

Review

## Adiponectin and atherosclerotic disease

Kazunori Shimada\*, Tetsuro Miyazaki, Hiroyuki Daida

*Department of Cardiology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-k, Tokyo 113-8421, Japan*

Received 16 November 2003; received in revised form 8 February 2004; accepted 11 February 2004

### Abstract

Adiponectin has been identified as one of the “adipocytokines” that are derived only from adipose tissue, and are abundantly present in circulating blood. Adiponectin has protective actions in the initiation and progression of atherosclerosis through anti-inflammatory and anti-atherogenic effects. Adiponectin levels are decreased in obesity, type 2 diabetes, and patients with coronary artery disease (CAD). Adiponectin levels were negatively correlated with the CRP levels in patients with CAD. Adiponectin plays a crucial role in the association between obesity, type 2 diabetes, and insulin resistance. Mechanisms explaining the relationship between adiponectin and insulin resistance suggest that adiponectin and tumor necrosis factor (TNF)- $\alpha$  inhibited each other's expression and production in adipocytes. Thiazolidinediones, which are insulin-sensitizing agents, increased the production of adiponectin through directly enhancing its gene expression. The C-terminal globular domain of adiponectin may play a central role in the protective effects against atherosclerosis. Adiponectin receptors 1 (AdipoR1) and 2 (AdipoR2) are expressed ubiquitously in most organs, especially in skeletal muscle in AdipoR1, and liver in AdipoR2. With the prospect of future basic and clinical research on the molecular structure–receptor relationship, adiponectin could become a promising target for future investigations in reducing the morbidity and mortality of atherosclerotic disease.

© 2004 Elsevier B.V. All rights reserved.

*Keywords:* Adiponectin; Metabolic syndrome; Atherosclerotic disease; Adiponectin receptor; Anti-inflammatory effect; Anti-atherogenic effect

### 1. Introduction

Obesity, which is the accumulation of excess body fat, is associated with increasing risk for many common diseases, including dyslipidemia, hypertension, type 2 diabetes, and atherosclerotic cardiovascular disease [1,2]. In 1998, the World Health Organization (WHO) recognized the term “metabolic syndrome” for the clustering of metabolic risk factors [3]. In 2001, The National Cholesterol Education Program Adult Treatment Program (NCEP ATP

III) Guidelines defined metabolic syndrome, which includes abdominal obesity, atherogenic dyslipidemia, elevated blood pressure, insulin resistance, and prothrombotic and proinflammatory states, as a secondary target for cardiovascular risk reduction, after treatment of the primary target—low density lipoprotein (LDL)-cholesterol [4]. It has been well documented that cardiovascular disease and all-cause mortality are increased in patients with metabolic syndrome [5,6].

Adipose tissue, which accounts for more than 10% of the body weight, is currently considered to be not only a reservoir for energy storage, but also an active endocrine tissue [7]. Indeed, adipose tissue produces several proactive cytokines, the so-called “adipocyto-

\* Corresponding author. Tel.: +81-3-5802-1056; fax: +81-3-5689-0627.

*E-mail address:* shimakaz@med.juntendo.ac.jp (K. Shimada).

kines” [8]. Adiponectin has been identified recently as one of the adipocytokines with important metabolic effects [9–12]. It is derived only from adipose tissue and is abundantly present in circulating blood. Adiponectin circulates at high concentrations ranging from 2 to 30 mg/l, which is  $10^3$  higher than the concentrations of other major hormones (e.g. leptin and cortisone), and  $10^6$  higher than those of most inflammatory cytokines [e.g. tumor necrosis factor (TNF)- $\alpha$  and interleukin (IL)-6] (Fig. 1A) [13]. Adiponectin is a 244 amino acid protein produced by apM1 (adipose most abundant gene transcript) cDNA (Fig. 1B) [9], and is also known as GPB28 (gelatin-binding protein of 28 kDa) in humans. The mouse homolog of adiponectin has been cloned as AcrP30 (adipocyte complement-related protein of 30 kDa) and adipoQ [11,12]. The human adiponectin gene that is encoded by apM1 mRNA, is located on chromosome 3q27, consisting of three exons and two introns [14,15] Moreover, the cloning of complementary DNAs encoding adiponectin receptors 1 (AdipoR1) and 2 (AdipoR2) was reported in June 2003 [16]. Adiponectin is composed of two structurally distinct domains: a collagen-like fibrous domain and a com-

