

Adiponectin: A Regulator of Energy Homeostasis

Adiponectin, a protein produced exclusively in adipose tissue, occurs in serum in relatively high concentration. Its concentration is decreased in obese and in type 2 diabetic humans. When administered to mice, it enhances insulin sensitivity and glucose tolerance, and appears to increase free fatty acid oxidation in muscle. Adiponectin is likely to be involved in the regulation of energy homeostasis.

Key words: adiponectin, insulin, diabetes, glucose intolerance, adipose tissue, Pima Indians, obesity

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Adipose tissue has traditionally been regarded as a storage site for energy. Beginning in 1995, with the discovery of leptin,¹ adipose tissue has been recognized as an active endocrine organ, secreting peptide hormones that are collectively known as adipocytokines. Other important adipocytokines are tumor necrosis factor- α (TNF- α), interleukin-6, plasminogen activator inhibitor-1, resistin, and others.²

A new adipocytokine was discovered by Lodish's laboratory³ and was named adipocyte complement-related protein of 30 kD (Acrp30), or adiponectin. It is secreted exclusively by adipose tissue.³ It affects glucose homeostasis and insulin sensitivity, primarily through action on muscle and liver. Thus, adiponectin mRNA expression is reduced in obese and diabetic mice.⁴ Its surprisingly high concentration in human serum (5–10 $\mu\text{g/mL}$ ⁵ or 0.01% of serum protein) was found to be significantly decreased in obese humans, in patients with type 2 diabetes, and in patients that were insulin resistant.⁶

Pima Indians in Arizona suffer from a high prevalence of type 2 diabetes, which is associated with obesity. Their levels of serum adiponectin are significantly depressed.⁶ In fact, an early decline in plasma adiponectin was found to be a risk factor for subsequent diabetes in that population.⁷ Human hypoadiponectinemia was more closely related to the level of insulin resistance and hyperinsulinemia than to the degree of adiposity and glucose intolerance.⁶

A single injection of purified, recombinant adiponectin in mice caused a transient decrease in basal

serum glucose levels, reversed hyperglycemia and insulin resistance in obese and in diabetic mice,⁵ and suppressed hepatic gluconeogenesis.⁸ Conversely, serum adiponectin levels were decreased in wild-type mice fed a high-fat diet and in *db/db* (genetically obese) mice.⁹ Berg et al.⁵ describe adiponectin as “a potent insulin enhancer.”

The adiponectin molecule (Figure 1), which has a molecular mass of 30 kD (247 amino acids), consists of an amino-terminal signal sequence; this sequence is followed by a non-conserved domain of 28 amino acids and 22 glycine-x-tyrosine triplets (termed “collagen repeats”) and ends in the carboxy-terminal globular domain, which is homologous to the globular complement factor C1q. The protein exists in plasma in trimeric, hexameric, and higher-order polymeric structures.¹¹ A fragment (Figure 1) of a bacterially produced recombinant adiponectin molecule, made by trypsin digestion and consisting of the carboxy-terminal end, was found to be much more active than the intact molecule.¹² When injected intravenously, it caused a rapid increase in muscle free fatty acid oxidation and prevented the rise in plasma free fatty acids and triglycerides upon feeding a high-fat diet.¹² Chronic intravenous administration of small amounts prevented diet-induced obesity in mice without influencing food intake. The authors¹² suggested that the adiponectin molecule in vivo might undergo proteolysis to produce the active C-terminal fragment, which then would be the signal from adipose tissue to muscle. By increasing influx of free fatty acids into muscle and their oxidation there, adiponectin could thereby control energy homeostasis.

A possible connection between adiponectin and the anti-diabetic action of the peroxisome proliferator-activated receptor γ (PPAR γ) was suggested by the work of Scherer's group.¹³ The authors refer to the well known therapeutic agent for diabetes, thiazolidinedione (TZD), an activator of the nuclear receptor PPAR γ . This PPAR γ agonist improves muscle glucose disposal and suppresses hepatic glucose output. Adiponectin action has exactly the same effect. To test their hypothesis that adiponectin is the mediator of PPAR γ action, the authors¹³ treated obese and diabetic mice (*db/db* mice) with TZD for 11 days. In consequence, their plasma adiponectin level increased greater than threefold, and they experienced a 50% drop in blood glucose. A similar result was obtained in fat-fed type 2 diabetic mice. In human patients, the authors found that in 3 subjects harboring a dominant-negative mutation in the PPAR γ gene, presenting with severe insulin resistance and type 2 diabetes, plasma adiponectin was fivefold lower compared with healthy subjects. Healthy subjects, on the other hand,

