Metabolism
Endocrinology of Adipocyte - Molecular Basis of Metabolic Effects

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A B S T R A C T

Adipocyte, once considered as an inert site for lipid storage, is now being increasingly recognized as a critical mediator of a variety of physiological and pathological responses including immune-mediated inflammation, vascular remodelling, and energy homoeostasis. Adipocytes secrete proteins collectively called adipokines, which control and regulate a wide array of physiological processes including feeding behaviour, energy homoeostasis, insulin sensitivity and action, lipid and glucose metabolism, as well as vascular tone and endothelial function. Most of these effects are mediated through paracrine, autocrine, and endocrine pathways. Two recently discovered proteins, resistin and adiponectin, play a significant role in glucose and lipid metabolism. Recent understanding of the molecular basis of metabolic effects of resistin and adiponectin, along with their pro- and antiinflammatory activities, has facilitated better comprehension of the link between obesity, diabetes, atherosclerosis, and cardiovascular disease. Mapping of a susceptibility locus for T2DM, insulin resistance, and metabolic syndrome has not only provided the basis of additional genome-wide scans in different ethnic groups, but may also facilitate identification of at-risk subjects as well as the future drug development programmes.

RESISTIN

Resistin is a member of the recently discovered cysteine-rich secretory protein family and is expressed in adipose tissue. The structure of resistin bears close similarity to proteins that are involved in the inflammatory process. Resistin is identical to FIZZ3 (Found in Inflammatory Zone 3). Likewise Resistin-Like Molecules-α and β (RELM-α and RELM-β) bear structural identify with FIZZ1 and FIZZ2 respectively. Both resistin and FIZZ1/RELM-α are expressed in adipose tissue. While FIZZ1/RELM-α is also expressed in the stromal component of the lung and is associated with pulmonary inflammation, FIZZ2/RELM-β is expressed in the epithelial cells of the intestine and shares some of the metabolic properties of resistin. Interestingly, the physiological functions of resistin are also shared by other proinflammatory cytokines, such as IL-6 and TNF-α. These three adipokines not only cause insulin resistance, but may also be causally related to inflammatory process associated with obesity and coronary heart disease. A recent study has shown, for the first time, a significant positive correlation of serum resistin to CRP which remained significant even after adjusting for BMI and type of diabetes, suggesting that proinflammatory properties of resistin may be partially independent of the class (degree) of obesity.

Along with TNF-α and IL-6, resistin constitutes the triad of adipokinetes that induce insulin resistance, and their serum...
concentration is increased in T2DM. Acute administration of resistin in rodents leads to an increase in hepatic glucose production and a rise in blood glucose.\textsuperscript{3} Serum resistin levels are elevated in obese mice and immunoneutralization of circulating resistin improves insulin sensitivity and glucose tolerance.\textsuperscript{3} Furthermore, treatment of normal mice with recombinant resistin results in impairment of glucose tolerance as well as of insulin action. Resistin expression was shown to be decreased by treatment of a murine adipocyte cell line with rosiglitazone, a peroxisome proliferator-activated receptor gamma (PPAR-\(\gamma\)) agonist.\textsuperscript{6} Likewise, administration of rosiglitazone to obese mice resulted in reduction of the elevated serum resistin. Finally, infusion of either resistin or RELM-\(\beta\) in rodents was shown to induce severe hepatic, but not peripheral insulin resistance.

In a recent study, mice deficient in resistin were generated by replacing the coding exons of the resistin gene (rstn) with the reporter gene lacZ. Mating of heterozygous animals resulted in normal-sized litters of all genotypes at the rstn locus (+/+), +/−, and −/−. The (−/−) mice (lacking resistin) appeared grossly normal, with normal size and distribution of adipose depots. However, blood glucose levels after 4–6 hours of fasting were 20% to 30% lower in the (−/−) mice. Gene expression of key gluconeogenic enzymes, glucose 6-phosphatase (G6Pase) and PEPCK was markedly decreased in the liver of clamped (−/−) mice. Administration of recombinant resistin was sufficient to raise blood glucose by 25%. In contrast, rstn (+/+S) mice on a high-fat diet demonstrated a strong correlation between body weight and blood glucose.\textsuperscript{7} Thus, resistin is considered an important regulator of glucose metabolism in mouse models that are commonly used to study obesity, insulin resistance, and T2DM.

