

Review

# Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease

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## Abstract

The increasing prevalence of obesity and metabolic syndrome/insulin resistance has attracted considerable interest due to their identification as risk factors for cardiovascular disease and, hence, targets for cardiovascular disease prevention. This review focuses on adiponectin, the most profusely secreted protein from adipose tissue, which itself is being increasingly recognised as an important and very active endocrine organ, secreting a wide range of biologically active substances known as adipokines or adipocytokines. Adiponectin has been demonstrated to have insulin sensitising effects, and secretion of adiponectin is reduced as adipose tissue mass increases. Adiponectin has also been demonstrated to have anti-inflammatory and anti-atherogenic properties, and is independently associated with cardiovascular disease. The evidence that suggests adiponectin plays a role in the relationship between obesity and insulin resistance, and also insulin resistance and cardiovascular disease, is examined. Variation in the adiponectin gene is one tool to determine whether this relationship is causal. The association of identified variants with human disease, specifically obesity and its consequences, type 2 diabetes and cardiovascular disease is reviewed. This data may enable patients at greater risk of the adverse effects of obesity to be identified and, as such, benefit from more targeted therapy of its consequences. © 2006 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Adiponectin; Single nucleotide polymorphism; Metabolic syndrome; Type 2 diabetes mellitus; Cardiovascular disease

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**1. Introduction**

Obesity is reaching epidemic proportions in developed nations [1]. This major adverse impact on health is partly due to the induction of metabolic derangements which form a cluster of cardiovascular risk factors. This cluster has been termed the metabolic syndrome. An attempt to define this syndrome was first made by the World Health Organisation in 1998, of which abnormal glucose tolerance was a key factor. A clinical definition of metabolic syndrome was produced by the National Cholesterol Education Program—adult treatment program III in 2001, which includes abdominal obesity, dyslipidaemia, hypertension, insulin resistance and prothrombotic and inflammatory states [2]. The metabolic syndrome was identified as a target for cardiovascular risk reduction, as both cardiovascular disease and all-cause mortality are increased in this syndrome [3]. There is, however, currently much debate if current definitions add any cardiovascular disease risk above the individual components or other primary risk factors not included in the definition. This would include factors such as smoking and LDL cholesterol which also tend to cluster with hypertension, etc. [4,5].

Excess body fat is stored in adipose tissue which forms over 10% of total body weight, but it is now clear that adipocytes have functions other than simple storage cells [6]. The most significant of these appears to be the secretory

capacity of the adipocyte. The adipocyte secretes a number of peptides that have been labelled adipocytokines or adipokines. Adipokines identified to date seem to function as modulators of metabolism, such as leptin and resistin, or of inflammation, such as tumour necrosis factor  $\alpha$  (TNF), interleukin 6, adipsin (also known as complement factor D), acetylation-stimulating protein, visfatin (also known as B-cell colony-enhancing factor), plasminogen-activator inhibitor type 1 as well as other complement components and interleukins [6,7]. The most abundantly secreted adipokine is adiponectin, making up 0.01% of circulating protein with serum concentration a thousand times greater than other hormones and  $10^6$  greater than other inflammatory cytokines [8].

**2. Adiponectin**

Adiponectin is induced during adipocyte differentiation and is secreted only by differentiated adipocytes, with higher levels secreted from subcutaneous fat compared to visceral fat [9]. The protein consists of 244 amino acids (30 kDa) and has three distinct domains as shown in Fig. 1, namely, a signal sequence with a variable region domain, a collagen triple helix and a globular head domain which is similar in structure to TNF and the complement factor C1q [10]. Adiponectin is part of a family of proteins which contain this globular C-

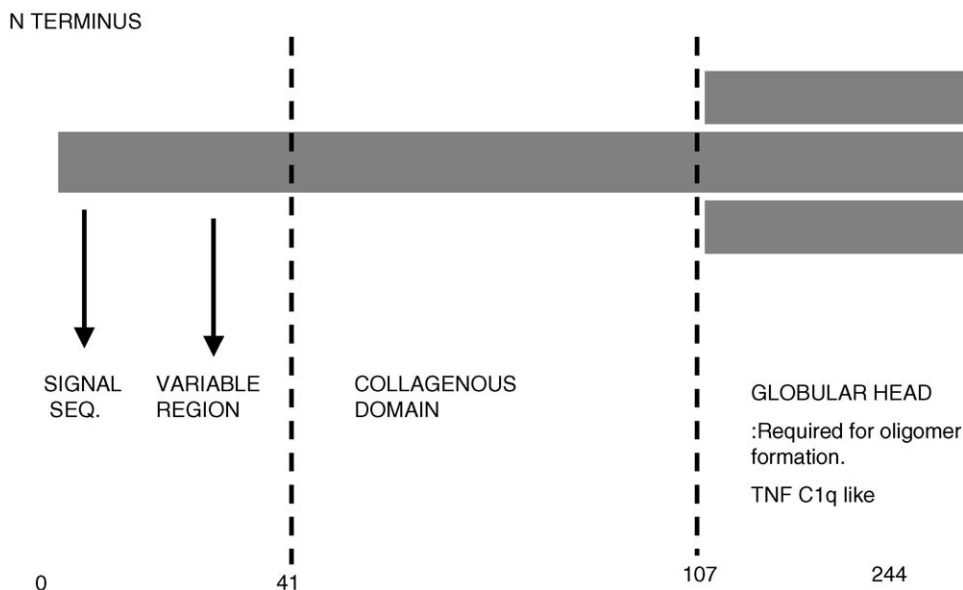


Fig. 1. The structural domains of the adipokine adiponectin.

