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Adiponectin – a key adipokine in the metabolic syndrome

J. P. Whitehead,[1] A. A. Richards,[1] I. J. Hickman,[1,3] G. A. Macdonald[1,2] and J. B. Prins[1,3]

[1]Centre for Diabetes and Endocrine Research, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia
[2]Departments of Gastroenterology & Hepatology, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia
[3]Departments of Diabetes & Endocrinology, University of Queensland, Princess Alexandra Hospital, Brisbane, Australia

Adiponectin is a recently described adipokine that has been recognized as a key regulator of insulin sensitivity and tissue inflammation. It is produced by adipose tissue (white and brown) and circulates in the blood at very high concentrations. It has direct actions in liver, skeletal muscle and the vasculature, with prominent roles to improve hepatic insulin sensitivity, increase fuel oxidation [via up-regulation of adenosine monophosphate-activated protein kinase (AMPK) activity] and decrease vascular inflammation. Adiponectin exists in the circulation as varying molecular weight forms, produced by multimerization. Recent data indicate that the high-molecular weight (HMW) complexes have the predominant action in the liver. In contrast to other adipokines, adiponectin secretion and circulating levels are inversely proportional to body fat content. Levels are further reduced in subjects with diabetes and coronary artery disease. Adiponectin antagonizes many effects of tumour necrosis factor-α (TNF-α) and this, in turn, suppresses adiponectin production. Furthermore, adiponectin secretion from adipocytes is enhanced by thiazolidinediones (which also act to antagonize TNF-α effects). Thus, adiponectin may be the common mechanism by which TNF-α promotes, and the thiazolidinediones suppress, insulin resistance and inflammation. Two adiponectin receptors, termed AdipoR1 and AdipoR2, have been identified and these are ubiquitously expressed. AdipoR1 is most highly expressed in skeletal muscle and has a prominent action to activate AMPK, and hence promote lipid oxidation. AdipoR2 is most highly expressed in liver, where it enhances insulin sensitivity and reduces steatosis via activation of AMPK and increased peroxisome-proliferator-activated receptor α ligand activity. T-cadherin, which is expressed in endothelium and smooth muscle, has been identified as an adiponectin-binding protein with preference for HMW adiponectin multimers. Given the low levels of adiponectin in subjects with the metabolic syndrome, and the beneficial effect of the adipokine in animal studies, there is exciting potential for adiponectin replacement therapy in insulin resistance and related disorders.

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Introduction

Appreciation of the role of adipose tissue as more than just an inert storage depot for triglyceride has increased enormously following the discovery of leptin by Friedman and colleagues [1]. The current view of the adipocyte is of a dynamic endocrine cell releasing free-fatty acids and secreting a number of factors, termed adipokines, including tumour necrosis factor-α (TNF-α), interleukins,

Correspondence:
Dr J. P. Whitehead, Associate Director/Prof J. B. Prins, Director, Centre for Diabetes and Endocrine Research, Department of Diabetes and Endocrinology, University of Queensland, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Brisbane QLD 4102, Australia.
E-mail:
jwhitehead@cder.soms.uq.edu.au/jprins@cder.soms.uq.edu.au

plasminogen-activator inhibitor type 1, leptin, resistin and adiponectin. These adipokines contribute to the regulation of a variety of processes ranging from appetite and insulin sensitivity to inflammation and atherogenesis. Adipokines exert their effects both centrally, at the level of the central nervous system, and peripherally in tissues such as skeletal muscle and the liver.

Adiponectin was originally identified and named by four independent groups, a decade ago. It was first discovered by Scherer and Lodish [2], who called it adipocyte complement-related protein of 30 kDa (Acrp30), in a subtractive hybridization screen comparing 3T3-L1 adipocytes with undifferentiated preadipocytes. A similar approach was employed by Spiegelman and colleagues [3], who termed the protein they isolated AdipoQ. These studies showed that mRNA for adiponectin was induced over 100-fold during differentiation and suggested that its expression was limited exclusively to adipocytes. Human adiponectin (which shares 83% similarity with the mouse polypeptide) was first cloned from adipose tissue by Matsuzawa and colleagues [4], who called it adipose most abundant gene transcript 1 (apM1). Finally, a very different approach was employed by Tomita and colleagues [5], who isolated adiponectin from human plasma by virtue of its affinity for gelatin, naming it gelatin-binding protein of 28 kDa (GBP28). Although there was a surprising lag following the original descriptions of this adipokine, the nomenclature and both the physiological and pathophysiological importance of adiponectin have been established over recent years.

Adiponectin is present in the circulation of healthy humans and mice at high concentrations. It accounts for approximately 0.01% of total plasma protein with plasma levels in the μg/ml range, around three orders of magnitude higher than leptin. Importantly, and in contrast to other adipokines whose levels increase with fat mass, adiponectin levels decrease in individuals with obesity [6]. In humans, and animal models, adiponectin levels are further reduced in individuals with type 2 diabetes and show strong negative correlations with multiple indices of insulin resistance [7,8]. Indeed, one study reported that adiponectin levels accounted for 73% of the variance in insulin sensitivity [9]. Similarly, decreased adiponectin levels correlate with reduced vascular function and increased coronary artery disease (CAD) [10].

Several recent reviews provide excellent commentaries on specific aspects of adiponectin biology and function [11–13]. In the current review, we present a broad and somewhat historical perspective on the current understanding of adiponectin.

Adiponectin Structure and Multimerization

Human adiponectin is a 244 amino acid protein of approximately 28 kDa, which belongs to the complement factor C1q family of proteins (figure 1a). It contains an amino-terminal signal peptide; a short non-helical, variable region that shows no homology to other proteins; 22 G-X-Y or G-X-X collagen repeats involved in collagen-triple helix formation; and a globular head at the carboxyl-terminus which shows a very high degree of homology with subunits of C1q and the globular domains of type VIII and type X collagens [2,5,14].

Biochemical analysis of adiponectin by a number of methods including size exclusion chromatography, velocity sedimentation analysis and non-reducing, non-heat denaturing sodium dodecylsulfate-polyacrylamide gel electrophoresis indicated that adiponectin existed in a number of forms essentially comprised of homotrimers, which further combine to make up larger multimeric complexes [2,5] (figure 1b). Adiponectin is found in serum in a number of complexes which include trimers and hexamers, collectively described as low-molecular weight (LMW) oligomers, and high-molecular weight (HMW) multimers (12-, 18-mers and possibly larger). Analysis of crystals of the globular domain of mouse adiponectin (residues 111–247) revealed a trimeric structure with unexpected homology to the TNF family and features common to all C1q family proteins [15]. Formation of trimers within the globular domain is thought to facilitate nucleation of the collagen triple-helices [15].

Adiponectin is subject to several post-translational modifications. Although our understanding of the roles and regulation of these modifications is incomplete, it is clear that they contribute to adiponectin multimerization and function. The cysteine residue situated in the amino-terminal variable domain (C36 in human and C39 in mouse adiponectin, respectively) is involved in disulphide bond formation which is essential for formation of oligomeric complexes greater than the basic homotrimer [16–18]. Other post-translational modifications include hydroxylation of highly conserved proline and lysine residues within the variable and collagenous domain [19,20]. These hydroxylated-lysine residues are further modified by the addition of a glucosylgalactosyl group [19,20]. Although the structural significance of these glycosylation events have not been reported, mutation of four of the five glycosylated lysine residues significantly attenuates the insulin-sensitizing action of recombinant adiponectin on primary rat hepatocytes [19]. Adiponectin has also been reported to undergo sialylation [21], although further characterization is