The role of resistin in the etiology of obesity, insulin resistance, and T2DM in humans remains debatable. It has been shown that resistin expression is increased in abdominal compared to thigh adipose tissue.\textsuperscript{8} Insulin has been shown to increase resistin protein secretion from human adipocytes \textit{in vitro} suggesting that increased resistin secretion may provide the connectivity between visceral adiposity, insulin resistance, hyperinsulinemia, and increased risk for T2DM.\textsuperscript{9} Adipose tissue resistin expression in obese nondiabetic humans was shown to be related to hepatic fat content and degree of insulin resistance as determined by the HOMA model.\textsuperscript{10} Thus, the link between visceral adipocyte, resistin, hepatic fat, hepatic insulin resistance and increased endogenous hepatic glucose production, may explain the metabolic profile of T2DM. Thiazolidinediones (TZD) have been shown to reduce hepatic fat content and improve hepatic insulin sensitivity in T2DM.\textsuperscript{11} To investigate whether this is mediated by a reduction in serum resistin, Bajaj et al studied 13 subjects with T2DM, prior to and following treatment with 45 mg Pioglitazone (PIO) daily for 14 weeks. As a result of the treatment, there was: (i) a significant decrease in hepatic fat content from 21.1 ± 3.5 to 11.2 ± 2.1%, and (ii) a significant decrease in levels of plasma resistin from 5.3 ± 0.6 to 3.5 ± 0.3 ng/ml. Both prior to, and following treatment with PIO, plasma resistin concentration remained positively correlated with hepatic fat content.\textsuperscript{12} Thus, there is evidence suggesting crosstalk between adipose tissue (energy storage) and liver, where insulin plays a significant role in the intermediary metabolism. It is interesting to speculate the possible role of RELM-\(\beta\) (from intestinal epithelium) through portal circulation, reinforcing the regulatory role of hepatic intermediary metabolism.

**ADIPONECTIN**

Adiponectin is a recently discovered novel adipose-specific 247-aminoacid protein, with high structural homology to TNF-\(\alpha\).\textsuperscript{13} While pharmacological doses of recombinant resistin hyperactivate gluconeogenesis through decreased hepatic insulin sensitivity, adiponectin inhibits gluconeogenesis by increasing insulin sensitivity. Adiponectin is considered as an antidiabetogenic and anti-atherogenic adipokine. Plasma levels of adiponectin are reduced in obese rodents\textsuperscript{14} and humans and as well as in humans with T2DM.\textsuperscript{15} It has been suggested that adiponectin might function as an adipostat in regulating energy balance and that its deficiency might contribute to the development of obesity and T2DM.