terminal C1q-like domain, often with a variable number of collagenous repeats, including seven other adiponectin like molecules (known as C1q/TNF related proteins [CTRP<sub>s</sub>]) [11] as well as various hibernating proteins, lung surfactant A, mannose-binding protein and the macrophage scavenger receptor family [12]. The globular domain also has structural homology with the TNF-ligand family and to genes expressed in activated T-cells [11]. This C1q/TNF superfamily, collectively known as the soluble collagen defence molecules, are mostly part of the innate immune system. The family is said to have derived from a recognition molecule of the ancient innate immune system that later diverged into the C1q and TNF lines [12]. Secreted monomeric adiponectin is not observed in the circulation, as the monomers associate at the globular domain via disulphide bonds between two cysteine residues [13]. There are two main circulating forms, a hexamer of 180 kDa (LMW Ad) and a larger multimer of approx 400 kDa (HMW Ad) [13]. A small amount of the very pharmacologically active globular domain, possibly resulting from proteolytic cleavage, has also been described [14]. The two multimeric forms may have different functions and binding properties which will be discussed later. Two specific adiponectin receptors have so far been identified [15], and adiponectin binding to T-cadherin has also been demonstrated [16]. The specific receptors have been designated AdipoR1 and AdipoR2 and their structure and function has recently been reviewed elsewhere [17]. Simply, these receptors are loosely related to the seven transmembrane spanning receptor family, although are unique in the fact that the N-terminus is intracellular, the significance of which is not known [15]. AdipoR1 has been identified in skeletal muscle with moderate expression elsewhere, with AdipoR2 mainly expressed in liver, but both receptors have been identified in endothelial cells [15].

### 3. Adiponectin and insulin resistance/metabolic syndrome

#### 3.1. Animal models

Adiponectin knockout mice have been developed, which when fed normal chow showed no difference in body weight or adiposity at 16 or 30 weeks, although subtle differences in fat metabolism could be detected including decreased clearance of free fatty acids. However, the adiponectin knockout mice developed severe insulin resistance when fed with a high sucrose/fat diet, despite no difference in weight gain compared with wild type mice fed the same diet [18]. The insulin resistance in obese and lipoatrophic mouse models can be partially normalised by administration of recombinant adiponectin [19]. Metabolic derangement in insulin resistant states is also a recognised cause of hepatomegaly and abnormal liver function tests (non-alcoholic fatty liver disease [NAFLD]). In Ob/Ob mice, a model of obesity due to leptin deficiency, the administration of adiponectin reduces

liver size, abnormal liver function tests and fat content on liver biopsy, compared to untreated mice [20]. Furthermore, in models of diabetes, hyperglycaemia can be reduced by the administration of adiponectin, an effect that does not depend on the modulation of insulin concentrations [19]. This effect appears to be modulated not only by upregulation of insulin signalling, but also by regulation of free fatty acid utilisation and transcription of gluconeogenic enzymes such as glucose-6-phosphatase and PEPCK [21]. Therefore, it appears that adiponectin has a role in the development of insulin resistance in some circumstances, particularly when nutrition is overloaded.

#### 3.2. Human studies

Adiponectin circulates at a concentration of between 3 and 30 µg/ml [8], increases with age [22] and is higher in women than men [13]. The difference between men and women is thought to be a direct effect of androgens on adiponectin synthesis [23]. Serum adiponectin is lower in those with obesity [8] and type 2 diabetes [24] and increases with weight reduction [25]. The adiponectin gene also contains response elements for peroxisome proliferator-activator receptor  $\gamma$  (PPAR $\gamma$ ) [26], a key regulator of glucose and lipid metabolism. Adiponectin transcription is also responsive to glucocorticoids, prolactin, growth hormone and catecholamines, via the  $\beta$  receptor. These endogenous hormones all have actions which include significant modulation of insulin sensitivity [27–29]. Serum adiponectin is negatively correlated with the adverse features of the metabolic syndrome as well as other associated features of insulin resistance and conventional cardiovascular risk factors. This includes serum insulin, total cholesterol, low density lipoprotein (LDL), apolipoprotein B-100, triglycerides, plasma glucose, HbA1c as well as lower high density lipoprotein (HDL) and smaller LDL particle size [30–45]. A similar relationship has been shown with liver function tests and hepatic fat content in a “healthy” obese cohort [46], and levels were also predictive of future hepatitis in NAFLD [47]. Serum adiponectin levels are negatively correlated with body mass index (BMI) [33,34,48], waist circumference [45], waist hip ratio [49], intra-abdominal fat [50] and percentage body fat [36,51]. Blood glucose levels in oral glucose tolerance testing [48], the insulin sensitivity indices HOMA [30,33,36,51] (HOMeostasis Model Assessment) and QUICKI [36] (QUAntitative Insulin Sensitivity Index) as well as insulin sensitivity markers in frequently sampled intravenous glucose tolerance testing [39,41,42] and clamp studies [48,52] are positively correlated with serum adiponectin. These relationships are independent of adiposity, BMI and hyperglycaemia. In humans, or human cell lines adiponectin has been found to increase insulin signalling efficiency [53], predict intra-hepatic [46] and muscle triglyceride content [54], decrease liver glucose production [21], increase the capacity for fat oxidation [21,55] and increase muscle glucose uptake and utilisation [21,50,56].