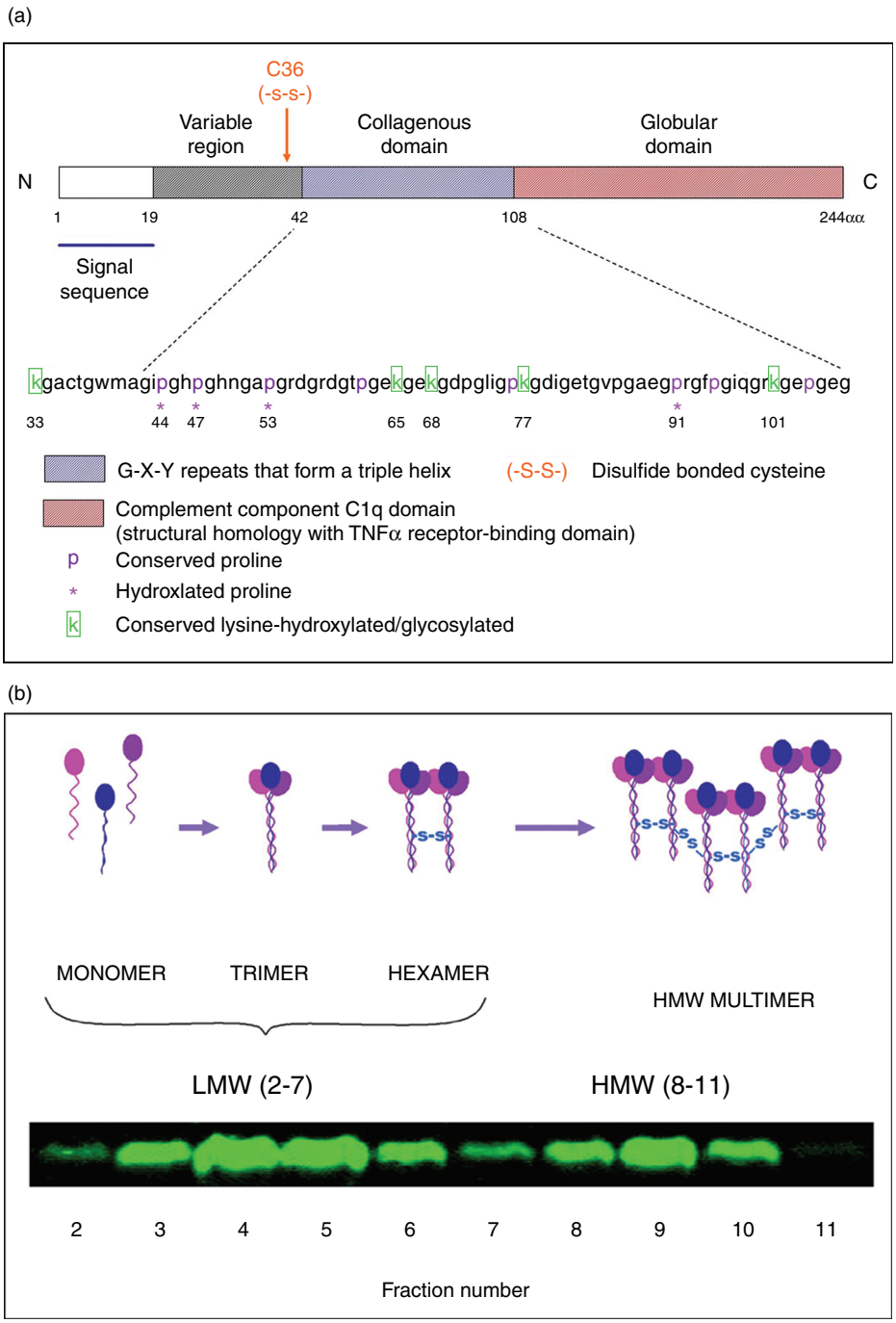

Fig. 1 (a) Domain structure of human adiponectin. Numbering is given for human adiponectin. (b) Adiponectin multimerization and analysis by velocity sedimentation. Separation of low-molecular weight (LMW) and high-molecular weight (HMW) multimers was performed by centrifugation on a 5–20% sucrose density gradient. Fractions 2–7 of the gradient contain the LMW multimers, while HMW multimers are found in fractions 8–11.

required to identify the site and the structural and functional significance of this modification.

Indirect evidence suggests that these post-translational modifications are important for oligomerization. When expressed in bacterial systems, which do not carry out post-translational modifications other than disulphide bond formation, HMW adiponectin complexes are virtually undetectable [22] or only found at low levels [18].

Adiponectin Expression, Secretion and Metabolism

Expression and Secretion

Current opinion, based on the early reports, is that adiponectin expression and secretion is restricted to adipocytes [2–4]. Adiponectin expression occurs from an intermediate stage of adipogenesis onwards [2,3] and expression appears common to adipocytes found in both white and brown adipose tissue [23,24]. Studies of isolated human explants or adipocytes suggest there are no differences in adiponectin secretion from visceral or subcutaneous depots [25], although secretion may be regulated differentially in different depots [26]. Early evidence suggesting that adiponectin expression may be reduced in states of obesity was provided by Spiegelman and colleagues [3], who observed decreased adiponectin mRNA in adipose tissue from obese (ob/ob) mice and humans. Matsuzawa's group went on to develop the first adiponectin ELISA, using this to show that plasma adiponectin levels were indeed reduced in obese subjects [6]. They also demonstrated that males had lower circulating adiponectin levels than females and that weight loss increased serum adiponectin [6,7]. Subsequent studies have confirmed these observations and have established further correlations between reduced serum adiponectin and disease states including insulin resistance and CAD [8,27]. These disease associations were independent of body mass index (BMI) [8,27]. Multiple additional factors, including age and pregnancy, also appear to affect adiponectin levels, and detailed longitudinal studies in mice suggest that adiponectin expression and secretion is under complex hormonal control, particularly at the post-transcriptional level [28,29].

Recently, there have been several reports suggesting adiponectin may be expressed from non-adipose cells. Adiponectin mRNA has been detected in mouse liver parenchyma cells following administration of carbon tetrachloride [30], and adiponectin mRNA and protein have been detected in human liver biopsies from patients with steatosis [31]. In the latter study, adiponectin protein was found by immunohistochemistry to be localized primarily to endothelial cells of portal vessels and liver sinusoids, rather than in hepatocytes. Adiponectin expression has been observed in skeletal muscle in cultured myotubes and *in vivo* [32,33]. Incubation of human myotubes with recombinant adiponectin, from mammalian cells, resulted in a dramatic increase in adiponectin mRNA levels (80-fold) and protein (eightfold) [32]. Moreover, adiponectin expression is induced in skeletal muscle in response to inflammatory cytokines in both the *in vivo* and *in vitro* settings, by a process that appears to involve nitric oxide production [33]. Regulation of adiponectin expression from non-adipose tissues, particularly muscle, appears to be different from that in adipose tissue [33]. Expression of adiponectin has also been detected in osteoblasts although the physiological relevance of this is unclear [34].

Regulation of Adiponectin Expression and Secretion

Investigations have begun to elaborate the mechanisms involved in the regulation of adiponectin expression and secretion. Studies have focused on the effects of hormonal and environmental factors on circulating adiponectin levels in the whole organism or on the expression and secretion of adiponectin at a cellular level.

In vivo investigations found that circulating adiponectin levels *per se* are not subject to acute regulation. However, adiponectin expression may be regulated relatively acutely (4–6 h) through fasting and refeeding, by mechanisms thought to involve the nuclear receptors C/EBPβ and nuclear factor Y [23,35]. In healthy normal weight subjects, circulating adiponectin levels appear to exhibit ultradian pulsatility and diurnal variation, with a decline of up to 30% at night [36]. Such diurnal variations are lost in individuals with obesity or diabetes [7] but are restored upon weight loss [37].

There is a sexual dimorphism in circulating adiponectin levels, with females having higher levels than males [6,38]. Interestingly, there is also sexual dimorphism with respect to oligomeric complexes, as males have a reduced proportion of HMW multimers compared to females [16]. In mice, differences in adiponectin levels only become apparent upon sexual maturation [29]. Several lines of evidence suggest both androgens and oestrogens play negative roles in the production of adiponectin protein. Neonatal castration of male rats results in adiponectin levels similar to those observed in females, whilst ovariectomy of adult females also increases circulating adiponectin levels [29]. Long-term (>3 months) caloric restriction results in increased circulating adiponectin, and insulin sensitivity, in mice [29,39]. A recent study in humans found no change in adiponectin levels after caloric restriction for 4 days [40].

Circulating adiponectin levels exhibit an inverse correlation with adipose tissue mass in adults [3,6,27,41]. However such a correlation does not exist in the newborn. Adiponectin levels in cord-blood of full-term infants are significantly higher than in adult subjects

and correlate positively with birth weight and BMI [42]. Whilst adiponectin levels remain largely unchanged in neonates (postnatal days 3–7) [42], reversal of these positive correlations occurs during childhood [43]. An explanation for these differences has not been established, but contributing factors may include alterations in adipose tissue distribution and adipocyte cell size during development [42]. The finding that circulating adiponectin levels are elevated in newborns is not conserved across species, as newborn mice display lower adiponectin levels than their mature counterparts [29]. Once again, differences in the extent and distribution of fat tissue may account for these species differences [42].

Although secretion of adiponectin from visceral and subcutaneous depots appears comparable [25], regulation of adiponectin secretion exhibits some depot specificity. For example, there is a strong negative correlation between adiponectin expression and secretion with BMI in isolated visceral adipocytes but not in subcutaneous adipocytes [26]. This observation may represent one feature of visceral adipose tissue (VAT) biology that leads to its strong association with negative health outcomes and may also explain the finding that even after controlling for BMI and fat mass individuals with higher VAT have lower adiponectin levels than subjects with less VAT [9].

Investigations at a cellular level, using 3T3-L1 adipocytes, suggested adiponectin is located in intracellular vesicles that are distinct from other cellular compartments such as those defined by the transferrin receptor (a marker for the endosomal pathway) and the insulin-responsive glucose transporter Glut4 [44]. Acute insulin treatment promotes increased adiponectin secretion from 3T3-L1 cells and this, like insulin-stimulated translocation of Glut4, is dependent on PI 3-kinase activity [2,44]. However, it remains to be determined whether such insulin-stimulated secretion of adiponectin is particular to adipocytes, which would be consistent with a dedicated secretory pathway, or can be recapitulated in other cell types. Relatively chronic insulin treatment (16–24 h) results in a decrease in adiponectin expression in 3T3-L1 adipocytes [45] but an increase in adiponectin mRNA in isolated human adipose tissue [46]. The explanation for these contrasting observations is unclear, but likely reflects intrinsic differences between the murine 3T3-L1 system and that of primary human adipose tissue. Given the role of hyperinsulinemia in the development of insulin resistance and type 2 diabetes, it will be important to further elucidate the effects of insulin on adiponectin production from isolated human adipocytes and adipose tissue.