**Molecular Biology**

Adiponectin is the most abundant protein in the adipocyte, and its expression is highly specific to adipose tissue. At the time of its discovery in 1995, its similarity to complement factor Clq was recognised (and hence the name Adipocyte Complement Related Protein of 30-kDa or in short ACRP 30).\textsuperscript{16} The spatial organization of adiponectin is complex, with a globular head domain (gAd) hinged with a collagen triple helix region which extends up to amino acid 110. The globular carboxy-terminal part bears structural homology with the complement protein Clq, and also with the proteins of the TNF family. A part of the collagen domain is highly conserved, exhibiting structural homology between mouse, bovine and human adiponectin. This region includes four lysine residues (at position 65, 71, 80 and 104) which are highly conserved between species. These lysine residues are hydroxylated and subsequently glycosylated together with proline 94, leading to six isoforms.\textsuperscript{17} Mutations at positions 111, and 112, located near the hinge between the collagen and globular domains, may alter the spatial organisation of the protein, and interfere with post-translational modifications such as glycation of lysine residues which are considered essential for the modulation of insulin sensitivity.\textsuperscript{18} A comprehensive discernment of structure-activity relationship is an essential prerequisite for the understanding of metabolic effects of adiponectin. It has been suggested that globular head domain (gAd) regulates energy homeostasis by enhancing mitochondrial FFA oxidation in the muscle, thereby reducing serum FFA, resulting in increased sensitivity to insulin and enhanced insulin-mediated glucose uptake by muscle and adipose tissue. Through increased \(\beta\)-oxidation of FFAs, gAd reduces the triglyceride content of the muscle. This in turn leads to increased insulin sensitivity in the muscle as shown by increased phosphorylation of insulin receptor and insulin receptor substrate-1.\textsuperscript{19} In contrast to the lack of any demonstrable inhibiting effect(s) of gAd on glucose production in primary hepatocytes, intraperitoneal administration of full-length recombinant adiponectin has been shown to produce a decrease in blood
glucose, indicating liver as a likely target of adiponectin, a fact later confirmed by in vitro studies on isolated hepatocytes.18 Using hyperinsulinemic-euglycaemic clamps it was shown that adiponectin lowers hepatic glucose production without affecting peripheral glucose uptake. The molecular basis of hepatic effects of adiponectin is through the reduced hepatic expression of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK), the gluconeogenic enzymes regulating endogenous glucose production and hepatic glucose output. Thus resistin and adiponectin affect the hepatic expression of key gluconeogenic enzymes in opposite direction: resistin enhancing the expression of G6Pase and PEPCK, while adiponectin reducing their expression in the liver.

The contrasting and differential hepatic effects of gAd and full-length Ad may possibly be due to the fact that hydroxylation and subsequent glycosylation of four conserved lysine residues in the collagenous domain is an essential prerequisite for activity at the hepatocyte. As gAd lacks these post-translational modifications, it is unable to inhibit glucose production in primary hepatocytes.

**Adiponectin Receptors**

Recent studies on cloning of adiponectin receptors indicate the presence of two types of such receptors (adiponectin receptor 1, Adipo R1; and adiponectin receptor 2, Adipo R2). The genes of Adipo R1 and Adipo R2 are located on human chromosomes 1p36 and 12p13, respectively. It has been further demonstrated that Adipo R1 is mostly expressed in the skeletal muscle while Adipo R2 is predominantly expressed in the liver.20 These two adiponectin receptors are predicted to contain seven transmembrane domains; nevertheless, these are structurally and functionally distinct from G-protein coupled receptors. Consistent with the observation that gAd lacks demonstrable effect on primary hepatocytes, it has been shown that gAd binds more avidly than full-length adiponectin with myocyte and skeletal muscle membranes, and that this pattern of binding is reversed in hepatocytes and liver membranes. Human and murine cDNAs show high degree of homology for both receptor subtypes: 96.8% homology for Adipo R1 and 95.2% for Adipo R2. It is also remarkable that Adipo R1 and Adipo R2 receptor proteins are conserved from yeast (Saccharomyces cerevisiae) to human.21 More notable is the fact that the yeast homologue has a principal role in lipid metabolic pathways that regulate fatty acid oxidation.