plement C1q-like globular domain (Fig. 1B) [10]. Adiponectin belongs to the soluble collagen superfamily, and has structural homology with collagen VIII, X, complement factor C1q [9], and TNF family [17]. Both C1q and TNF family play important roles in inflammation, the immune system, and atherosclerosis. Recent reports suggested that adiponectin may have anti-inflammatory and anti-atherogenic properties. Moreover, a recent study demonstrated that the C-terminal globular domain of adiponectin protects against atherosclerosis [18]. In this review, we focus on the role of adiponectin in atherosclerotic disease and discuss the potential uses of adiponectin-associated therapies in the treatment and prevention of atherosclerotic disease.

## 2. Anti-inflammatory and anti-atherogenic property of adiponectin

### 2.1. Atherosclerosis is an inflammatory disease

Inflammation is an important factor in the initiation and development of atherosclerosis [19]. The first

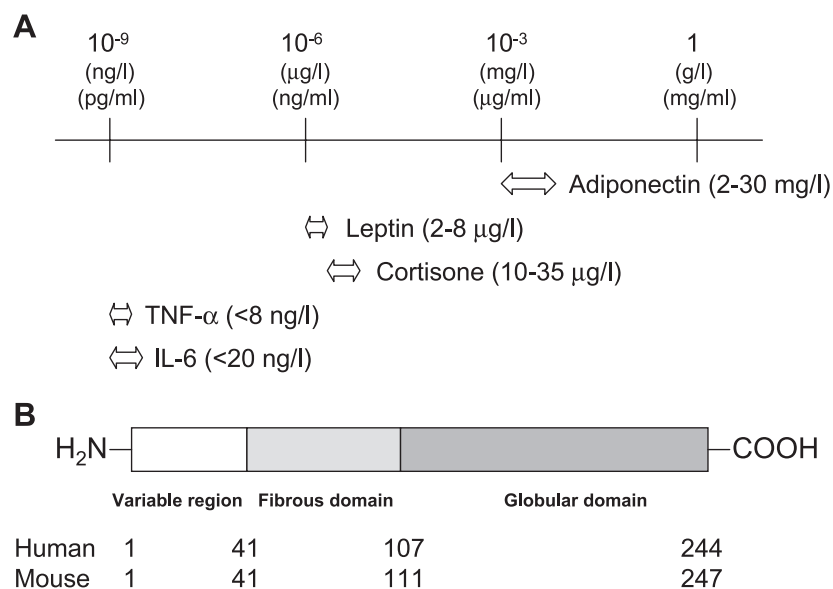


Fig. 1. Structure and circulating concentration of adiponectin protein. (A) The circulating adiponectin is abundant with concentrations ranging from 2 to 30 mg/l. This level is  $10^3$  higher than the concentrations of other major hormones (e.g. leptin and cortisone), and  $10^6$  higher than those of most inflammatory cytokines (e.g. TNF- $\alpha$  and IL-6). (B) Adiponectin consists of a globular domain, fibrous domain, and variable region. TNF- $\alpha$ : tumor necrosis factor- $\alpha$ , IL-6; interleukin-6.

change that precedes the formation of lesions of atherosclerosis is endothelial injury, which is mediated by various inflammatory stimuli, including TNF- $\alpha$ . Secondary, leukocytes adhere to the endothelium, and migrate into the arterial wall, where they can transform to macrophages. Subsequently, the macrophages and migrated smooth muscle cells take up modified LDL and transform into lipid-laden foam cells. The scavenger receptors play important roles in this lipid accumulation and foam cell formation.