TNF-α

In vitro studies indicate that TNF-α, which is elevated in conditions of obesity, may be partially responsible for decreased adiponectin production in obesity. TNF-α treatment (for 24 h) reduced the expression and secretion of adiponectin from 3T3-L1 adipocytes [45,47], whilst a significant inverse relationship between adiponectin and TNF-α expression has been reported in human adipose tissue [27]. It is tempting to speculate that TNF-α may contribute to the observed time-dependent reduction in adiponectin expression from isolated human adipocytes, however, strategies that block TNF-α failed to inhibit the reduction in adiponectin mRNA [46]. Adenoviral expression of recombinant adiponectin reduced TNF-α levels in mice lacking adiponectin further highlighting the reciprocal relationship between TNF-α and adiponectin [48].

Other factors that have been shown to reduce adiponectin gene expression, and may therefore contribute to associated metabolic and vascular complications, include glucocorticoids (dexamethosone) [45,46], interleukin-6 [49], chronic exposure to endothelin-1 [50] and β-adrenergic agonists [23].

Thiazolidinediones

The insulin-sensitizing thiazolidinediones (TZDs), which serve as agonists for the transcription factor PPARγ, have positive effects on multiple aspects of adiponectin production. Long-term (12–16 week) [47,51,52] or relatively acute (14–21 days) [53,54] administration of TZDs elevated serum adiponectin in humans. Importantly, in the latter studies, changes in circulating adiponectin levels were achieved without changes in fat mass or other circulating adipokines suggesting that TZD-induced alterations in serum adiponectin may be responsible for the insulin-sensitizing effects of these agents. TZD treatment also increased circulating adiponectin in several mouse models of insulin resistance, each with varying degrees of obesity and glucose intolerance [47,53,55].

TZDs act directly on adipocytes, increasing adiponectin production from isolated human adipocytes [26,51,52] and 3T3-L1 adipocytes [47,55]. In addition, TZDs ameliorate the effects of TNF-α on adiponectin expression in 3T3-L1 adipocytes, suggesting one mode of action of the TZDs [47]. However, TZD treatment increased adiponectin levels in lean mice without a concomitant reduction in TNF-α expression, indicating TZDs can promote adiponectin expression through TNF-α independent mechanisms [47].

The adiponectin promoter contains a functional PPARγ response element, which binds the PPARγ/RXR dimer resulting in increased promoter activity, providing a direct link between the TZDs and increased adiponectin production [47,56]. Further, albeit indirect, evidence of the importance of PPARγ in adiponectin gene expression comes from the finding that patients with a dominant-negative mutation in PPARγ associated with severe insulin resistance, display a fivefold reduction in circulating adiponectin levels [53].

The effects of TZDs are not simply limited to increasing adiponectin expression. Scherer's group recently reported that TZDs promote a shift in the multimer profile of adiponectin, with TZD treatment increasing the amount of circulating HMW adiponectin, even in individuals where there is no increase in total serum adiponectin [24]. In a series of *in vivo* studies, they found that following TZD treatment, the change in proportion of HMW adiponectin (Δ HMW/total adiponectin) or ΔS_A showed a stronger correlation with improved insulin sensitivity than change in total adiponectin levels. They also provided evidence that the multimeric composition of intracellular adiponectin differs to that of circulating adiponectin, with adipocytes containing a much greater proportion of HMW adiponectin than is present in the circulation [24]. Moreover, TZD treatment promoted increased formation and subsequent secretion of HMW complexes [24]. Together these observations suggest that multimerization of adiponectin can be regulated at an intracellular level and afford further insight into the mode of action of the TZDs. Although the mechanisms underlying the TZD induced increase in HMW adiponectin remain to be elucidated a role for intracellular chaperones has been proposed [24].

Further evidence in support of an important role for the HMW form of adiponectin comes from two recent studies. In the first, relatively short-term treatment (21 days) with the TZD pioglitazone increased adiponectin levels twofold in patients with type 2 diabetes [54]. Despite this, there was no significant correlation between the percentage increase in adiponectin levels with pioglitazone and either the percentage decrease in endogenous glucose production (EGP) or the percentage increase in glucose uptake. In contrast, there was a strong correlation between the percentage increase in S_A (there was a twofold increase in the percentage of HMW adiponectin, which represents a fourfold increase in circulating levels in absolute terms) and the percentage change in EGP [54]. The second study examined adiponectin complexes in individuals following weight loss and in patients with CAD [57]. Weight loss promoted a shift in the distribution of adiponectin, with a relative increase in the proportion of HMW adiponectin and reduction in the LMW adiponectin forms. In addition, patients with CAD had reduced levels of HMW but not LMW adiponectin compared with control subjects.

Like TZDs, a novel insulin sensitizer, IκB kinase β inhibitor, has recently been shown to increase adiponectin levels in mice without affecting whole body weight [58]. In *in vitro* studies, the inhibitor blocked TNF-α-induced reduction in adiponectin secretion by a mechanism which may involve Akt [58].

Adiponectin Metabolism and Excretion

Relatively little is known about how plasma adiponectin is metabolized and ultimately cleared from the circulation, with much of our current understanding coming from studies in mice. It appears that once in the circulation, adiponectin oligomers are extremely stable and do not undergo exchange from one form to another, even in the face of an insulin or glucose challenge [16]. That said, circulating HMW adiponectin has a longer half-life than LMW adiponectin (9 vs. 4.5 h) although HMW multimers may be cleared more rapidly than LMW adiponectin following metabolic challenges [16]. A recombinant form of adiponectin (C39S), that is unable to form oligomers larger than trimers and is sensitive to proteolytic cleavage following secretion, appears to be more biologically active than HMW adiponectin and subject to more rapid clearance from the circulation. Together with the above observations that suggest an important role for HMW adiponectin in insulin sensitivity, these findings have led to the proposal that HMW adiponectin may be converted to a biologically active form, in response to metabolic challenge, through reduction and proteolytic cleavage [24]. Reduction, followed by proteolysis is an emerging mechanism of ligand activation [59]. In support of such a model for adiponectin activation, a small amount of a C-terminal globular domain-containing fragment of adiponectin has been detected in human serum following immunoprecipitation [60]. Moreover, leucocyte elastase, which is secreted by monocytes, has recently been identified as an enzyme capable of cleaving the collagenous domain of adiponectin to produce such fragments [61].

Although little is known about the process of adiponectin clearance, adiponectin has been detected in urine from type 2 diabetic subjects and healthy males [62] (JPW and JBP, unpublished observations). Urinary adiponectin levels, which are around three orders of

magnitude lower than serum levels, were significantly elevated in patients with macro-albuminuria and correlated with urinary albumin. These observations support a model where leakage of circulating adiponectin, through the damaged kidneys of these patients, is largely responsible for the high urinary adiponectin, although additional physiological and pathophysiological factors may also be involved [62]. Intriguingly, serum adiponectin levels were also elevated in patients with macro-albuminuria suggesting that there may be a compensatory mechanism to assuage microvascular damage in the advanced stages of diabetic nephropathy by increased production of adiponectin.

Adiponectin and Insulin Sensitivity

Adiponectin as a Diabetogene

It is well established that circulating adiponectin levels are reduced in obese and insulin resistant states [8,41,63]. Studies in rhesus monkeys, which provide good models of human obesity and type 2 diabetes, have confirmed these findings and have also provided evidence that adiponectin levels decrease in parallel with the progression of insulin resistance and type 2 diabetes [64]. Increased plasma adiponectin is strongly and independently associated with reduced risk of type 2 diabetes in healthy individuals [65]. Adiponectin is encoded by the *APM1* gene which maps to chromosome 3q27, a region identified as a susceptibility locus for the metabolic syndrome and type 2 diabetes [66,67]. Together these observations make the *APM1* gene a candidate type 2 diabetes susceptibility gene.

Several common single-nucleotide polymorphisms (SNPs) have been identified in the *APM1* gene and promoter region in Japanese, French Caucasian and Swedish Caucasian populations [68–70]. In some cases, but not all, these SNPs associate with adiponectin levels and or type 2 diabetes [68–70]. A recent study found little evidence to support the hypothesis that genetic variation in the *APM1* gene represents a major contributor to type 2 diabetes in French Caucasians, rather it found evidence of a diabetes susceptibility locus at 3q27 that influences genetic predisposition to type 2 diabetes and reduced serum adiponectin in patients with type 2 diabetes [71]. Missense mutations in adiponectin have been identified in subjects with type 2 diabetes and hypoadiponectinemia, and several of these mutations have been shown to inhibit adiponectin multimerization and secretion, consistent with a causative role in diabetes [18,72].

Lessons from Adiponectin Administration

Administration of recombinant forms of adiponectin affects glucose and lipid homeostasis at multiple points, providing insight into the actions of adiponectin at both a cellular level as well as in the whole animal. When considering the effects of recombinant adiponectin, it is important to bear in mind the expression system used and the form of the recombinant protein employed. Such features have major implications as they determine whether post-translational modifications such as glycosylation (which does not occur in bacteria) and multimerization will be similar to that of the endogenous protein.