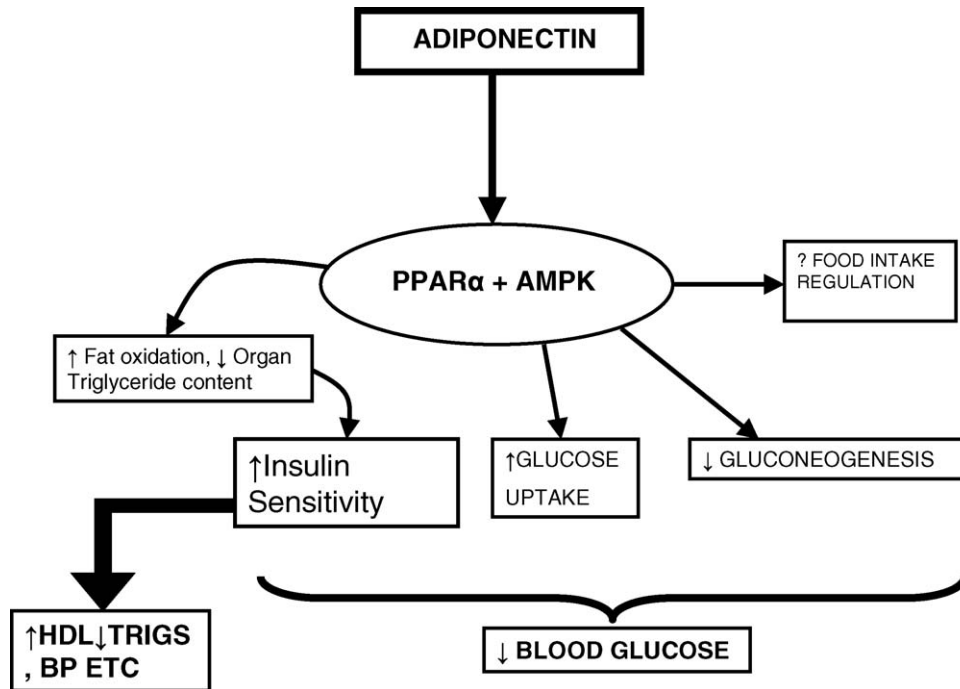


Fig. 2. A summary of the action of adiponectin on metabolism via its effect on AMPK and PPAR- $\alpha$ .

### 3.3. Mechanism of action

Studies from animal models and human subjects confirm that adiponectin is an insulin sensitising hormone that is negatively regulated by obesity. The production of adiponectin may be inhibited by a paracrine action of TNF whose secretion increases with increasing fat mass [57]. A significant number of the metabolic actions of adiponectin are dependent on the activation of AMP-dependent kinase (AMPK) [21,55]. AMPK is a fuel-sensing enzyme [58] and is activated by adiponectin, probably through a cAMP-dependent pathway [21]. AMPK-dependent fuel-sensing systems have been identified in myocytes, hepatocytes, skeletal muscle and parts of the central nervous system [59]. AMPK is activated when ATP is required, and one of its main stimulators is the AMP/ATP ratio, although it has been suggested that AMP kinase activation is the final common pathway of a number of insulin sensitizers including leptin and metformin [59]. As shown in Fig. 2, activation increases glucose uptake, reduces hepatic glucose production and increases fatty acid oxidation with the aim of increasing ATP production. The reduction of hepatic and skeletal muscle triglyceride that occurs increases insulin sensitivity [19]. The addition of a specific AMPK inhibitor to animal models or cell experiments significantly reduces any effect of adiponectin observed [21].

Adiponectin also enhances the transcription of other genes involved in fatty acid metabolism, most notably peroxisome proliferator-activated receptor- $\alpha$  (PPAR $\alpha$ ) [15], the target of the fibrate group of lipid lowering agents. Activation of PPAR $\alpha$  leads to an increase in levels of molecules involved in free fatty acid transport, such as CD36, and energy dissipa-

tion, such as uncoupling protein-2 [19] which also increases fatty acid oxidation. Total energy expenditure, especially under hyperinsulinaemic conditions, increases with serum adiponectin [50]. The rate of fat oxidation appears to play an important role in determining body weight as it is lower in pre-obese or formerly obese people when compared to controls of the same weight [60,61]. Intra-organ fat deposition appears to predict those at highest risk of the consequences of obesity. In obese children, those who had impaired glucose tolerance had higher intra-muscular and visceral fat than those who did not, despite similar BMI [62]. The accumulation of intra-muscular and visceral fatty acids, which has been implicated in reducing insulin sensitivity as well as other cellular functions, is known as “lipotoxicity” [6].

Hence, most if not all of the metabolic effects of adiponectin can be explained directly or indirectly by increased fatty acid oxidation and reduction in intra-organ triglyceride. Whilst the mechanism of action of adiponectin is partially understood little is known about the exact physiological role of adiponectin in metabolism [63]. The secretion of adiponectin is influenced by adipose tissue mass, and it may be that adiponectin is part of a system to regulate adipocyte size or adipose tissue mass. In the lean state adiponectin induces uptake of fatty acids into storage depots, whilst, when the adipocyte reaches a certain size, secretion is switched off to prevent further lipid accumulation [56]. Further intake or availability of fatty acids means that they will be stored elsewhere, with the following adverse effects on metabolism. This overnutrition can also cause activation of the innate immune system, impairment of insulin signalling and hyperglycaemia [64]. It is possible modula-

tion of adiponectin secretion is also part of this chain of events.

#### 4. Atheroma and inflammation

The mechanisms responsible for the atheromatous occlusion of the coronary and other arteries have yet to be fully elucidated. A detailed discussion is beyond the scope of this review and has recently been reviewed elsewhere [65]. However, inflammation and endothelial injury caused by exposure to cardiovascular risk factors, such as the features of the metabolic syndrome, appear to be important initiating events inducing endothelial injury, possibly by a mechanism inducing oxidative stress. Endothelial injury is followed by adherence and migration of leukocytes into the injured arterial wall. Inflammatory cytokines and cell signalling via adhesion molecules play important roles in this process. Transformation of these cells into macrophages and foam cells, and accompanying changes to the surrounding matrix and smooth muscle, lead to plaque formation. Stress signalling plays a significant role in the maintenance of inflammation, and may be important in a number of steps in the development of coronary artery disease (CAD). Adiponectin may modulate this process indirectly via its effect on conventional risk factors, but as it is derived from ancient immune system molecules, it is plausible that it would also be able to modulate this inflammatory response and have a direct anti-atherogenic effect.