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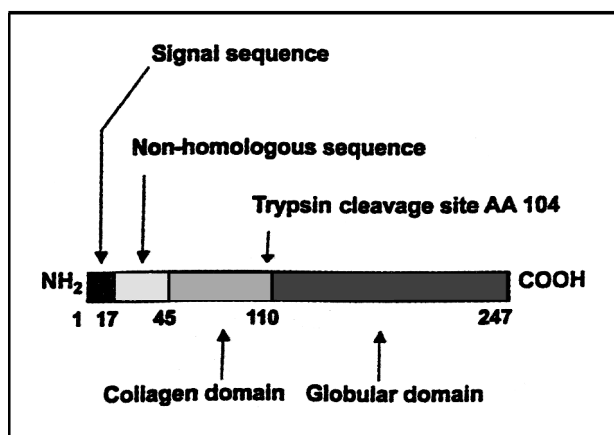


Figure 1. Schematic structure of adiponectin. Adapted from reference 10, with permission.

when treated with TZD, showed a 130% increase in their plasma adiponectin. The authors,¹³ therefore, proposed the hypothesis that PPAR γ may either directly affect the adiponectin gene or that activation of PPAR γ may increase adipogenesis, thereby indirectly increasing adiponectin production and secretion (Figure 2).

Ultimately, the following question arises: what is the physiologic function of adiponectin, a protein present in such abundance in serum, about 1000 times the concentration of leptin? The “gold standard” in the determination of the function of a particular protein is through disruption of the gene responsible for its production followed by a study of the physiologic consequences (loss of function). This was done for adiponectin by a number of investigators but with surprisingly different outcomes.

Maeda et al.¹⁵ developed adiponectin-knockout (KO) mice by replacing exon 2, which contains the translation initiation site of the *adiponectin* gene, with the *neomycin resistance* gene. Confirming tests showed that the KO mice lacked adiponectin mRNA in their fat tissue and adiponectin protein in their plasma. The KO mice had identical body fat, fasting plasma glucose, and insulin levels, and gave the same glucose tolerance tests, as wild-type mice. Muscle fatty acid-transport protein 1 (FATP-1) mRNA was decreased in the KO mice, whereas adipose tissue TNF- α mRNA and plasma TNF- α levels were dramatically increased. The effects on FATP-1 mRNA and on TNF- α could be reversed by infection of the KO mice with an adenovirus engineered to produce full-length adiponectin in the animals *in vivo*.

Striking differences appeared when the KO mice were fed a high-fat/high-sucrose (HFS) diet for 2 weeks: plasma glucose levels were double the wild-type HFS controls, plasma insulin was more than threefold higher, and insulin resistance was severe. TNF- α mRNA in fat tissue increased greatly in the KO mice given this diet. Again, these severe effects of the *adiponectin* gene

disruption in the HFS mice were reversible by infection with the adiponectin-producing adenovirus. Surprisingly, even after 6 weeks of eating the HFS diet, body weights and adiposity were the same in the KO and the wild-type mice.

By using muscle cells (C2C12 myocytes) in culture, the authors¹⁵ showed opposite effects of adiponectin and TNF- α on insulin stimulation of the insulin receptor substrate IRS-1-associated PI3 kinase, glucose uptake, and FATP-1 protein, all stimulated by adiponectin and inhibited by TNF- α . The authors¹⁵ suggested that adiponectin, an insulin-sensitizing hormone, acts in opposition to TNF- α , which is thought to cause insulin resistance in obesity.¹⁶ They proposed the hypothesis that “two adipocytokines, adiponectin and TNF- α , suppress each other’s production locally in adipose tissue and suppress each other’s function remotely in muscle.”¹⁵ They draw attention to the homology of the crystal structure of the C-terminal globular domain of adiponectin to that of TNF- α and suggest that these two adipocytokines might bind to each other’s receptors or to a common signaling protein.