Lodish and colleagues employed bacterially expressed full-length adiponectin or globular adiponectin, produced by proteolytic cleavage of full-length adiponectin (pcgAd – aa's 104–247) [60] or by expression of the globular domain itself (gAd – aa's 107–247) [73]. Acute administration of pcgAd to mice (3×25 μg) reduced plasma free-fatty acids and glucose after a high-fat meal or following injection of intralipid. *In vitro* studies, performed on isolated muscle strips and C2C12 cells, suggested that these effects were mediated by activation of AMP-activated protein kinase (AMPK) leading to increased fatty acid oxidation and glucose uptake in muscle [60,73]. Daily administration of pcgAd also promoted weight reduction in mice fed a high-fat/sucrose diet without affecting food intake [60]. Administration of full-length adiponectin at similar or higher dose (3×50 μg) was without effect [60,73].

Bacterial expression systems were also employed by Kadowaki's group to produce full-length adiponectin or gAd [55]. In an animal model of lipoatrophy, prolonged administration of low-dose gAd (2.5 μg/day) promoted partial restoration of insulin sensitivity and complete reversal of insulin resistance when combined with leptin administration. The effects appeared to be due to increased expression of molecules involved in fatty acid transport (CD36), combustion (acyl-CoA oxidase) and energy dissipation (UCP2) in skeletal muscle, with reduced expression of CD36 in the liver. These changes correlated with decreased triglyceride content in both skeletal muscle and liver as well as improved insulin signalling. Similarly, insulin resistance in obese mice was improved by administration of gAd which promoted fatty acid oxidation in skeletal muscle. Throughout these studies, full-length adiponectin was less efficacious and was used at a higher dose (50 μg/day) to elicit similar effects to gAd [55]. They concluded that gAd, and to a lesser extent full-length adiponectin, elicited its primary effects at the level of skeletal

muscle, possibly through induction of PPARγ/PPARα. In addition to these effects, Goldstein and colleagues [74] found that bacterially produced gAd stimulated AMPK activity and glucose uptake in primary rat adipocytes suggesting a possible paracrine effect.

Scherer and colleagues purified full-length adiponectin from mammalian HEK293 cells, which would be expected to secrete mature adiponectin exhibiting a typical post-translational glycosylation pattern [39]. They found that a single dose of adiponectin (28 μg/g body weight), resulting in a two to threefold increase in circulating adiponectin, promoted a reduction in basal blood glucose levels in healthy, obese and non-obese diabetic mice, without affecting insulin levels. In contrast, a single dose of bacterially produced gAd (aa's 110–247), similar to that used by Lodish, was without effect [39]. *In vitro* studies, using isolated primary hepatocytes, demonstrated that full-length adiponectin-improved insulin's ability to suppress hepatic glucose output (HGO) [39]. Further *in vivo* investigations, using the pancreatic insulin clamp technique, found that acute infusion of adiponectin inhibited glucose production without affecting glucose uptake [75]. These findings led to the proposal that suppression of HGO may represent the main mechanism by which adiponectin acutely lowers plasma glucose [39,75].

Work from Cooper and colleagues provided further evidence in support of a direct role of full-length adiponectin on hepatic tissue [19,76]. *In vitro* studies confirmed the observations of Scherer's group, that full-length adiponectin increased insulin-induced inhibition of glucose output in primary hepatocytes. They also showed that such effects were limited to adiponectin produced from mammalian cells as full-length bacterially produced adiponectin was without effect [19]. Administration of full-length adiponectin, generated in HEK293 cells, also ameliorated hepatic abnormality in murine models of alcoholic (ASH) and non-alcoholic (NASH) steatohepatitis [76]. Induction of ASH, by chronic alcohol consumption, was associated with an increase in TNF-α levels and reduced serum adiponectin concentrations. Adiponectin treatment of these animals decreased hepatic expression of TNF-α and reduced fatty acid synthesis (↓ acetyl-CoA carboxylase and fatty acid synthase activities) whilst increasing hepatic fatty acid oxidation (↑ carnitine palmitoyltransferase I activity) [76].

In a recent study, Cooper and co-workers compared the effects of recombinant full-length adiponectin purified from bacteria with endogenous adiponectin purified from foetal bovine serum [20]. Acute administration of endogenous bovine adiponectin (40 μg/g body weight) reduced blood glucose and lipid clearance following an oral lipid challenge. Similarly, chronic treatment (using osmotic pumps for delivery) with bovine adiponectin improved insulin sensitivity and glucose tolerance in high-fat fed mice. In contrast, bacterially expressed recombinant adiponectin administered at the same dose failed to elicit any effects [20].

Adiponectin also appears to have important central effects. Intracerebroventricular administration of adiponectin decreased body weight, and improved insulin sensitivity, as a result of elevated energy expenditure through increased thermogenesis [77]. Full-length wild-type adiponectin, C39S adiponectin and gAd were all effective.

One consensus from these studies is the lack of efficacy of full-length adiponectin when produced in bacteria. One possibility is that bacterially produced full-length adiponectin may have increased susceptibility to proteolytic degradation and/or enhanced clearance from the circulation, although the latter would not contribute to its lack of activity *in vitro*. A more likely explanation is that the lack of post-translational modifications and/or multimerization of such bacterially produced adiponectin compromise its activity. As mentioned, bacterially produced adiponectin forms HMW oligomers with limited efficiency [18,22]. In this context, it is intriguing that both bacterially produced gAd and a C39S mutant generated in mammalian cells [24] appear to be active, even though oligomerization of both proteins is limited to trimers. Whilst the physiological relevance of gAd and putative proteolytic adiponectin products, have recently been questioned [11,20], the former studies highlight its potential benefits as a pharmacological therapeutic.

Lessons from Adiponectin Knockout (KO) Mice and Transgenics

Three groups have investigated the effects of deletion of the *APM1* gene on insulin sensitivity [48,78,79]. Kadowaki's and Matsuzawa's groups both found that adiponectin KO mice were insulin resistant, although there were some minor differences in the observations from the two groups [48,78]. In both cases, KO mice exhibited severe insulin resistance when challenged with a high-fat diet. In Kadowaki's laboratory, adiponectin$^{+/-}$ mice displayed insulin resistance and glucose intolerance, even when maintained on a standard chow diet, and this was further exacerbated in KO mice suggesting a dose-dependent effect [78]. Matsuzawa's group observed severe insulin resistance

coupled with defects in insulin signalling only after high-fat feeding of their KO mice [48]. However, they were able to detect changes in the expression of molecules associated with fatty acid metabolism (fatty-acid transport protein 1) and insulin resistance (TNF-α) in KO animals fed a standard diet [48]. They also showed that adenoviral production of adiponectin reversed the changes in expression of these molecules and ameliorated insulin resistance. Somewhat perplexingly, Chan and colleagues failed to identify evidence of insulin resistance in their KO mice, even after high-fat feeding, and found that fatty acid oxidation was increased in muscle and liver from KO animals [79]. The reasons for this remain obscure.

In addition, Kadowaki's group reported that adiponectin KO mice displayed greater neointimal formation in response to external vascular cuff injury than did wild-type mice, providing evidence to support the antiatherogenic properties of adiponectin [78]. Using the KO mice, generated by Matsuzawa's group, Walsh and colleagues were able to show that adiponectin was required for angiogenesis in response to tissue ischemia and that this was through stimulation of AMPK [80].

Scherer's group generated a transgenic mouse that displays elevated circulating adiponectin levels [81]. Their initial observation was that low-level expression of an adiponectin mutant lacking a portion of the collagenous domain, termed ΔGly-adiponectin, resulted in increased secretion of endogenous adiponectin whilst the mutant itself failed to be secreted. Using this information, they produced a transgenic mouse expressing ΔGly-adiponectin from an aP2 promoter, conferring adipose-specific expression. This resulted in a threefold increase in serum adiponectin. This model of hyperadiponectinaemia exhibited increased insulin sensitivity through improved carbohydrate and lipid metabolism associated with increased activation of AMPK in liver and expression of PPARγ in WAT. The transgenic mice were also resistant to diet-induced decreases of insulin sensitivity. On a cautionary note, elevated adiponectin also produced expansion of BAT-like tissue, particularly in the interscapular region and bilateral exophthalmia as well as orbital fat pads. With age, these adipose deposits expanded wrapping around the neck and stretching around regions of the head [81].

Collectively, these studies confirm the importance of adiponectin in the maintenance of metabolic control and insulin sensitivity, although the latter report also provides the caveat that too much adiponectin may bring its own complications.

Adiponectin Receptors

AdipoR1 and AdipoR2

To identify adiponectin receptors, Kadowaki and colleagues screened a human skeletal muscle library for gAd binding, using retroviral expression in Ba/F3 cells [82]. They discovered a single cDNA that encoded a protein they termed AdipoR1. Database searches revealed a second homologous open reading frame, derived from a distinct gene, which they termed AdipoR2. Northern blot analysis suggested ubiquitous expression of AdipoR1 mRNA that was most abundant in skeletal muscle. AdipoR2 expression appeared more limited with abundant expression in liver, although this differential expression of AdipoR2 was more apparent in tissue from mouse than human [82,83]. Within the liver, AdipoR2 expression occurs principally on hepatocytes [31].