##### 4.1. Adiponectin and animal models of atheroma

When the adiponectin gene is over expressed in atheroma models, Ob/Ob and the Apo E deficient mice, rates of CAD are reduced by 30% and rates of diabetes are also reduced [66]. Adiponectin knockout mice have higher systolic blood pressure when fed with a high sucrose/high fat diet and demonstrate increased stenosis after mechanical injury and decreased vasodilatation to nitric oxide (NO) donors compared to wild type mice [67]. Knockout mice also mimic the pathological myocardial remodelling seen in obesity. There is an exaggerated response to pressure load/angiotensin II infusion, with increased left ventricular thickness, intraventricular thickness, concentric hypertrophy and mortality observed in knockout mice, all of which is attenuated if the adiponectin gene is knocked back in [68]. These effects were independent of any other effects on weight, adipose tissue or metabolic parameters, and suggest that adiponectin has a direct anti-atherogenic effect.

##### 4.2. Adiponectin, atheroma and human studies

There are a number of non-invasive tests of very early CAD which are predictive of future cardiovascular disease. One of these is brachial artery flow mediated dilatation (FMD) which measures the degree of impairment of arteries to dilate in response to high flow, and estimates

vascular health. Lower serum adiponectin was correlated with impaired endothelial-dependent and -independent FMD in healthy subjects [69,70] and with endothelial-dependent FMD in both subjects with hypertension [67] and in those with type 2 diabetes [71] (T2DM). Alternative vascular biology techniques predictive of future macrovascular events including pulse wave velocity [33] and carotid artery inter-medial thickness [37] have also been found to be negatively correlated with serum adiponectin, although the effect on pulse wave velocity was not independent of the changes to insulin sensitivity with variation in serum adiponectin.

Serum adiponectin is lower in patients with CAD, as well as in those with T2DM, compared to controls but it is even lower in patients with T2DM with CAD [24]. Clearance of adiponectin from the circulation is mainly renal-dependent, and levels increase with the development of chronic renal failure and in those undergoing renal replacement therapy [72]. However, even within subjects with reduced renal clearance, mean serum adiponectin is lower in those with CAD, and can predict length of survival on dialysis [73]. Serum adiponectin is independently, negatively correlated with C-reactive protein, interleukin-6, PA-1, tPA, uric acid, fibrinogen and blood pressure [33–35,40,43,74,73,75–78]. The relationship with lipid risk factors has been described but adiponectin is also related to other CAD risk factors. Subjects with lower levels of adiponectin have longer QT intervals on resting ECG, an independent predictor of CAD [79], and adiponectin levels are lowered by smoking [80], probably through a direct effect mediated through nicotinic acetylcholine receptors [81]. Epicardial fat, representative of visceral fat, removed at operation was found to have 40% lower adiponectin mRNA expression if the patient had CAD [82]. Serum adiponectin is lower at acute coronary syndrome presentation compared to patients with stable CAD [32], and the persistence of this low level is predictive of long-term outcome [83].

In case/control studies lower serum adiponectin was associated with higher urinary isoprostanes (a marker of oxidative stress damage thought to be a marker of early atheroma events) in subjects with normal glucose tolerance, although not in a cohort with abnormal glucose tolerance [84]. Lower serum adiponectin levels have been associated with cerebrovascular disease [85], peripheral vascular disease [86] and CAD [87–89]. A serum adiponectin level of less than 4.0  $\mu\text{g/ml}$  was associated with a doubling of CAD risk, an effect independent of known CAD risk factors [87]. In the prospective Health Professionals Follow Up study of healthy men, those in the highest adiponectin quintile had a significantly lower risk of myocardial infarction, even when corrected for differences in lipid profile, family history or glycaemic status [88]. The protective effect of adiponectin was also seen in a prospective study of subjects with type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Complications study [89] identified a 70% reduction in risk between 1st and 4th quartile. This reduction in risk was independent of conventional risk factors, markers of insulin resistance and markers of inflammation. A comparison of the impact of

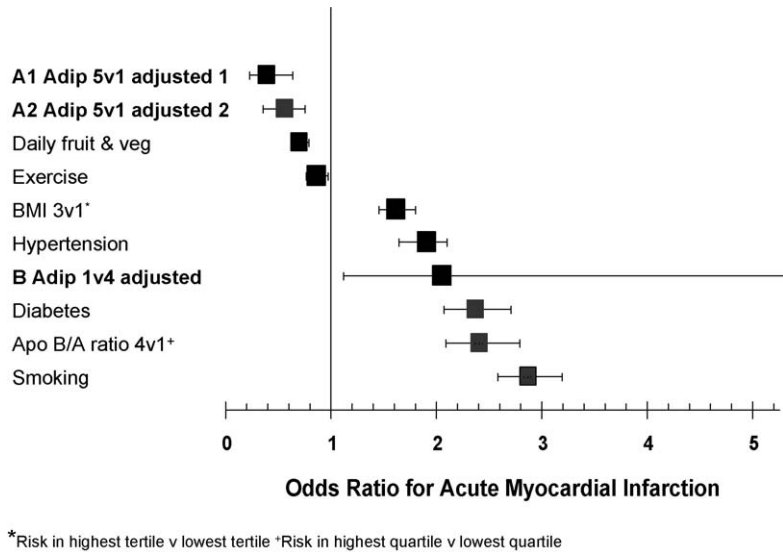


Fig. 3. A comparison of the effects of adiponectin on coronary artery disease risk with conventional risk factors as studied in the standardised case/control INTERHEART study of 15152 cases and 14820 controls [135]. (A1) Relative risk of myocardial infarction in the fifth quintile compared to first adjusted for age and smoking status only and (A2) additionally adjusted for BMI, LDL, HDL, blood pressure, history of diabetes or early myocardial infarction (N= 18225) [88]. (B) Odds ratio of CAD adjusted for dyslipidemia, diabetes, hypertension, smoking habit and BMI (N= 550) [87].