**Mechanism of Adiponectin Action**

The molecular basis of adiponectin action has recently been elucidated by focussing sharply on the possible signalling pathway(s) underlying its metabolic effects. 5-AMP- activated protein kinase (AMPK) is known to activate insulin-independent glucose uptake by muscle, in addition to downregulating hepatic expression of G6Pase and PEPCK, and stimulating the phosphorylation of β-isomer of acetyl coenzyme A carboxylase (ACC – β), thereby inhibiting ACC activity and increasing fatty acid oxidation. As adiponectin also exhibits all of these biological activities, it was hypothesised that these effects of adiponectin may be mediated through AMPK pathway. It has now been convincingly demonstrated that phosphorylation and activation of AMPK are indeed stimulated with gAd and full-length Ad in the skeletal muscle, while only with full-length Ad in the liver. In vitro gAd treatment of myocytes or muscle resulted in transient phosphorylation of AMPK, peaking at 5 minutes post-treatment, leading to ACC inhibition by 15 minutes, resulting in a decrease of malonyl CoA, depression of carnitine palmitoyltransferase-1 activity, and a significant increase in fatty acid oxidation in the muscle. In contrast, hepatic AMPK activation required full-length Ad. Evidence for a preferential binding of gAd to muscle membranes, and of full length Ad to liver membranes suggested that the two tissues (muscle and liver) may display distinct receptors on their respective cell surface.22

**Adiponectin, Hepatic fat, and Insulin resistance**

Recent studies have provided evidence that increased hepatic fat content is an important determinant of hepatic insulin resistance in type 2 diabetic patients.11 As fatty liver is common in type 2 diabetic patients, it has been suggested that fatty liver results from accelerated fatty acid mobilization from expanded visceral fat stores and their deposition in the liver, as well as decreased hepatic fatty acid oxidation thereby causing an increase in the hepatic fat content. Thiazolidinediones have been shown to reduce hepatic fat content and improve hepatic insulin sensitivity in patients with T2DM.11 The thiazolidinediones initiate their action by binding PPARγ, primarily located on adipocytes,23 and thereby increasing plasma adiponectin levels. Indirect evidence suggests that adiponectin might mediate some of the insulin-sensitizing effects of PPARγ agonists.

The first clinical study aimed at investigating the effect of long term (14 weeks) administration of 45 mg pioglitazone daily in subjects with T2DM resulted in a three-fold increase in plasma adiponectin which correlated inversely with endogenous (hepatic) glucose production. There was also a significant inverse correlation of plasma adiponectin with hepatic fat content. Higher the plasma adiponectin levels lower the hepatic fat content. Thus the increase in plasma adiponectin following pioglitazone therapy is strongly associated with a decrease in hepatic fat content and enhanced hepatic and peripheral insulin sensitivity.24 Put together with a similar study on resistin referred to earlier,12 there is unequivocal evidence that pioglitazone (thiazolidinedione) treatment of subjects with T2DM increases plasma adiponectin and decreases plasma resistin levels, resulting in a decrease in hepatic fat content and a reduction in hepatic glucose production. Indicators of net therapeutic benefit included a decrease in fasting plasma glucose as well as a lowering of the HbA1c and serum triglyceride levels.24

**Genetics of Adiponectin**

A year after the discovery of adiponectin, the corresponding cDNA was cloned in 1996 and named ‘AdiPose Most abundant gene transcript 1 (APM 1)’.25 The gene spans 16 Kb at the long arm of human chromosome 3 (3q27). Recent studies have provided evidence for linkage between 3q27 and T2DM in French-Caucasian and Japanese populations.

A significant association of the levels of circulating adiponectin with T2DM was found in French-Caucasian population with two SNPs located in 5’ flanking region of the gene: -11391 G-A and -11377 C-G.26 In the Japanese population, the variant G-allele
at +45 and the wild-type G-allele at +276 were risk alleles for T2DM. An association between the clinical components of IRS and SNPs +45, +276, and their haplotype frequencies, has been observed in a cohort of Italian population. These genetic studies, although suggestive, need to be confirmed in several population subgroup of different ethnicity.

The \textit{APM1} region has also been linked with metabolic syndrome (IRS). \textit{APM1} knockout mice show diet-induced features of insulin resistance including delayed clearance of free fatty acids (FFAs) and higher levels of TNF\(\alpha\). Overexpression of adiponectin (via adeno viral expression) in \textit{APM1} knockout mice remedied these metabolic changes and reversed the insulin-resistant phenotype.