Structural predictions for AdipoR1 (375 aa's, 42.4 kDa) and AdipoR2 (311 aa's, 35.4 kDa) suggest that both are seven transmembrane domain proteins. Investigations of transiently expressed, epitope-tagged AdipoR1 and AdipoR2 constructs indicate that both are type IV-A proteins, with an intracellular N-terminus, and that they may form homo-/hetero-oligomers [82]. They lack significant homology with other mammalian proteins, being only distantly related to G-protein-coupled receptors, however, they are conserved from yeast to humans. Indeed, the yeast homologue has a principal role in metabolic pathways regulating lipid metabolism such as fatty acid oxidation. Of further interest is that the yeast homologue, PHO36, is the receptor for osmotin. Osmotin has no sequence homology with adiponectin, but has structural similarity and is able to bind and activate AdipoR1 and AdipoR2 [84]. This raises the possibility that there may be other mammalian ligands for the receptors.

Exogenous expression of either AdipoR1 or AdipoR2, in 293T cells or C2C12 myocytes, increased binding of globular and full-length adiponectin. Fatty acid oxidation was stimulated in response to gAd in C2C12 myocytes, and this effect was potentiated in cells overexpressing AdipoR1 or AdipoR2. In contrast, stimulation of fatty acid oxidation in response to full-length adiponectin was only observed in cells overexpressing AdipoR2. Detailed studies investigating binding of adiponectin to endogenous receptors in C2C12 myocytes, coupled with siRNA approaches, shed some insight into these observations. AdipoR1 seems to serve as a high-affinity receptor for gAd and a low-affinity receptor for full-length adiponectin. AdipoR2 appears

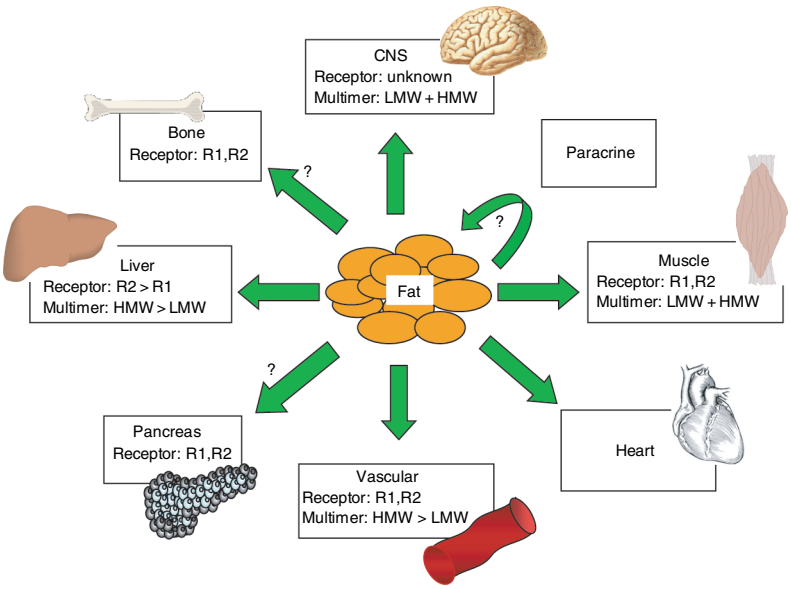

Fig. 2 Summary of adiponectin receptor expression and multimer responsiveness in various target tissues.

to act as an intermediate-affinity receptor for full-length and gAd [82].

Given the apparent differences in tissue expression and binding affinities of AdipoR1 and AdipoR2 (figure 2), Yamauchi and colleagues went on to compare the effects of globular and full-length adiponectin in different cell types [82]. Whilst globular and full-length adiponectin bound to C2C12 myocytes and promoted PPARα ligand activity and fatty acid oxidation, only gAd stimulated glucose uptake and this appeared to be through AdipoR1. AMPK, acetyl CoA carboxylase (ACC) and p38MAPK were phosphorylated in response to both globular and full-length adiponectin and inhibition of these pathways, using DN-AMPK or the p38MAPK inhibitor SB203580, reduced fatty acid oxidation and glucose uptake. In contrast to C2C12 myocytes, primary hepatocytes bound only full-length adiponectin and this stimulated AMPK and ACC phosphorylation as well as PPARα ligand activity. Inhibition of AdipoR2 expression, by siRNA, reduced binding of full-length adiponectin and PPARα ligand activity in hepatocytes.

It is noteworthy that the majority of these studies were performed using bacterially expressed adiponectin and that full-length adiponectin was typically required at an order of magnitude higher than gAd to elicit a similar response.

Kadowaki and colleagues recently reported on the regulation of expression of AdipoR1 and AdipoR2 [85]. Levels of both receptors decreased in response to physiological and pathophysiological increases in insulin [85]. The insulin stimulated decrease in AdipoR1 and AdipoR2 appeared to be mediated by PI 3-kinase dependent inhibition of Foxo1, as these effects were blocked by inhibition of PI 3-kinase or adenoviral expression of constitutively active Foxo1. In the continued absence of specific antibodies for AdipoR1 and AdipoR2, these studies were limited to measurements of mRNA. However, evidence in support of a concomitant reduction in receptor protein levels came from studies of the ob/ob mouse, which is a model of obesity-induced insulin resistance and extreme hyperinsulinemia. Skeletal muscle from ob/ob mice displayed decreased AdipoR1 and AdipoR2 mRNA expression and reduced binding of globular and full-length adiponectin. Full-length adiponectin failed to promote phosphorylation of AMPK leading to the suggestion that these mice may be adiponectin resistant [85]. A teleological explanation for the observation that insulin decreases AdipoR1 and AdipoR2 expression has not yet been proposed, whilst others have failed to recapitulate significant effects of insulin on adiponectin receptor expression [86,87].

A small study by Ravussin and colleagues found a positive relationship between expression of AdipoR1 and AdipoR2 in skeletal muscle and insulin sensitivity in non-diabetic Mexican Americans [83]. Even though subjects with a family history of type 2 diabetes were matched with control subjects for BMI and body fat, the expression levels of both receptors, as well as adiponectin, were lower in those subjects with a family history of diabetes. Thus, the impaired expression of the receptors, in combination with lower concentrations of the circulating hormone, may predispose these subjects to

the disease. A second study by Häring and colleagues failed to detect such associations between adiponectin receptor expression and insulin sensitivity [86]. However, this latter study measured adiponectin receptor expression in cultured myotubes, so it is conceivable that changes in AdipoR1 and AdipoR2 expression may have occurred during culture. Both studies did find that expression of AdipoR1 was only moderately higher than AdipoR2 (approximately 1.8 fold) in human muscle, which is consistent with the original data from Kadowaki's group [82], suggesting that the difference in expression of AdipoR1 and AdipoR2 in human skeletal muscle may not be as pronounced as that in mouse muscle.

Recent reports have also examined the expression of AdipoR1/R2 in various tissues and cell types. Kadowaki's group reported AdipoR1 and AdipoR2 expression in WAT and BAT, with expression of both markedly reduced in the ob/ob mouse [85]. Both receptors are also expressed in mature 3T3-L1 adipocytes [87]. AdipoR1 is expressed constitutively whereas AdipoR2 expression is low in preadipocytes and induced during differentiation. Treatment with growth hormone increased AdipoR2, but not AdipoR1, expression [87].

Expression of AdipoR1 and AdipoR2 in primary human and murine osteoblasts, which are of mesenchymal origin like adipocytes, has also been reported [34] and is an area of considerable interest in the bone field. These authors also found evidence of expression and secretion of adiponectin from osteoblasts and have previously reported that leptin and leptin receptors are expressed in human osteoblasts [88]. Collectively, these observations suggest a paracrine role for adiponectin in adipose tissue, and possibly bone, which may be subject to regulation or perturbation by other circulating factors.

Transcripts for AdipoR1 and AdipoR2 have been detected in human primary monocytes [89]. Treatment of macrophages with PPARα and PPARγ agonists increased AdipoR2 expression whilst a liver X receptor (LXR) agonist increased levels of AdipoR1 and AdipoR2 [89]. Incubation with adiponectin reduced macrophage cholesterol content, and this effect was potentiated by simultaneous treatment with a PPARα ligand. Expression of both receptors was also found in vascular cells, including human aortic smooth muscle cells and human microvascular endothelial cells, and at moderately increased levels in regions of atherosclerotic lesions [89]. Together these data provide insight into the anti-atherosclerotic and anti-atherogenic effects of adiponectin. AdipoR1 and AdipoR2 have also been detected in human and rat islets and beta cells [90]. Treatment of these cells with globular or full-length adiponectin failed to affect fatty acid oxidation although gAd promoted a modest increase in lipoprotein lipase (LPL) expression. The significance of adiponectin receptors in islets and beta cells is currently unclear.