serum adiponectin on risk of heart disease, before and after adjustment for conventional risk factors, can be seen in Fig. 3.

4.3. Mechanism of anti-inflammatory action

The anti-inflammatory actions of adiponectin may be via inhibition of stress signalling pathways directly e.g. nuclear

factor-κB (NFκB) binding or due to interference with TNF production and intracellular signalling (or a combination of both mechanisms). These are both impaired in the presence of adiponectin [90]. Adiponectin has been shown to affect CAD development at each stage as indicated in Fig. 4. Arterial injury results in accumulation of adiponectin at the site of injury, with adiponectin binding preferentially

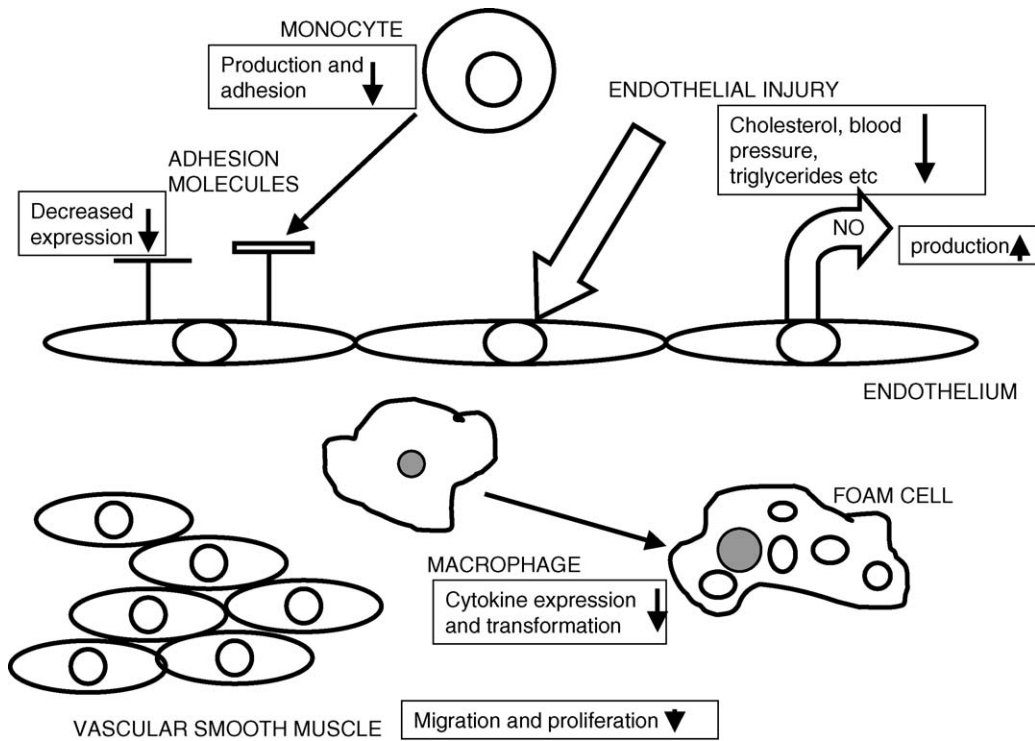


Fig. 4. A summary of the anti-atherogenic actions of adiponectin.

to injured endothelium compared to undamaged vessel wall [91]. Adiponectin also enhances endothelial function with an effect on eNOS, an enzyme responsible for NO synthesis leading to increased NO levels [92]. In large vessels endothelial cell death is also reduced by adiponectin, although the mechanism is unknown [93], whilst in capillary endothelial cells adiponectin induces apoptosis and inhibits migration, proliferation and angiogenesis [94]. A reduction in adiponectin secretion may be required to allow the increase in angiogenesis required for expansion of fat mass. The association of obesity with many forms of malignancy may, in part, be due to loss of regulation of tumour neovascularisation with lower adiponectin levels [94]. Expression of the adhesion molecules VCAM-1, E-selectin and ICAM-1 is reduced by adiponectin [43,95–97], which also reduces proliferation of myelomonocytic cell lines leading to reduced monocyte adhesion. This appears to be a very specific effect on myelomonocytic cell lines, and is due to reduction in the activity of the anti-apoptosis gene Bcl-2, resulting in increased cell death within the bone marrow [12]. Under the influence of adiponectin the monocytes that do adhere produce less inflammatory cytokines, especially TNF, and are less likely to transform into foam cells, due to reduced phagocytosis and lipid accumulation, and reduced expression of class A scavenger receptor [98]. Vascular smooth muscle proliferation and migration is also reduced, as adiponectin reduces ERK kinase activity, probably mediated through inhibition of binding of platelet derived growth factor [99].