As \textit{APM1} region has also been shown to be linked with quantitative traits associated with the metabolic syndrome, and hyperadiponectemia is associated with insulin resistance, T2DM, obesity and dyslipidemia, the \textit{APM1} has been considered a good candidate gene for these disorders. This is supported by the demonstration of decreased expression of \textit{APM1} gene in the omental and subcutaneous adipose tissue from subjects with T2DM. Studies in \textit{APM1} knock out mice lend further credence to this hypothesis. A genomewide scan for coronary heart disease performed in an Indo-Mauritian population, with previously demonstrated clustering of metabolic disorders associated with insulin resistance, identified a susceptibility locus on chromosome 16p13 and linkage with the metabolic syndrome on chromosome 3q27 which is also the region of \textit{APM1}. \textsuperscript{28} This is of particular significance and relevance due to the fact that: (i) in Mauritius, the prevalence of CHD, T2DM, and IRS is amongst the highest in the world, and (ii) the studies were conducted in 99 families of North-Eastern Indian origin. Similar studies in Bihar, Orissa and West Bengal may generate data of considerable interest.

In addition to its metabolic effects, adiponectin has also been shown to modulate endothelial inflammatory response through TNF-\(\alpha\)-induced expression of endothelial adhesion molecules. \textsuperscript{29} \textit{In vitro} studies in human aortic endothelial cells have shown that human recombinant adiponectin, not only suppresses endothelial expression of adhesion molecules but also decreases the proliferation of vascular smooth muscle cells, and reduces lipid accumulation in macrophages, thereby modulating transformation of macrophages to foam cells. \textsuperscript{30}

Two clinical studies published earlier this year provide interesting data linking the metabolic and anti-inflammatory roles of adiponectin. In a study of 77 subjects who had diabetes or were at high risk to develop diabetes, there was a significant negative correlation between circulating levels of adiponectin and CRP, PAI-1, and tissue plasminogen activator (tPA). \textsuperscript{31} These negative associations remained significant after adjusting for gender and BMI. This study reinforces earlier observation regarding the protective role of adiponectin against inflammation and endothelial dysfunction, and provides evidence of its negative association with tPA, which is known to play a role in impaired fibrinolysis. A similar study in women with prior gestational diabetes mellitus (pGDM) who are known to be at higher risk of developing T2DM and associated cardiovascular complications showed that plasma adiponectin was significantly lower in pGDM as compared to women with normal glucose tolerance during pregnancy. The differences remained statistically significant even after adjustment for body fat mass. Equally significant were the differences in the levels of PAI-1 and ultrasensitive CRP which were higher in the pGDM group. It was concluded that lower plasma adiponectin concentrations characterize women with previous GDM independently of the prevailing glucose tolerance, insulin sensitivity or the degree of obesity and are associated with subclinical inflammation and atherogenic parameters. \textsuperscript{31}

Thus, the role of adiponectin as an integrator of metabolic and inflammatory signals underlying obesity, T2DM, and coronary heart disease has assumed considerable significance, both in terms of its potential as a part of preventive strategies, and also as a prototype molecule for the development of new analogues and related compounds aimed at therapeutic intervention. Further, development of PPAR-\(\gamma\) agonists which increase endogenous adiponectin may be equally promising and rewarding.

**PROLOGUE**

The last decade has witnessed a paradigm shift in the understanding of cell biology of adipocyte. From an inert storage depot, it has emerged as a dynamic endocrine organ, with additional paracrine and autocrine functions. With resistin and adiponectin emerging as main links between obesity, T2DM, and atherosclerosis, their connectivity with IRS and its constituents is being extensively investigated. Thiazolidinediones, which are insulin sensitizing PPAR-\(\gamma\) agonists, increase the expression of adiponectin and reduce the expression of resistin, thus indicating new molecular targets for the development of future therapies.

**REFERENCES**