T-cadherin

Lodish and colleagues employed retroviral expression of a C2C12 myoblast cDNA library in Ba/F3 cells to identify adiponectin-binding proteins [91]. A major difference between this study and that of Kadowaki's group was the way the ligand, namely adiponectin, was produced and presented. In the former [82], recombinant adiponectin (globular and full-length) was expressed in bacteria. In the latter, adiponectin (FLAG-tagged) was produced by expression in mammalian (HEK293) cells, and Ba/F3 cells expressing adiponectin-binding protein(s) on their cell surface were enriched by sequential sorting using adiponectin-coated beads [91]. Subsequent DNA analysis revealed T-cadherin as the adiponectin-binding protein. Expression of T-cadherin conferred binding of hexameric and HMW multimers but not trimeric adiponectin. Globular or full-length adiponectin produced in bacteria failed to bind T-cadherin, leading to the suggestion that post-translational modifications other than disulphide bonds may be required for the interaction. Further investigations demonstrated that binding of adiponectin to T-cadherin required divalent cations and could be blocked by EDTA. T-cadherin and wild-type adiponectin could be co-immunoprecipitated from co-expressing cells. This interaction appeared to be specific for hexameric/HMW multimers as mutation of C39, which limits multimerization to the formation of trimers, prevented the interaction. Taken together, these results suggest that T-cadherin may serve as a binding protein specific for hexameric/HMW adiponectin.

Adiponectin and Inflammation

Inflammation within tissues is now well recognized to have an important role in the development of multiple pathologies including vascular disease, liver disease, cardiomyopathy and renal disease. The inflammatory response correlates with multiple metabolic markers including obesity (particularly visceral), dyslipidaemia, hypertension and insulin resistance. A prominent systemic marker of this low-grade tissue inflammation is C-reactive protein (CRP) [92]. Circulating and adipose levels of CRP are reciprocally associated with

adiponectin levels [92]. The role of obesity in the initiation and acceleration of tissue inflammation has been well studied. Excess adipose tissue can contribute to inflammation in two ways – (i) ectopic fat storage induces lipotoxicity, promoting an intracellular inflammatory response and (ii) altered adipokine production in obesity contributes to the inflammatory response. It is now recognized that adiponectin has a role in both of these processes.

A role for adiponectin in inflammation was immediately apparent, when it was recognized that the protein had sequence and/or structural homology to immune proteins such as complement and TNF-α. It soon became apparent that adiponectin had a predominantly anti-inflammatory action, contrasting it with most other adipokines [11,93]. Epidemiological studies reproducibly showed an inverse association between serum adiponectin concentrations and (vascular) inflammatory markers and manifestations of the metabolic syndrome including CRP, fibrinogen, hypertension and endothelial function [94–97]. Further support for an anti-inflammatory role of adiponectin came from genetic studies demonstrating a relationship between the I164T adiponectin polymorphism and low-serum adiponectin, hypertension [94], CAD and the metabolic syndrome [98].

In parallel with these studies, *in vitro* and transgenic studies provided support and mechanistic insight into the anti-inflammatory role of adiponectin. The adiponectin KO mouse has increased atherogenesis, and an exaggerated neo-intimal and vascular smooth muscle proliferative response to vascular injury. The latter response can be attenuated with adiponectin administration [99]. Adiponectin administration also significantly reduces atherogenesis in the apo-E-deficient mouse [100].

Adiponectin has been shown to have a role in hepatic inflammation and steatosis. Hypo-adiponectinaemia is associated with NASH [101], and adiponectin has been shown to have beneficial anti-inflammatory effects in liver, reducing steatosis, hepatomegaly and inflammation in mouse models of alcoholic and non-alcoholic fatty liver disease [76].

Endothelial cells and the vasculature are targets of adiponectin actions. *In vitro* studies demonstrate that adiponectin adheres to injured vascular endothelium [102] and inhibits TNF-α-induced monocyte adhesion to endothelial cells [103]. It also decreases the expression of endothelial cell adhesion molecules [103], and TNF-α-induced NFκB activation [104]. Adiponectin has direct *in vitro* effects on macrophages including inhibition of macrophage to foam-cell progression [105], phagocytic activity and lipopolysaccharide-induced TNF-α production [106].

In normal tissues, angiogenesis is a controlled process and this control is perturbed in many tumours and their metastases. Adiponectin has anti-angiogenic activities in this setting, through dual mechanisms of inhibition of endothelial cell proliferation and migration and the promotion of endothelial cell apoptosis [107]. In contrast, injured vasculature is characterized by accelerated endothelial cell turnover and apoptosis and reduced angiogenesis, especially in the setting of ischaemia. In these settings, adiponectin regulates the endothelial response to injury [108] by suppression of endothelial cell apoptosis [57] and stimulation of angiogenesis [80]. The clinical relevance of the differing molecular weight multimers is again demonstrated by the more potent vascular-protection afforded by the HMW isoforms [57].

The contrasting actions of adiponectin in 'normal' and 'diseased' tissue suggest that adiponectin expression forms part of the normal anti-inflammatory/reparative response to injury. In addition to studies in vascular tissue [108], support for this concept comes from studies in liver and skeletal muscle. Serum adiponectin has been shown to be reduced in NASH compared to age, sex and BMI-matched controls [109]. Kaser *et al.* [31] studied human liver tissue and found that hepatic adiponectin (and AdipoR2) expression is reduced in NASH compared with hepatic steatosis. This study did not include any histologically normal liver, and adiponectin protein was localized predominantly in endothelial cells rather than hepatocytes. Overall, the finding of low-adiponectin 'expression' in liver is consistent with the low levels of circulating adiponectin seen in non-alcoholic fatty liver disease (NAFLD) [101,110] and may simply reflect the association between NASH, insulin resistance and obesity. Alternatively, it could represent paracrine effects related to the disease process in hepatocytes and/or the inflammatory infiltrate in the hepatic lobule; or an attenuated or subnormal adiponectin response to the initial insult, leading to the situation where individuals who are unable to appropriately increase adiponectin production in response to injury get progressive disease. In a complementary study, Delaigle *et al.* [33] show that adiponectin expression can be induced in skeletal muscle by exposure to inflammatory cytokines. The authors postulate that the adiponectin response could be viewed as local anti-inflammatory protection and a means of providing energy supply to the myocytes.

To summarize, adiponectin has anti-inflammatory actions in a variety of tissues. These actions include direct effects on monocyte/macrophages, endothelial

cells, hepatic and muscle cells plus indirect effects via inhibition of TNF-α production and action.

Therapeutic Potential and Conclusions

The strong association between hypoadiponectinaemia and metabolic dysfunction [28,94–97] provides the perfect scenario for adiponectin (replacement) therapy [93]. Cellular and animal studies provide proof of concept of the benefit of adiponectin therapy in multiple disease states [57,76,111,112]. In man, such therapy could involve identification of individuals with metabolic dysfunction and hypoadiponectinemia followed by restoration of lean 'healthy' adiponectin levels by administration of recombinant hormone.

An alternative approach is to provide therapies that have an effect to increase adiponectin levels. The TZD class of compounds is in widespread clinical use for Type 2 diabetes, and one of the effects of these drugs is to increase adiponectin production and circulating levels [54,113]. These drugs are given orally, but it is not clear to what extent the efficacy of the compounds is adiponectin mediated. An additional question is whether provision of supra-physiological (or pharmacological) concentrations of adiponectin will be even more beneficial than providing normal levels.

A further complexity arises when one considers what form of adiponectin to provide as therapy. As outlined above, HMW multimers appear to provide more 'metabolic' benefit than do the LMW oligomers. Therefore, one strategy might be to change the distribution of existing adiponectin rather than increase total circulating levels. Interestingly, the TZDs appear to preferentially promote production of the beneficial HMW forms [24].

It is not yet clear whether the distribution of adiponectin multimers (which gives the S_A) or the absolute amount of specific adiponectin complexes is the most important parameter. For example, it may be that a low-circulating concentration of adiponectin, which is predominantly HMW, multimer provides greater metabolic advantage than a high-circulating concentration of adiponectin, with relatively low amounts of the HMW form. This question needs to be addressed in more detail, although work by Matsuzawa's group suggests that the absolute amount of HMW multimer may be the important parameter [57].

Overall, the prospect of adiponectin therapy, or regulation of adiponectin multimer distribution, production, clearance or action is an exciting one for the treatment of many facets of the metabolic syndrome.

References

1 Zhang Y, Proenca R, Maffei M *et al.* Positional cloning of the mouse obese gene and its human homologue. Nature 1994; **372**: 425–432.

2 Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995; **270**: 26746–26749.

3 Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996; **271**: 10697–10703.

4 Maeda K, Okubo K, Shimomura I *et al.* cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 1996; **221**: 286–289.

5 Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem (Tokyo) 1996; **120**: 803–812.

6 Arita Y, Kihara S, Ouchi N *et al.* Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999; **257**: 79–83.

7 Hotta K, Funahashi T, Arita Y *et al.* Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000; **20**: 1595–1599.

8 Weyer C, Funahashi T, Tanaka S *et al.* Hypoadiponectinemia in Obesity and Type 2 Diabetes: Close Association with Insulin Resistance and Hyperinsulinemia. J Clin Endocrinol Metab 2001; **86**: 1930–1935.

9 Bacha F, Saad R, Gungor N, Arslanian SA. Adiponectin in youth. relationship to visceral adiposity, insulin sensitivity, and beta-cell function. Diabetes Care 2004; **27**: 547–552.