The activation of the adiponectin receptor leads directly to a number of changes in fuel sensing, stress and cell signalling pathways [17], although the exact physiological role

of adiponectin in metabolism or inflammation has yet to be defined, partly due to the variation in its circulating forms. The most biologically active form appears to be the trimer, although this has a very short half-life [13]. The effects on AMPK and ERK kinase phosphorylation seem to be dependent on the trimer or the globular C-terminus alone [100], which, despite being not conclusively identified in the circulation has very powerful pharmacological activity [14]. However, some functions seem to be dependent on the hexamer such as T-cadherin [16], NFκB binding [100] and endothelial cell apoptosis [93]. The hexamer has a much longer half-life, and it has been suggested that circulating hexamers act as a precursor pool, broken into smaller units by a serum reductase, at the appropriate metabolic trigger [100]. Interpretation of experiments is also complicated by the fact that post-translational modification may be required for some functions [101], and this does not occur in bacterial-produced recombinant adiponectin.

## 5. Variation in the adiponectin gene in humans

Adiponectin protein is the product of Adipose most abundant gene transcript 1 (*APM1*) gene, also known as the adiponectin encoding adipocyte C1q and collagen containing domain (ACDC), which is found on chromosome 3q27 [102]. The gene was identified through a systemic survey of active genes in adipose tissue using complimentary DNA sequencing and spans 16kb and three exons [103]. This area of the genome has been identified by whole genome linkage studies to be a susceptibility locus for risk for the

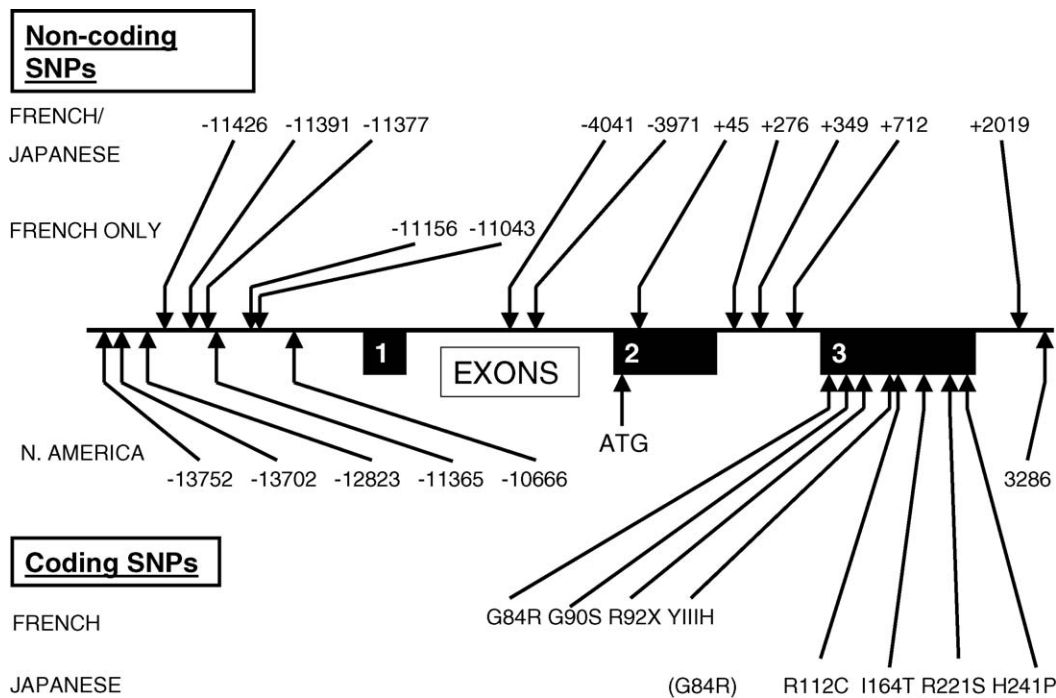


Fig. 5. Variation in the *APM1* gene and the populations in which they were originally identified.

metabolic syndrome, type 2 diabetes (T2DM) and cardiovascular disease [104,105]. Serum adiponectin shows strong heritability of between 55 and 93% [38,106,107]. Functional single nucleotide polymorphisms (SNPs) and missense mutations have been identified in European, North American and Japanese populations and are shown in Fig. 5.

### 5.1. *APM1* variation, conventional coronary artery disease risk factors, metabolic syndrome and type 2 diabetes

The observed actions of adiponectin make *APM1* a good candidate gene for T2DM. Early studies, one in a Japanese and the other in a European sample, first screened the *APM1* gene to identify variation. Following this the association of the identified variants with adverse metabolic markers and obesity and diabetes traits has been extensively studied, mainly in the form of case/control studies.

### 5.2. Screening for variation in the *APM1* gene

The Japanese study included 480 controls and 384 cases [108]. Ten relatively frequent SNPs were detected and four rare missense mutations were also identified (Fig. 5). The only two SNPs that were identified as having different frequency in cases versus controls were at position +45 T>G (risk allele +45G) and +276 G>T (risk allele +276G). Haplotype analysis suggested that either a combination of both SNPs was responsible for this effect or that both were in linkage disequilibrium with an unidentified and truly functional SNP. The impact on serum adiponectin was studied for this variant; overall levels may have been lower in +276G carriers ( $p=0.08$ ), however, a statistically significant result was observed in the higher BMI group ( $p=0.84, 0.09, 0.01$  for trend in serum adiponectin versus +276 genotype in the BMI tertiles, respectively). No other SNPs were found to be associated with serum adiponectin levels.

