pancreatic islet cells and on other enteroendocrine cells may complicate the picture.

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Adiponectin Sparks an Interest in Calcium

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Adiponectin and its receptors, AdipoR1 and AdipoR2, regulate glucose and fatty acid metabolism via activation of AMP-activated protein kinase. Recent work in *Nature* (Iwabu et al., 2010) demonstrates that adiponectin induces a marked Ca^{2+} influx in skeletal muscle via AdipoR1 to control mitochondrial biogenesis, reduce oxidative stress, and enhance endurance capacity.

The term adipokine was coined to describe signaling messengers that are secreted from adipocytes, of which the two most abundant are leptin and adiponectin. Because adiponectin levels are decreased in human obesity and type 2 diabetes, this adipokine has generated enormous interest within the scientific community. It has previously been shown that adiponectin-deficient mice exhibited insulin resistance (Maeda et al., 2002) and that exogenous administration of adiponectin lowered circulating glucose and ameliorated insulin resistance (Berg et al., 2001; Fruebis et al., 2001) via a process thought to involve activation of AMP-activated protein kinase (AMPK). Recent work published in *Nature* (Iwabu et al., 2010) now identifies a novel mechanism by which adiponectin protects against skeletal muscle insulin resistance (Figure 1).

In 2003, Kadowaki and colleagues reported the cloning of complementary DNAs encoding adiponectin receptors 1 and 2 (Adipor1 and Adipor2) (Yamauchi et al., 2003). They found that AdipoR1 is abundantly expressed in skeletal muscle and liver, whereas AdipoR2 is predominantly expressed in the liver. Intriguingly, both receptors were predicted to contain seven-transmembrane domains but were unlike G protein coupled in structure. Nonetheless, these novel receptors maintained functionality because subsequent studies identified that disruption of the adiponectin receptors AdipoR1 and AdipoR2 abrogated adiponectin's metabolic actions (Yamauchi et al., 2007).

As insulin resistance has been linked to mitochondrial dysfunction, many strategies have been employed to improve mitochondria function (e.g., upregulate fatty acid oxidation rates, increase fatty acid uptake, etc.) or increase mitochondria number (mitochondrial biogenesis). AMPK is activated when the energy charge of the cell is low, such as during fasting or following exercise. AMPK is well known to stimulate an increase in fatty acid oxidation via phosphorylation of acetyl-CoA-carboxylase (ACC) and to upregulate mitochondrial biogenesis through the master regulator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) (Jäger et al., 2007). The metabolic effects of AMPK and adiponectin are similar in insulin-sensitive tissues, and it was no surprise that, indeed, adiponectin was found to regulate glucose utilization and fatty acid combustion through AMPK (Yamauchi et al., 2002). Another cellular energy sensor, the nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase silent information regulator of transcription (SIRT1), is also able to activate PGC-1α by deacetylation (Lagouge et al., 2006). Because SIRT1 is able to activate an upstream activating AMPK kinase, LKB1, strong links between AMPK, SIRT1, PGC-1α, and consequently mitochondrial biogenesis and function are emerging.

The recent study published in *Nature* (Iwabu et al., 2010) has added to our understanding of the mechanism of action of adiponectin and adipoR1 in skeletal muscle. These authors provide detailed