## 2.2. In vitro studies of adiponectin

Physiological concentrations of adiponectin inhibited TNF- $\alpha$ -induced monocyte adhesion and expression of endothelial-leukocyte adhesion molecule-1 (E-selectin), vascular cell adhesion molecule-1 (VCAM-1), and intracellular adhesion molecule-1 (ICAM-1), on the endothelium (Fig. 2) [20]. It has

been suggested that the intracellular signal by which adiponectin suppressed adhesion molecule expression is inhibition of endothelial NF- $\kappa$ B signaling through the activation of cAMP protein kinase A [21]. Adiponectin suppressed macrophage to foam cell formation through the inhibition of class A macrophage scavenger receptor (SR-A) (Fig. 2) [22]. In addition, adiponectin has inhibitory effects on the proliferation of myelomonocytic lineage cells, and on the function of matured macrophages, such as phagocytosis and TNF- $\alpha$  production [23]. Moreover, adiponectin suppressed the proliferation and migration of smooth muscle cells induced by platelet-derived growth factor (PDGF)-BB through binding with PDGF-BB directly, and inhibited p42/44 extracellular signal-related kinase (ERK) phosphorylation in PDGF-BB-stimulated smooth muscle cells (Fig. 2) [24]. A recent study demonstrated that adiponectin suppressed the expression of heparin-binding epidermal growth factor

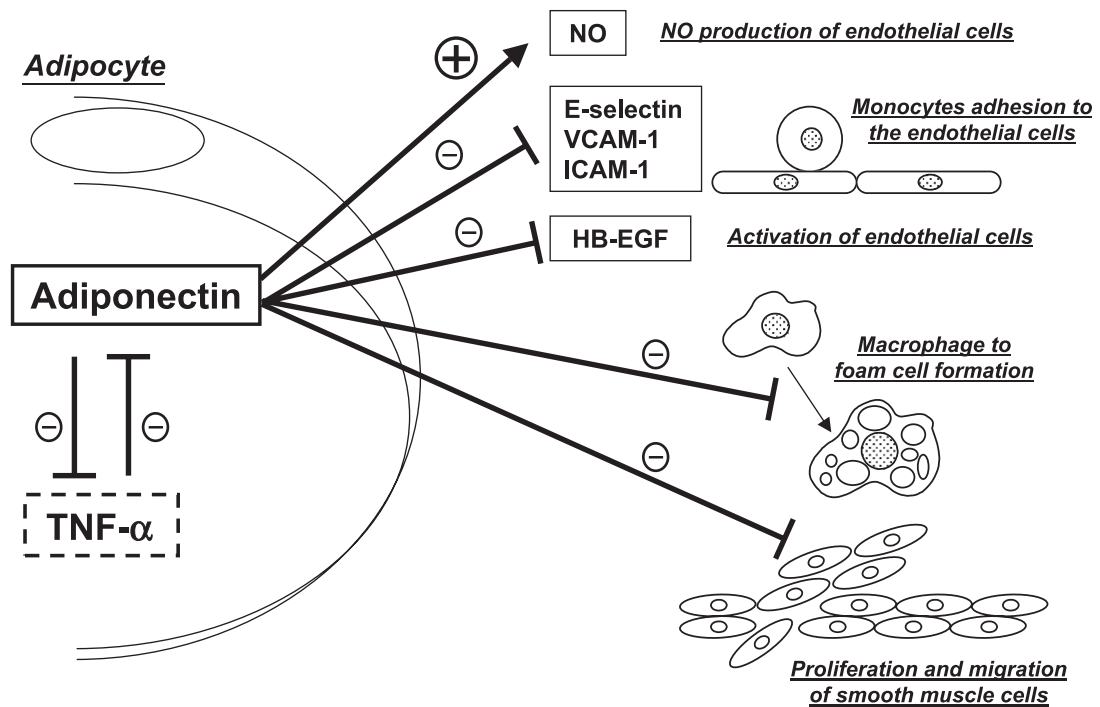


Fig. 2. Protective action of adiponectin in the initiation and progression of atherosclerosis through anti-inflammatory and anti-atherogenic effects. Adiponectin modulates the inflammatory response of endothelial cells, activation of monocyte/macrophage, transformation from macrophages to foam cells, and proliferation and migration of smooth muscle cells in the arterial wall. TNF- $\alpha$ : tumor necrosis factor- $\alpha$ , NO: nitric oxide, VCAM-1: vascular cell adhesion molecule-1, ICAM-1: intracellular adhesion molecule-1, HB-EGF: heparin-binding epidermal growth factor-like growth factor.