The European study was performed using a cohort of 1373 healthy French Caucasians and 148 multiplex T2DM families [106]. Two new SNPs and four new missense mutations were discovered (Fig. 5). Genotype frequencies differed widely from the Japanese study. Serum adiponectin level was found to vary with genotype at position -11391, -11377, +45, +276, +2019 and with a pooled analysis of “at least one versus none” of the missense mutations. However, haplotype analysis of the effects of the +45 and +276 SNPs, which showed the weakest association, suggested that most of their effect was due to linkage disequilibrium with SNPs -11391 and -11377. The effect of the +2019 SNP also appeared to be mainly due to its linkage disequilibrium with two of the missense mutations. No relationship between SNPs and insulin resistance indices was described (clamp studies were not performed), although the promoter gene variants -11391A, -11377G and “at least one mutation” were associated with T2DM. Although the results of these studies differ, they do support the hypothesis that

variation in the *APM1* gene contribute to genetic risk to T2DM.

### 5.3. The common +45T>G and +276G>T variants

These variants are the most common and have been the most widely studied, both separately and as haplotypes as shown in Table 1. The results are conflicting, with the association dependent on both the location and type of sample.

The +45T (wild type) has been associated with lower serum adiponectin in samples from France [106] (as above), and Canada [109] (healthy volunteers). Low allele-specific mRNA expression of the +45T has also been demonstrated in adipose tissue taken from healthy volunteers from Taiwan [110]. In these cohorts the +45T was associated with T2DM, adverse lipid profiles in obesity and obesity, respectively. However, the other allele, +45G has been associated with lower serum adiponectin in the Amish [107] (Healthy), and with markers of insulin sensitivity in Japan [108] (T2DM/Healthy controls), Greece [111] (women with polycystic ovarian syndrome), Germany [112] (Healthy with family history T2DM), Sweden [113] (women), Spain [49] (Healthy) and the East Coast of Italy [114] (T2DM/healthy controls). The +45G was also associated with obesity in Sweden [113], although not in Finland [50], and in the prospective risk of T2DM in France [115] (DESIR study) and in a Multicentre study in Europe and Canada [116] (STOP-NIDDM study).

The +276G>T SNP also shows different associations in different samples. The wild type G allele was associated with lower serum adiponectin (France, Greece, Spain), worse markers of insulin sensitivity (Japan, Greece, Sweden, Spain, Italy-East Coast), obesity (Sweden) and T2DM (Japan) in the above studies [49,106,108,111,113,114] and also low serum adiponectin in the Health professionals follow up study [117] (51,529 men, USA). However, the variant T allele has been associated with lower serum adiponectin and higher HOMA [118] (healthy volunteers, Italy-Lazio), adverse lipid parameters [109] (Canada) and T2DM [116] (STOP-NIDDM).

Where these SNPs have been combined [114] (Italy-East Coast) to generate a risk haplotype the two SNPs were found to be in strong linkage disequilibrium. A risk haplotype was generated and much stronger associations were observed when carriers were compared with non-carriers (+45/+276: TG/XX versus XX/XX). The haplotype was associated with higher body weight, waist circumference, blood pressure and HDL/total cholesterol ratio. The haplotype was also associated with lower serum adiponectin and higher risk of T2DM in an American sample [114].

The reason for this variation is unclear, although transcription enhancers have been described in adiponectin gene introns [119], the variability of the associations with the +45 and +276 SNPs and their position and effect on transcription (silent G15G and intron 2, where no regulatory sites have been described) suggests that the SNPs are either in linkage disequilibrium with another functional SNP for which they

Table 1

Populations in which an association between adiponectin gene variants and serum adiponectin, conventional risk factors, markers of the metabolic syndrome and cardiovascular disease have been identified

Risk allele	Sample	Associations		References					
		Location	Type <sup>a</sup>		Low serum adiponectin	Obesity/adverse metabolic markers	T2DM	CAD	
–11426A>G	G	Sweden	T2/GT/HV		+			[121]	
		France	T2/FH/HV			+		[124]	
–12823G>A	G	Pima	HV	+				[123]	
–11391G>A	A	France	HV			+		[115]	
		France	T2/FH/O/HV	+	+	+		[106,120]	
–11377C>G	C	Japan	T2/HV			+		[122]	
		France	O/HV/T2/FH	+	+	+		[106,120]	
		Sweden	T2/GT/HV		+			[121]	
		Amish	HV	+				[107]	
+45T>G	T	France	T2/FH/HV	+				[106]	
		Taiwan	HV	+	+			[110]	
		Canada	HV	+	+			[111]	
		Germany	FH		+			[112]	
		Sweden	HV		+			[113]	
		France	HV		+	+		[115]	
		Greece	PCO		+			[111]	
	G	Japan	T2/HV			+		[108]	
		Spain	HV		+			[49]	
		Amish	HV	+				[107]	
		Europe	GT		+	+		[116]	
		Italy-East Coast	T2/HV		+			[114]	
		France/Swiss	CD/HV				+	[128]	
+276G>T	G	France	T2/FH/HV	+	+			[106]	
		Greece	PCO	+	+			[111]	
		Japan	T2/HV	+	+	+		[108]	
		USA	HV	+			+	[117]	
		USA	T2/HV			+		[114]	
		Sweden	HV		+			[113]	
		Spain	HV	+	+			[49]	
		Italy-East Coast	T2 + CD/T2		+		+	[114,129]	
	T	Canada	HV		+			[109]	
		Europe	GT			+		[116]	
		Italy-Lazio	CD/HV	+	+		+	[118,127]	
+2019DeIA	DeIA	France	T2/FH/HV	+				[106]	
		Amish	HV	+				[107]	
I164T “At least one” mutation	T	Japan	T2/CD/HV	+	+		+	[75,108,125]	
		France	T2/FH/HV	+		+		[106]	

Negative associations and the studies with only negative associations [50,132] have not been included.