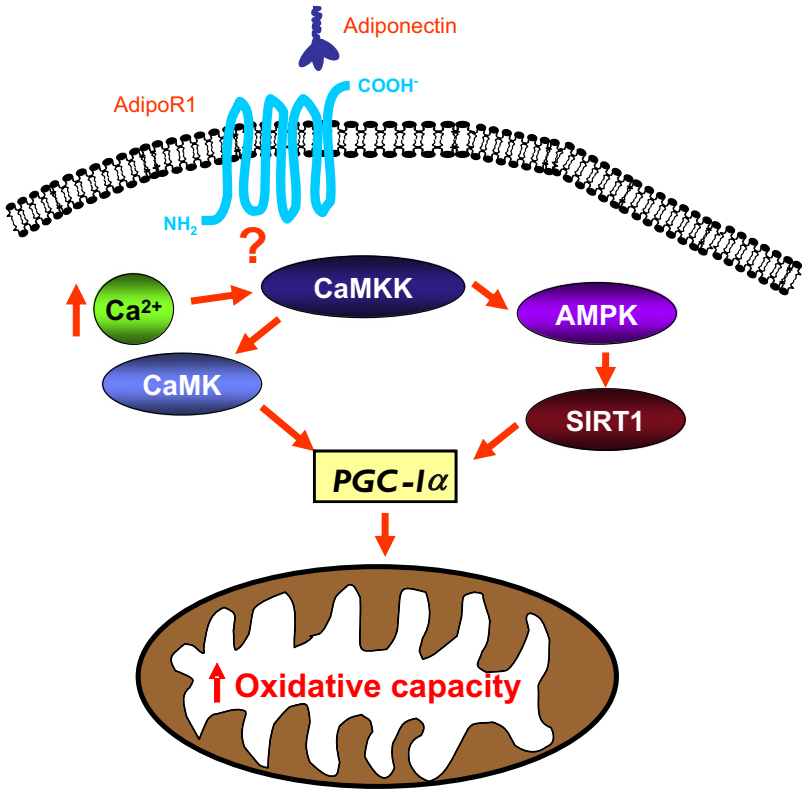

Figure 1. Adiponectin-Mediated Oxidative Phenotype via AdipoR1 and Calcium
Iwabu et al. (2010) propose that, in skeletal muscle, adiponectin induces extracellular Ca^{2+} influx by AdipoR1. This calcium influx sets off a chain of events whereby PGC1-α can be activated through both the CaMK pathway and via AMPK and SIRT1, leading to increased oxidative capacity. The mechanism by which adiponectin and AdipoR1 increase Ca^{2+} influx into cells remains to be fully determined.

evidence connecting adiponectin and AdipoR1 to AMPK, SIRT1, and PGC-1α. Most interesting is the observation that adiponectin induces calcium (Ca^{2+}) influx into myotubes via AdipoR1, which activates Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ), AMPK, SIRT1, and PGC-1α to subsequently increase mitochondrial biogenesis. Furthermore, muscle-specific disruption of AdipoR1 in mice in vivo suppressed these effects, resulting in insulin resistance and decreased exercise endurance capacity.

Muscle-specific disruption of AdiopR1 (muscle-R1KO) mice produced a phenotype that was consistent with impaired oxidative capacity. This was characterized by decreased mitochondria DNA content, oxidative type 1 myofibers, expression levels of oxidative phosphorylation and mitochondrial genes, pAMPK and PGC-1α protein expression, β-oxidation, and a decrease in exercise endurance performance compared with control animals. Analysis of 24 hr oxygen consumption revealed a decrease in whole-body oxygen consumption. Intriguingly, given such a strong oxidative phenotype, the respiratory quotient (RQ), a measure of substrate utilization, was not different when comparing muscle-R1KO with control mice. As skeletal muscle oxidative capacity and insulin sensitivity are inextricably linked, it seemed likely that the muscle-R1KO mice would have metabolic abnormalities. Indeed, glucose and insulin homeostasis was disrupted in these animals, as measured by oral glucose tolerance test, insulin tolerance test, and hyperinsulinaemic euglycaemic clamp studies. Signaling in the muscle suggested that the decrease in insulin sensitivity was due to impaired tyrosine phosphorylation of IRS-1 and decreased phosphorylation of Akt. Because body weight, lean body mass, or fat mass were not reported in this study, it is not clear whether the decreased insulin action in muscle-R1KO mice was linked to obesity.

Iwabu and colleagues next performed in vitro studies in the skeletal muscle C2C12 cells treated with adiponectin. Adiponectin treatment increased PGC1-α and mitochondrial DNA content. In a series of studies using specific short interfering RNAs (siRNAs), suppression of AdipoR1, CaMKKβ, AMPKα1 and 2 (in combination), PGC1α, or SIRT1 blunted the ability of adiponectin to increase mitochondrial DNA, whereas siRNAs of AdipoR1 and CaMKKβ inhibited the ability of adiponectin to induce PGC1-α mRNA expression. Most importantly, adiponectin appears to be necessary to signal with AdipoR1 to initiate Ca^{2+} influx. Calcium plays a pivotal role in the exercise response, particularly with contraction-induced glucose uptake, and is also involved in fatty acid oxidation. Adiponectin-induced Ca^{2+} influx in the C2C12 cells was required for CaMKK and AMPK activation and for PGC1-α expression. In light of these results, the authors revisited the muscle-R1KO mice and observed impairment in adiponectin-induced calcium influx. As such, it would appear that Ca^{2+} influx is driving the adiponectin/adipoR1-stimulated intracellular signaling cascade. One important yet-to-be determined question is what is the mechanism by which adiponectin and AdipoR1 increase Ca^{2+} influx into the myotube? Is this a direct function of AdipoR1's unusual seven-transmembrane domain with an intracellular amino terminus? Or is/are second messenger molecule(s) needed downstream of the receptor, and if so, which ones? More studies are required to delineate this mechanism.