10 Goldstein BJ, Scalia R. Adiponectin. A Novel Adipokine Linking Adipocytes and Vascular Function. J Clin Endocrinol Metab 2004; **89**: 2563–2568.

11 Nawrocki AR, Scherer PE. The delicate balance between fat and muscle: adipokines in metabolic disease and musculoskeletal inflammation. Curr Opin Pharmacol 2004; **4**: 281–289.

12 Haluzik M, Parizkova J, Haluzik MM. Adiponectin and its role in the obesity-induced insulin resistance and related complications. Physiol Res 2004; **53**: 123–129.

13 Shimada K, Miyazaki T, Daida H. Adiponectin and atherosclerotic disease. Clin Chim Acta 2004; **344**: 1–12.

14 Zhang Z, Henzel WJ. Signal peptide prediction based on analysis of experimentally verified cleavage sites. Protein Sci 2004; **13**: 2819–2824.

15 Shapiro L, Scherer PE. The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. Curr Biol 1998; **8**: 335–340.

16 Pajvani UBX, Combs TP *et al.* Structure-Function Studies of the Adipocyte-secreted Hormone Acrp30/

Adiponectin. Implications for metabolic regulation and bioactivity. J Biol Chem 2003; **278**: 9073–9085.
17 Tsao T-S, Tomas E, Murrey HE *et al.* Role of Disulfide Bonds in Acrp30/Adiponectin Structure and Signaling Specificity: Different oligomers activate different signal transduction pathways. J Biol Chem 2003; **278**: 50810–50817.
18 Waki H, Yamauchi T, Kamon J *et al.* Impaired multimerization of human adiponectin mutants associated with diabetes: Molecular structure and multimer formation of adiponectin. J Biol Chem 2003; **278**: 40352–40363.
19 Wang Y, Xu A, Knight C, Xu LY, Cooper GJS. Hydroxylation and Glycosylation of the Four Conserved Lysine Residues in the Collagenous Domain of Adiponectin. Potential role in the modulation of its insulin-sensitizing activity. J Biol Chem 2002; **277**: 19521–19529.
20 Wang Y, Lu G, Wong WP *et al.* Proteomic and functional characterization of endogenous adiponectin purified from fetal bovine serum. Proteomics 2004; **4**: 3933–3942.
21 Sato C, Yasukawa Z, Honda N, Matsuda T, Kitajima K. Identification and adipocyte differentiation-dependent expression of the unique disialic acid residue in an adipose tissue-specific glycoprotein, adipo Q. J Biol Chem 2001; **276**: 28849–28856.
22 Tsao TS, Murrey HE, Hug C, Lee DH, Lodish HF. Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). J Biol Chem 2002; **277**: 29359–29362.
23 Zhang Y, Matheny M, Zolotukhin S, Tumer N, Scarpace PJ. Regulation of adiponectin and leptin gene expression in white and brown adipose tissues: influence of beta 3-adrenergic agonists, retinoic acid, leptin and fasting. Biochim Biophys Acta 2002; **1584**: 115–122.
24 Pajvani UB, Hawkins M, Combs TP *et al.* Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione -mediated improvement in insulin sensitivity. J Biol Chem 2004; **279**: 12152–12162.
25 Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004; **145**: 2273–2282.
26 Motoshima H, Wu X, Sinha MK *et al.* Differential Regulation of Adiponectin Secretion from Cultured Human Omental and Subcutaneous Adipocytes: Effects of Insulin and Rosiglitazone. J Clin Endocrinol Metab 2002; **87**: 5662–5667.
27 Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes 2003; **52**: 1779–1785.
28 Cnop M, Havel PJ, Utzschneider KM *et al.* Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. Diabetologia 2003; **46**: 459–469.
29 Combs TP, Berg AH, Rajala MW *et al.* Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. Diabetes 2003; **52**: 268–276.
30 Yoda-Murakami M, Taniguchi M, Takahashi K *et al.* Change in expression of GBP28/adiponectin in carbon tetrachloride-administrated mouse liver. Biochem Biophys Res Commun 2001; **285**: 372–377.
31 Kaser S, Moschen A, Cayon A *et al.* Adiponectin and its receptors in non-alcoholic steatohepatitis. Gut 2005; **54**: 117–121.
32 Staiger H, Kausch C, Guirguis A *et al.* Induction of adiponectin gene expression in human myotubes by an adiponectin-containing HEK293 cell culture supernatant. Diabetologia 2003; **46**: 956–960.
33 Delaigle AM, Jonas JC, Bauche IB, Cornu O, Brichard SM. Induction of adiponectin in skeletal muscle by inflammatory cytokines: in vivo and in vitro studies. Endocrinology 2004; **145**: 5589–5597.
34 Berner HS, Lyngstadaas SP, Spahr A *et al.* Adiponectin and its receptors are expressed in bone-forming cells. Bone 2004; **35**: 842–849.
35 Park S-k, Oh S-Y, Lee M-Y *et al.* CCAAT/enhancer binding protein nuclear factor-y regulate adiponectin gene expression adipose tissue. Diabetes 2004; **53**: 2757–2766.
36 Gavrila A, Peng CK, Chan JL *et al.* Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. J Clin Endocrinol Metab 2003; **88**: 2838–2843.
37 Calvani M, Scarfone A, Granato L *et al.* Restoration adiponectin pulsatility severely obese subjects after weight loss. Diabetes 2004; **53**: 939–947.
38 Yang WS, Lee WJ, Funahashi T *et al.* Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 2001; **86**: 3815–3819.
39 Berg AH, Du Combs TPX, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001; **7**: 947–953.
40 Imbeault P, Pomerleau M, Harper ME, Doucet E. Unchanged fasting and postprandial adiponectin levels following a 4-day caloric restriction in young healthy men. Clin Endocrinol (Oxf) 2004; **60**: 429–433.
41 Abbasi F, Chu JW, Lamendola C *et al.* Discrimination between obesity insulin resistance relationship with adiponectin. Diabetes 2004; **53**: 585–590.
42 Kotani Y, Yokota I, Kitamura S *et al.* Plasma adiponectin levels in newborns are higher than those in adults and positively correlated with birth weight. Clin Endocrinol (Oxf) 2004; **61**: 418–423.
43 Stefan N, Bunt JC, Salbe AD *et al.* Plasma adiponectin concentrations in children: relationships with obesity

and insulinemia. J Clin Endocrinol Metab 2002; **87**: 4652–4656.
44 Bogan JS, Lodish HF. Two compartments for insulin-stimulated exocytosis in 3T3-L1 adipocytes defined by endogenous ACRP30 and GLUT4. J Cell Biol 1999; **146**: 609–620.
45 Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2002; **290**: 1084–1089.
46 Halleux CM, Takahashi M, Delporte ML *et al.* Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. Biochem Biophys Res Commun 2001; **288**: 1102–1107.
47 Maeda N, Takahashi M, Funahashi T *et al.* PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001; **50**: 2094–2099.
48 Maeda N, Shimomura I, Kishida K *et al.* Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002; **8**: 731–737.
49 Fasshauer M, Kralisch S, Klier M *et al.* Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2003; **301**: 1045–1050.
50 Clarke KJ, Zhong Q, Schwartz DD *et al.* Regulation of adiponectin secretion by endothelin-1. Biochem Biophys Res Commun 2003; **312**: 945–949.
51 Phillips SA, Ciaraldi TP, Kong AP *et al.* Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. Diabetes 2003; **52**: 667–674.
52 Tiikkainen M, Hakkinen A-M, Korsheninnikova E *et al.* Effects of Rosiglitazone and Metformin on Liver Fat Content, Hepatic Insulin Resistance, Insulin Clearance, and Gene Expression in Adipose Tissue in Patients With Type 2 Diabetes. Diabetes 2004; **53**: 2169–2176.
53 Combs TP, Wagner JA, Berger J *et al.* Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. Endocrinology 2002; **143**: 998–1007.
54 Tonelli J, Li W, Kishore P *et al.* Mechanisms early insulin-sensitizing effects thiazolidinediones type 2 diabetes. Diabetes 2004; **53**: 1621–1629.
55 Yamauchi T, Kamon J, Waki H *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001; **7**: 941–946.
56 Iwaki M, Matsuda M, Maeda N *et al.* Induction of adiponectin, a fat-derived antidiabetic antiatherogenic factor, by nuclear receptors. Diabetes 2003; **52**: 1655–1663.
57 Kobayashi H, Ouchi N, Kihara S *et al.* Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. Circ Res 2004; **94**: 27–31.
58 Kamon J, Yamauchi T, Muto S *et al.* A novel IKK[beta] inhibitor stimulates adiponectin levels and ameliorates obesity-linked insulin resistance. Biochem Biophys Res Comms 2004; **323**: 242–248.
59 Hogg PJ. Disulfide bonds as switches for protein function. Trends Biochem Sci 2003; **28**: 210–214.
60 Fruebis J, Tsao TS, Javorschi S *et al.* Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 2001; **98**: 2005–2010.
61 Waki H, Yamauchi T, Kamon J *et al.* Generation of globular fragment of adiponectin by leukocyte elastase secreted by monocytic cell line THP-1.Endocrinology 2004; **146**: 790–796.
62 Koshimura J, Fujita H, Narita T *et al.* Urinary adiponectin excretion is increased in patients with overt diabetic nephropathy. Biochem Biophys Res Comms 2004; **316**: 165–169.
63 Tschritter O, Fritsche A, Thamer C *et al.* Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 2003; **52**: 239–243.
64 Hotta K, Funahashi T, Bodkin NL *et al.* Circulating Concentrations of the Adipocyte Protein Adiponectin Are Decreased in Parallel With Reduced Insulin Sensitivity During the Progression to Type 2 Diabetes in Rhesus Monkeys. Diabetes 2001; **50**: 1126–1133.
65 Spranger J, Kroke A, Mohlig M *et al.* Adiponectin and protection against type 2 diabetes mellitus. Lancet 2003; **361**: 226–228.
66 Kissebah AH, Sonnenberg GE, Myklebust J *et al.* Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. Proc Natl Acad Sci USA 2000; **97**: 14478–14483.
67 Vionnet N, Hani El H, Dupont S *et al.* Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. Am J Hum Genet 2000; **67**: 1470–1480.
68 Hara K, Boutin P, Mori Y *et al.* Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 2002; **51**: 536–540.
69 Vasseur F, Helbecque N, Dina C *et al.* Single-nucleotide polymorphism haplotypes in the both proximal promoter and exon 3 of the APM1 gene modulate adipocyte-secreted adiponectin hormone levels and contribute to the genetic risk for type 2 diabetes in French Caucasians. Hum Mol Genet 2002; **11**: 2607–2614.
70 Gu HF, Abulaiti A, Ostenson CG *et al.* Single nucleotide polymorphisms in the proximal promoter region of the adiponectin (APM1) gene are associated with type 2 diabetes in Swedish caucasians. Diabetes 2004; **53** (Suppl. 1): S31–S35.