<sup>a</sup> T2=type 2 diabetes, GT=impaired glucose tolerance, CD=coronary artery disease, HV=healthy volunteers, FH=HV+family history of T2, PCO=polycystic ovarian syndrome, O=obese.

act as markers or they are being influenced by the environment. While the linkage disequilibrium is different between racial groups it is unlikely to be so in subjects from within Europe. Interaction between genotype and environmental factors, such as obesity should, therefore, be considered in interpreting these results.

#### 5.4. Other variants

A number of SNPs have been identified in the promoter, but the results of association studies using these have also been inconsistent. The –11377G SNP was associated with

low serum adiponectin in the French [106,120] and Amish [107], and T2DM in French [106,120] and Swedish [121] (T2DM/impaired glucose tolerance/controls) samples, whilst the –11377C was associated with T2DM in a Japanese sample [122]. The –11391G forms a risk haplotype in the French sample which strengthened these associations [106,120] although prospective risk of T2DM was lower in the presence of this variant in a second French sample [115]. Other SNPs studied include –12823G>A associated with variation in serum adiponectin in Pima Indians [123] and –11426A>G associated with variation in insulin sensitivity in French [124] and Swedish samples [121]. Examining the

impact of genetic variation in the promoter region has not yet conclusively identified a functional variant but identified variants are again associated with adverse metabolic features.

Only two of the missense mutations found in the Japanese population have been found to alter adiponectin levels (R112C, I164T) and one of these, I164T, has been found to be an independent predictor of blood pressure [75] and has a higher allele frequency in T2DM compared to controls [125]. The Y111H genotype was not associated with body weight in a sample of Swedish obese subjects [113]. The missense mutation proteins associated with diabetes or adiponectin levels as described all appear to have difficulty associating into trimers or multimers. This appears to be responsible for the impaired function, as mutations that can still form multimers (H241P, R221S) do not appear to associate with T2DM [126].

### 5.5. *APM1* gene variants and cardiovascular disease

Variation in the *APM1* gene and cardiovascular disease has been studied in five samples, one European [127] (Italy-Lazio), one Japanese [125] case/control study and two European T2DM samples (French/Swiss [128], Italian-East Coast [129]) as well as the Health Professionals follow up study [117] (prospective). As shown in Table 1 the results mirrored the finding of the metabolic studies, in that the allele associated with adverse metabolic profiles also associated with CAD (+276T in Lazio; +276G in the east coast). The latter also associated with CAD in the Health Professionals Study while no association was found for the +45 variant. Conversely, in the French/Swiss study the +45G allele was associated with CAD, an effect independent of components of the metabolic syndrome, with no association with +276. The Japanese studied the I164T mutation and found a higher frequency among CAD cases. In both cases and controls lower serum adiponectin levels were seen than in those who did not carry the mutation [125]. No association was found in this cohort between CAD and the SNPs +45 and +276. No CAD association with promoter variants has been described.

## 6. Conclusion

Adipocytes have an increasingly recognised role in the endocrine and paracrine control of metabolism and inflammation. One of the most abundant of the adipokines is adiponectin which appears to play a role in fatty acid and glucose metabolism through a change in insulin sensitivity and activation of fuel oxidation by AMPK and PPAR $\alpha$ . Adiponectin has a plethora of anti-inflammatory and anti-atherogenic actions including inhibition of the TNF signalling cascade, essentially acting as a TNF antagonist. Hence, serum adiponectin independently predicts the prospective risk of myocardial infarction. Variation in the adiponectin gene is associated with conventional cardiovascular risk factors, markers of the metabolic syndrome, Insulin resistance and cardiovascular disease. Although there is a

possibility that some negative association studies may have not been published it is very likely that they contribute to the risk of these disorders.

However, sampling of different populations has given inconsistent results so the precise contribution of each variant in different populations has yet to be determined. There are a number of possible reasons for this, firstly, the SNPs identified may only be markers of the functional SNP and different gene frequencies and hence varying linkage disequilibrium (Japanese versus European [108,106]) will give conflicting results. The two Italian samples [114,118] (Italy Lazio/East Coast) are close together but they had different +276 alleles conferring risk, they are, however, different types of sample (T2DM versus healthy controls). The regulation of the *APM1* gene and the influence of SNP's within it may be different in different metabolic environments including hyperglycaemia and obesity. Secondly, the results are further confounded as transcription of the gene may vary with environmental influences. Serum adiponectin varies with dietary fibre intake, exercise and glycaemic index of the diet [130,131], so different dietary trends and activity levels in different samples may influence the impact of genotype. The impact of genotype can certainly be modified by intervention, in a sample from Korea the +45 and +276 SNPs did not associate with type 2 diabetes [132] but did influence the response to rosiglitazone, a PPAR $\gamma$  agonist, dependent, in part, on upregulation of the adiponectin gene as its mechanism of action [133]. The majority of the studies performed so far are cross sectional studies and as such they have been unable to fully address the role of gene–environmental interaction in explaining the conflicting associations so far described. Variation in other areas of the genome may influence the results, the *APM1* locus is only one of five areas identified as associated with variation in serum adiponectin [107], and variation in the PPAR $\gamma$  gene has been shown to influence serum adiponectin levels [134].

There is also a lack of consistent differences in serum adiponectin by genotype, even when associations with disease are seen, and the most likely explanation for this is the failure of current assays to differentiate between types of circulating adiponectin, as not all the circulating forms may be functional. Further investigation is required to identify the actual functional changes responsible for this increased risk, and an accurate identification and measurement of the active circulating form is required before any variant can be said to be truly functional.

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## Appendix A

Papers were identified from Medline and Pubmed using the using the words “adipokines” and “adiponectin” with

“insulin resistance”, “diabetes”, “the metabolic syndrome”, “gene” and “cardiovascular disease” in turn.

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