Physical activity programs are successful in influencing weight loss and improving insulin sensitivity in human obesity, but such programs are only mildly successful due to low continual compliance rates. As such, developing drugs that act as "exercise mimetics" have long been an aim of many researchers. Of note, adiponectin is one of many factors that increase with exercise. Iwabu et al. (2010) exercise trained the muscle-R1KO mice to examine whether simultaneous activation of Ca^{2+} signaling and AMPK/SIRT1, pathways that are known to be activated by exercise, could rescue the phenotype. Following the training period, muscle-R1KO mice were more insulin sensitive and had increased mitochondrial DNA content and citrate synthase activity levels. This experiment emphasized two points: (1) just how

effective exercise is in reversing perturbed insulin sensitivity and oxidative capacity and (2) that because exercise activates Ca^{2+}, AMPK, SIRT1, and PGC1-α, the possibility of targeting the adiponectin/adipoR1 pathway as a therapeutic is tangible. It must be emphasized, however, that discovering any single pharmacological agent that will mimic the broad range of exercise-related health benefits is indeed remote, and, alas, we should not put our joggers into mothballs just yet! However, identifying and targeting specific common biochemical and molecular components of the exercise response are likely to lead to viable drug therapies and prove beneficial in the battle against such pathophysiologies as mitochondrial dysfunction in obesity and insulin resistance.

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Autophagy: A Potential Link between Obesity and Insulin Resistance

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Dysregulation of autophagy contributes to aging and to diseases such as neurodegeneration, cardiomyopathy, and cancer. The paper by Yang et al. (2010) in this issue of *Cell Metabolism* indicates that defective autophagy may also underlie impaired insulin sensitivity in obesity and that upregulating autophagy can combat insulin resistance.

Autophagy is responsible for the turnover of long-lived proteins and of intracellular structures that are damaged or functionally redundant. The process is essential for the maintenance of cellular homeostasis and is activated by starvation (to supply ATP-producing substrates, e.g., amino acids) and other stress-inducing conditions. Its dysregulation is involved in many disorders and in aging (Meijer and Codogno, 2009). In this issue of *Cell Metabolism,* Yang et al. (2010) now show that hepatic autophagy is defective in obesity and diabetes and that its upregulation improves insulin sensitivity.

During autophagy, part of the cytoplasm is surrounded by a double membrane, presumably formed from the endoplasmic reticulum (ER), to form an autophagosome that then fuses with lysosomes, after which the sequestered material is degraded. This process requires the participation of autophagy-related (ATG) proteins (see Meijer and Codogno, 2009 for review).

Autophagy is inhibited by the insulin-amino acid-mTOR signaling pathway via both short-term and long-term regulation mechanisms. Short-term inhibition can be produced by the mammalian target of rapamycin (mTOR) complex 1, which causes phosphorylation and the inhibition of ULK1 (the human homolog of yeast ATG1). Long-term regulation occurs via the transcription factors FoxO1 and FoxO3 (Liu et al., 2009), which control the transcription of *atg* genes and become phosphorylated and inhibited by insulin-induced activation of protein kinase B (Figure 1).

Recent evidence indicates that dysregulation of autophagy is implicated in obesity (characterized by ER stress, insulin resistance, and glucose intolerance; Hotamisligil, 2010) and in diabetes. Mice fed a high-fat diet (HFD) have reduced hepatic autophagy (Liu et al., 2009). However, autophagy, which is essential for maintaining the structure and function of β cells, is increased in β cells during this period of HF feeding (Ebato et al., 2008). In contrast, the