(EGF)-like growth factor (HB-EGF) in TNF- $\alpha$  induced activated endothelial cells and also the proliferation and migration of smooth muscle cells stimulated by basic fibroblast growth factor (bFGF), PDGF, EGF, and HB-EGF (Fig. 2) [25]. These data suggest that adiponectin modulates the inflammatory response of endothelial cells, activation of monocyte/macrophage, transformation from macrophages to foam cells, and proliferation and migration of smooth muscle cells, in consequence of protective action in the initiation and progression of atherosclerosis through anti-inflammatory and anti-atherogenic effects (Figs. 2 and 3). A recent report demonstrated that adiponectin has the direct action of stimulating the production of nitric oxide (NO) in endothelial cells (Fig. 2) [25,26]. This direct stimulation depends on the pathway of phosphatidylinositol-3-kinase (PI3K) involving phosphorylation of endothelial NO synthase (eNOS) at Ser1179 by AMPK [27].

### 2.3. Experimental studies of adiponectin in animals

Results with experimental animal models investigating the association between adiponectin and ath-

erosclerosis suggested that adiponectin might play a pivotal role in protecting against atherosclerosis. In a balloon-injured rat carotid artery, immunohistochemical analysis detected that adiponectin accumulates in the arterial walls of the injured vessels but not in non-injured walls [28]. Experiments with adiponectin-deficient mice demonstrated the acceleration of neointimal proliferation in response to injury of the external vascular cuff model [29]. Moreover, adiponectin-deficient mice exhibited augmented neointimal thickening and proliferation of smooth muscle cells in response to wire-injured arteries [25]. Conversely, supplementation with adenovirus expressing adiponectin improved neointimal proliferation in this wire injury model [25]. Taken together, these pieces of evidence with *in vivo* studies, suggest that therapeutic administration of adiponectin could reduce the development of atherosclerosis *in vivo*. Adenovirus treated increase plasma adiponectin significantly reduced the progression of atherosclerotic lesions in a well-established animal model of atherosclerosis: apolipoprotein E-deficient mice (apoE-KO) [30]. Immunohistochemical analysis showed adenovirus-mediated adiponectin actually migrated to the foam cells in atherosclerotic

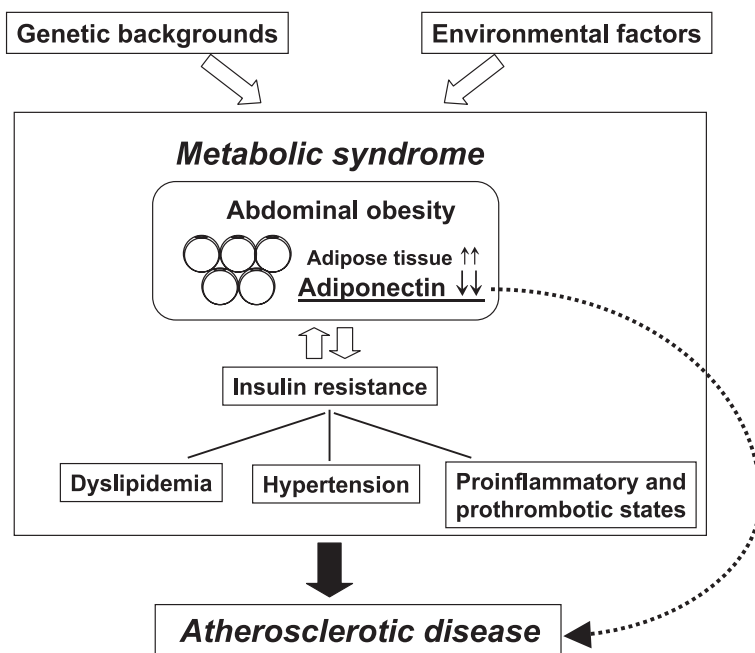


Fig. 3. Mechanisms of metabolic syndrome and atherosclerotic disease. Accumulation of adipose tissue leads to hypo adiponectinemia and the development of insulin resistance.

lesion [30]. In addition, the treatment with adenovirus expressing adiponectin suppressed the expression of VCAM-1, SR-A, and TNF- $\alpha$  in the aorta [30].