Almost simultaneously, Kubota et al.¹⁷ reported the production of adiponectin-KO mice by a method very similar to that of Maeda et al.¹⁵ These authors¹⁷ confirmed that body weight gains were similar in the KO and wild-type mice, even after feeding a high-fat diet. They found that the heterozygotes (*adiponectin*^{+/-}) developed mild insulin resistance, whereas the KO-mice (homozygotes, *adiponectin*^{-/-}) showed moderate insulin resistance when tested by the glucose-lowering effect of insulin administration.

Clearly, there was a discrepancy between the results of Maeda et al.¹⁵ and Kubota et al.;¹⁷ the former¹⁵ detected “severe” insulin resistance and glucose intolerance in the adiponectin-KO mice, and the latter¹⁷ found

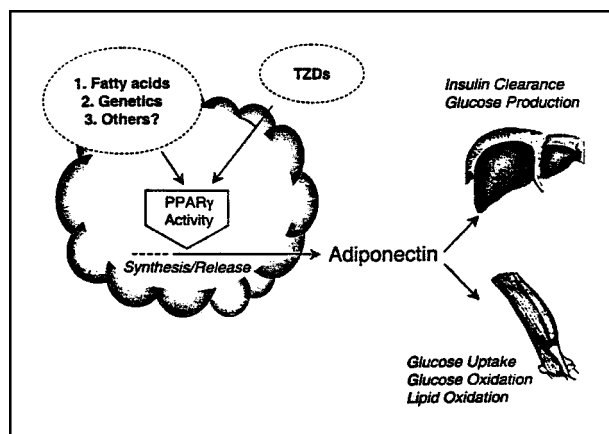


Figure 2. Role of PPAR γ and adiponectin in metabolism—action in liver and muscle. TZDs = thiazolidinediones PPAR γ = peroxisome proliferator-activated receptor γ . Adapted from reference 14, with permission.

only “modest” insulin resistance and glucose intolerance. Furthermore, the latter¹⁷ could not confirm the opposing effects of adiponectin and TNF- α .

A third attempt to determine the physiologic function of adiponectin by disruption of the *adiponectin* gene, reported recently by Ma et al.,¹⁸ added to the confusion. They found that adiponectin-KO mice suffered neither from insulin resistance nor glucose intolerance, even after feeding a high-fat/high-sucrose diet for 7 months. Whereas the Lodish laboratory¹² observed that injection of a protease-generated active fragment of the adiponectin protein stimulated free fatty acid oxidation by muscle (gain of function), Ma et al.¹⁸ showed that *absence* of adiponectin in their KO-mice (loss of function) *also* stimulated free fatty acid oxidation! Oxidation of ¹⁴C-palmitic acid by isolated soleus muscle of the KO-mice was increased by 47% more than that from wild-type mice. To explain this discrepancy, the authors¹⁸ suggested that the recombinant adiponectin injected by the Lodish group,¹² and in particular the protease-generated fragment thereof, may not represent the physiologic form of the protein. A recent report¹¹ demonstrated that adiponectin exists in plasma as a trimer, a hexamer, and a high-molecular weight complex. Tsao et al.¹⁹ provide evidence that the hexamer of adiponectin is its active form. Other explanations of discrepancies occurring between different experiments with KO-mice may be due to different genetic backgrounds of the strains.

In summary, adiponectin is a protein secreted exclusively by adipose tissue, present in abundance in plasma. Its level is decreased in the plasma of obese humans. Upon administration to mice it increases insulin sensitivity and glucose tolerance, suppresses gluconeogenesis by the liver, and causes weight loss in obese animals. Genetically adiponectin-deficient (adiponectin-KO) mice show mild insulin resistance and glucose intolerance, defects that are accentuated in animals fed a high-fat diet. Adiponectin may mediate the enhancement of insulin sensitivity brought about by activation of PPAR γ . Adiponectin appears to cause removal of free fatty acids from plasma, possibly by stimulating their oxidation by muscle, though this notion has been contradicted by experiments with adiponectin-KO mice.

In light of the uncertainties surrounding the experiments with KO-mice, it is difficult to assign a single, exclusive physiologic function to adiponectin. It is certainly involved in energy homeostasis. It has been suggested that as such it may function in combination with leptin.⁹

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