71 Gibson F, Froguel P. Genetics of the APM1 Locus and Its Contribution to Type 2 Diabetes Susceptibility in French Caucasians. Diabetes 2004; **53**: 2977–2983.
72 Kondo H, Shimomura I, Matsukawa Y *et al.* Association of adiponectin mutation with type 2 diabetes: a candidate gene for the insulin resistance syndrome. Diabetes 2002; **51**: 2325–2328.
73 Tomas E, Tsao TS, Saha AK *et al.* Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci USA 2002; **99**: 16309–16313.
74 Wu X, Motoshima H, Mahadev K *et al.* Involvement of AMP-Activated Protein Kinase in Glucose Uptake Stimulated by the Globular Domain of Adiponectin in Primary Rat Adipocytes. Diabetes 2003; **52**: 1355–1363.
75 Combs TP, Berg AH, Obici S, Scherer PE, Rossetti L. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 2001; **108**: 1875–1881.
76 Xu A, Wang Y, Keshaw H *et al.* The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 2003; **112**: 91–100.
77 Qi Y, Takahashi N, Hileman SM *et al.* Adiponectin acts in the brain to decrease body weight. Nat Med 2004; **10**: 524–529.
78 Kubota N, Terauchi Y, Yamauchi T *et al.* Disruption adiponectin causes insulin resistance neointimal formation. J Biol Chem 2002; **277**: 25863–25866.
79 Ma K, Cabrero A, Saha PK *et al.* Increased beta -oxidation but no insulin resistance or glucose intolerance in mice lacking adiponectin. J Biol Chem 2002; **277**: 34658–34661.
80 Shibata R, Ouchi N, Kihara S *et al.* Adiponectin stimulates angiogenesis in response to tissue ischemia through stimulation of AMP-activated protein kinase signaling. J Biol Chem 2004; **279**: 28670–28674
81 Combs TP, Pajvani UB, Berg AH *et al.* A transgenic mouse with a deletion in the collagenous domain of adiponectin displays elevated circulating adiponectin and improved insulin sensitivity. Endocrinology 2004; **145**: 367–383.
82 Yamauchi T, Kamon J, Ito Y *et al.* Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003; **423**: 762–769.
83 Civitarese AE, Jenkinson CP, Richardson D *et al.* Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. Diabetologia 2004; **47**: 816–820.
84 Narasimhan ML, Coca MA, Jin J *et al.* Osmotin Is a Homolog of Mammalian Adiponectin and Controls Apoptosis in Yeast through a Homolog of Mammalian Adiponectin Receptor. Mol Cell 2005; **17**: 171–180.
85 Tsuchida A, Yamauchi T, Ito Y *et al.* Insulin/foxo1 pathway regulates expression levels adiponectin receptors adiponectin sensitivity. J Biol Chem 2004; **279**: 30817–30822.
86 Staiger H, Kaltenbach S, Staiger K *et al.* Expression of adiponectin receptor mrna in human skeletal muscle cells is related to in vivo parameters of glucose and lipid metabolism. Diabetes 2004; **53**: 2195–2201.
87 Fasshauer M, Klein J, Kralisch S *et al.* Growth hormone is a positive regulator of adiponectin receptor 2 in 3T3-L1 adipocytes. FEBS Lett 2004; **558**: 27–32.
88 Reseland JE, Syversen U, Bakke I *et al.* Leptin is expressed in and secreted from primary cultures of human osteoblasts and promotes bone mineralization. J Bone Miner Res 2001; **16**: 1426–1433.
89 Chinetti G, Zawadski C, Fruchart JC, Staels B. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPAR[alpha], PPAR[gamma], and LXR. Biochem Biophys Res Comms 2004; **314**: 151–158.
90 Kharroubi I, Rasschaert J, Eizirik DL, Cnop M. Expression of adiponectin receptors in pancreatic beta cells. Biochem Biophys Res Commun 2003; **312**: 1118–1122.
91 Hug C, Wang J, Ahmad NS *et al.* T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. PNAS 2004, 0403382101.
92 Ouchi N, Kihara S, Funahashi T *et al.* Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. Circulation 2003; **107**: 671–674.
93 Chandran M, Phillips SA, Ciaraldi T, Henry RR. Adiponectin: more than just another fat cell hormone? Diabetes Care 2003; **26**: 2442–2450.
94 Iwashima Y, Katsuya T, Ishikawa K *et al.* Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension 2004; **43**: 1318–1323.
95 Ryo M, Nakamura T, Kihara S *et al.* Adiponectin as a biomarker of the metabolic syndrome. Circ J 2004; **68**: 975–981.
96 Schulze MB, Rimm EB, Shai I, Rifai N, Hu FB. Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. Diabetes Care 2004; **27**: 1680–1687.
97 Shimabukuro M, Higa N, Asahi T *et al.* Hypoadiponectinemia is closely linked to endothelial dysfunction in man. J Clin Endocrinol Metab 2003; **88**: 3236–3240.
98 Ohashi K, Ouchi N, Kihara S *et al.* Adiponectin I164T mutation is associated with the metabolic syndrome and coronary artery disease. J Am Coll Cardiol 2004; **43**: 1195–1200.
99 Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. Obesity, adiponectin and vascular inflammatory disease. Curr Opin Lipidol 2003; **14**: 561–566.
100 Okamoto Y, Kihara S, Ouchi N *et al.* Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 2002; **106**: 2767–2770.
101 Targher G, Bertolini L, Scala L *et al.* Decreased plasma adiponectin concentrations are closely associated with

nonalcoholic hepatic steatosis in obese individuals. Clin Endocrinol (Oxf) 2004; **61**: 700–703.

102 Okamoto Y, Arita Y, Nishida M *et al.* An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res 2000; **32**: 47–50.

103 Ouchi N, Kihara S, Arita Y *et al.* Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999; **100**: 2473–2476.

104 Ouchi N, Kihara S, Arita Y *et al.* Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000; **102**: 1296–1301.

105 Ouchi N, Kihara S, Arita Y *et al.* Adipocyte-derived plasma protein, adiponectin, suppresses lipid accumulation and class A scavenger receptor expression in human monocyte-derived macrophages. Circulation 2001; **103**: 1057–1063.

106 Yokota T, Oritani K, Takahashi I *et al.* Adiponectin, a new member of the family of soluble defense collagens, negatively regulates the growth of myelomonocytic progenitors and the functions of macrophages. Blood 2000; **96**: 1723–1732.

107 Brakenhielm E, Veitonmaki N, Cao R *et al.* Adiponectin-induced antiangiogenesis and antitumor activity involve caspase-mediated endothelial cell apoptosis. Proc Natl Acad Sci USA 2004; **101**: 2476–2481.

108 Matsuda M, Shimomura I, Sata M *et al.* Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J Biol Chem 2002; **277**: 37487–37491.

109 Hui JM, Hodge A, Farrell GC *et al.* Beyond insulin resistance in NASH. TNF-alpha or adiponectin? Hepatology 2004; **40**: 46–54.

110 Chitturi S, Abeygunasekera S, Farrell GC *et al.* NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002; **35**: 373–379.

111 Shibata R, Ouchi N, Ito M *et al.* Adiponectin-med modulation hypertrophic signals heart. Nat Med 2004; **10**: 1384–1389.

112 Yamauchi T, Kamon J, Waki H *et al.* Globular adiponectin protected ob/ob mice from diabetes and ApoE-deficient mice from atherosclerosis. J Biol Chem 2003; **278**: 2461–2468.

113 Moller DE, Berger JP. Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. Int J Obes Relat Metab Disord 2003; **27** (Suppl. 3): S17–S21.

