+} channels, and did not occur in Ca^{2+} free solution. The suppression of AMPK activity and increases in insulin and $[Ca^{2+}]_i$ induced by adiponectin were comparable to those evoked by a physiological high glucose stimulation (Table). The effect of adiponectin on insulin secretion was potentiated in the presence of AICAR and KN-62.



	pAMPK/tAPMK	Insulin	$[Ca^{2+}]_i$
Adiponectin (3mM glucose)	48 \pm 10% ($p=0.01$)	303 \pm 51% ($p=0.001$)	142 \pm 14% ($p=0.03$)
25mM glucose	68 \pm 6% ($p=0.01$)	173 \pm 12% ($p=0.001$)	161 \pm 11% ($p=0.01$)

In conclusion, we show that the fat hormone adiponectin is capable of increasing pancreatic β cell Ca^{2+} , giving rise to insulin secretion. Adiponectin also suppresses phosphorylation of AMPK to augment insulin production. The decrease in adiponectin in obesity likely impairs this system. These results reveal the potential of adiponectin as a novel therapeutic target against obesity-linked type 2 diabetes.

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