### 3. Adiponectin and atherosclerotic disease in clinical studies

#### 3.1. Adiponectin and early atherosclerotic disease

Vascular endothelial dysfunction plays an important role in pathogenesis of atherosclerosis. The measurement of forearm blood flow (FBF) during reactive hyperemia is one of the sensitive methods available for evaluating endothelial function. A recent report showed that peak FBF predicts the risk of cardiovascular events in patients with coronary artery disease [31]. Another recent report showed that peak FBF was correlated with the severity of obesity, such as waist circumference and body mass index (BMI). Multivariate analysis demonstrated that peak FBF was correlated with adiponectin levels in healthy subjects [32].

The intima-media thickness (IMT) of the carotid artery is associated with not only the prevalence of cardiovascular disease [33], but also an increased risk of cardiovascular events [34]. Carotid IMT was significantly greater and the levels of adiponectin were significantly lower in individuals with insulin resistance than those in normal subjects [35]. Moreover, carotid IMT was correlated with adiponectin in this study [35]. These results indicate that adiponectin, which is associated with endothelial dysfunction and carotid atherosclerosis, might be a useful marker of identifying the early stage of atherosclerosis.

#### 3.2. Adiponectin and coronary artery disease

Plasma levels of adiponectin are significantly decreased in obese patients, and the levels of adiponectin are strongly and negatively correlated with BMI [13]. Moreover, type 2 diabetic patients were found to have lower values of plasma adiponectin concentrations than non-diabetic subjects, independent of BMI [36]. Plasma levels of adiponectin were significantly decreased in patients with coronary artery disease (CAD) than in age- and BMI-adjusted control subjects [20]. Intriguingly, in patients with type 2 diabetes, plasma adiponectin levels were shown to be promi-

nently lower in patients with CAD than in patients without CAD [36]. In this study, the presence of microangiopathy, such as retinopathy and microalbuminuria, did not affect the plasma adiponectin levels. Therefore, adiponectin levels may be particularly associated with macroangiopathy in patients with type 2 diabetes. A recent study reported that hypoadiponectinemia was significantly and independently correlated with CAD even after adjustment for several coronary risk factors [37]. In this study, male subjects with hypoadiponectinemia (<4.0 mg/l) had a 2-fold increase in CAD prevalence, independent of other coronary risk factors. The definition of hypoadiponectinemia (<4.0 mg/l) was defined by 25th percentile in this study arbitrarily, because of the lack of established cut-off points for high and low levels of adiponectin in clinical setting. We need to establish the cut-off points for hypoadiponectinemia in a large population among various races and disorders.

It is well established that atherosclerosis is an inflammatory disease [19]. Among inflammatory markers, C-reactive protein (CRP) is one of the most beneficial and reliable biomarkers in cardiovascular risk assessment [38–41]. It has been reported recently that plasma adiponectin levels were negatively correlated with the CRP levels in patients with CAD [42]. This study also showed that not only was CRP mRNA expressed in human adipocyte, but also the levels of CRP mRNA in human adipose tissue were correlated negatively with the levels of adiponectin mRNA in that tissue [42]. Another study in patients with acute myocardial infarction (AMI) reported that the reduction in plasma adiponectin during the early time course of AMI, was negatively correlated with the plasma CRP levels [43]. CRP is generally produced in the liver, under regulation by cytokines, such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$  [41], however, a recent study showed the presence of CRP mRNA in atherosclerotic plaques [44]. Therefore, the expression of CRP may be negatively regulated by adiponectin in adipose tissue. Indeed, the reciprocal relationship was found between adiponectin and TNF- $\alpha$  on their local production in adipose tissue. CRP production modulated by TNF- $\alpha$  might be regulated by adiponectin in adipose tissue.

Restenosis after percutaneous coronary intervention (PCI) remains a major clinical problem. At the site of coronary angioplasty, an inflammatory re-

sponse can induce a chain reaction that causes restenosis. Adiponectin has anti-atherogenic and anti-inflammatory functions. Indeed, adiponectin accumulates in injured vascular walls after balloon injury in rat carotid artery [28], and adiponectin-deficient mice showed severe neointimal formation, which was attenuated by adenovirus-mediated supplementation of adiponectin [25]. Therefore, a preventive effect of adiponectin on restenosis after PCI has emerged. It was reported that the levels of adiponectin at PCI failed to predict angiographic in-stent restenosis after elective coronary stenting [45]. However, it is too early yet to conclude to negative effect of adiponectin on restenosis prevention in the clinical setting, because this study employed a small sample size, and most patients had hypoadiponectinemia.

Cigarette smoking is one of several major coronary risk factors for CAD, however, the atherogenic mechanisms of smoking have not been fully determined [46]. It was demonstrated recently that plasma levels of adiponectin were associated with smoking status in patients with CAD [47]. Increasing in the activity of the sympathetic nervous system, which was affected by nicotine to stimulate post-ganglionic sympathetic nerves, was found to decrease the plasma levels of adiponectin directly. In fact, it was reported that adrenergic agonist and cyclic AMP analogues inhibit the gene expression of adiponectin [48].

Whether the decreased level of adiponectin in patients with CAD is the cause or the consequence of atherosclerosis, is an important question in any discussion on the relationship between adiponectin and atherosclerosis. The possible mechanisms are (1) decreased production in adipocyte, (2) increased consumption in blood stream, or (3) both. It is clear that most patients with CAD have metabolic syndrome, which is closely related with insulin resistance [6]. Hyperinsulinemia associated with an insulin-resistant state may lead to decrease of production of adiponectin (cf. next paragraph 4). Meanwhile, the evidence which adiponectin accumulates in the arterial walls of the injured vessels but not in non-injured walls, might lead to the possibility of consumption of circulating adiponectin in patients with CAD [28]. Hypoadiponectinemia was found recently to be an independent predictor of cardiovascular events in patients with end-stage renal disease [49]. Although

the precise mechanism of the increase in plasma adiponectin remains unclear, the plasma levels of adiponectin in end-stage renal disease patients are relatively higher than general population. Future prospective large-scale studies with intervention which modulates the levels of adiponectin, may not only lead to the determination of the role of hypoadiponectinemia as a candidate risk factor for CAD, but also provide the precise explanation as to whether or not hypoadiponectinemia is a cause or consequence of atherosclerosis.

#### **4. Adiponectin in obesity, type 2 diabetes, and metabolic syndrome**

Insulin resistance induced by an excess of adipose tissue is one of the major risk factor for diabetes and cardiovascular disease. Recent studies have confirmed an association between adiponectin and insulin resistance (Fig. 3). In animal models, the plasma levels of adiponectin were found to be decreased, and also correlated with insulin resistance in rhesus monkeys, which spontaneously develop obesity and type 2 diabetes [50]. Moreover, insulin resistance of lipoatrophic mice was reversed by the administration of adiponectin [51]. The Pima Indians of Arizona have the highest known prevalence of obesity, type 2 diabetes, and insulin resistance [52]. The plasma levels of adiponectin are significantly lower in Pima Indians than in Caucasians, and positively correlated with the indices of insulin resistance measured by hyperinsulinemic clamp in both Pima Indians and Caucasians [53]. Multivariate analysis demonstrated that indices of insulin resistance, but not 2-h glucose concentration of 75 g oral glucose tolerance test, were significant independent determinants of adiponectin levels, explaining a variance level of 47% [53]. A prospective study in Pima Indians showed that individuals with low levels of adiponectin are more likely to develop type 2 diabetes than those with high levels of adiponectin [54]. Moreover, a prospective analysis of Pima Indian children demonstrated that plasma levels of adiponectin decreased with increasing adiposity [55]. These results indicate that adiponectin plays a crucial role in the association between obesity, type 2 diabetes, and insulin resistance.

The mechanisms that could explain the relationship between adiponectin and insulin resistance remain obscure. TNF- $\alpha$ , which inhibits the insulin signaling molecules, has been shown to potently induce insulin resistance [56]. An intriguing study demonstrated that 3T3-L1 adipocytes treated with insulin or TNF- $\alpha$  decreased mRNA expression of adiponectin [57]. Adiponectin-deficient mice showed delayed clearance of free fatty acid induced by inhibition of fatty-acid transporter protein 1 (FATP-1) in muscle, high level of TNF- $\alpha$  mRNA in adipocytes, high levels of TNF- $\alpha$  in plasma, and reduction of insulin-receptor substrate 1 (IRS-1)-associated phosphatidylinositol 3 kinase (PI3-kinase) in muscle [58]. This study also demonstrated that TNF- $\alpha$  reduced the expression of FATP-1 mRNA and the activity of IRS-1-associated PI3-kinase in muscle, whereas adiponectin increased the expression and activity of these compounds [58]. These findings suggest that TNF- $\alpha$  may be one of the candidate molecules responsible for the regulation of adiponectin expression and production of adipocytes. Moreover, adiponectin and TNF- $\alpha$  inhibit each other's production in adipocytes.

Glucocorticoids, thyroid hormones, growth hormone, and angiotensin II have been shown to impair glucose tolerance and/or insulin resistance. Among these hormones, only glucocorticoids, but not thyroid hormones, growth hormone or angiotensin II, suppressed the gene expression of adiponectin in adipocytes [57]. Moreover,  $\beta$ -adrenergic agonist, such as isoproterenol and CL316,243, and cAMP also decreased the expression of adiponectin [48,59,60]. These results indicate that decreased expression and production of adiponectin could also be associated with glucocorticoid- or catecholamine-induced insulin resistance.

The region of chromosome 3q27, which is encoded by apM1 mRNA, has also been found to contain a susceptibility locus for type 2 diabetes and metabolic syndrome [61,62]. Several single-nucleotide polymorphisms (SNPs) and missense mutations in the apM1 gene were identified by several groups. A silent SNP in exon 2 (45T  $\rightarrow$  G) was associated with BMI and insulin sensitivity in nondiabetic German subjects, however, these associations was observed only in individuals without familial predisposition for type 2 diabetes [63]. In Japanese studies, one report revealed a positive relationship between

this polymorphism and type 2 diabetes [64]; however, this variant was not found to be associated with the presence of obesity in another study [15]. Identical negative results were observed in a German population study that investigated metabolic parameters and diabetes [65], in a Swedish population study that investigated obesity [66], and in a French Caucasian study that investigated diabetes [67]. Another SNP in exon 2 (276G  $\rightarrow$  T) may be susceptible to type 2 diabetes. SNP276G  $\rightarrow$  T is associated with the insulin resistance index and plasma adiponectin levels in Japanese population [64]. The haplotype defined by two SNPs in exon 2 (45T  $\rightarrow$  G and 276G  $\rightarrow$  T) was strongly associated with metabolic syndrome, including body weight, waist circumference, blood pressure, fasting glucose, insulin level, homeostasis model assessment (HOMA), and the total to high-density cholesterol ratio in an Italian population. Moreover, this haplotype is a significant determinant of plasma adiponectin levels [68]. Currently, four missense mutations (R112C, I164T, R221S, and H241P) in exon 3 were identified. In these mutations, the I164T mutation is associated with plasma adiponectin level and type 2 diabetes in Japanese subjects [69]. Two SNPs ( $-11391A \rightarrow G$ ,  $-11377C \rightarrow G$ ) in the 5' sequences were associated with plasma adiponectin levels and type 2 diabetes in a French population [67].

## 5. Adiponectin as a therapeutic target

### 5.1. The increment therapies of adiponectin

Hypoadiponectinemia has been demonstrated in human subjects with obesity, type 2 diabetes, and CAD. Recent studies examined whether or not plasma adiponectin levels were increased by intervention therapies including body weight reduction, exercise training, and drug administration.

Body weight reduction increased plasma adiponectin levels in both diabetic and non-diabetic subjects [36], in obese patients who received gastric partition surgery [70,71] and in premenopausal obese women [70]. In addition, the changes in plasma adiponectin levels were significantly correlated with the changes in BMI [70]. However, regular exercise training without reduction of body weight did not

alter the adiponectin levels in healthy subjects, although insulin sensitivity significantly improved [72]. Therefore, the improvement of insulin sensitivity induced by exercise training might not be mediated by adiponectin.

The peroxisome proliferator-activated receptor (PPAR)- $\gamma$  is the main regulator of adipocyte differentiation and adipocyte gene expression involved in fatty acid metabolism and insulin sensitization [73]. Thiazolidinediones, which are synthetic ligands to PPAR- $\gamma$ , are a new class of insulin-sensitizing agent for the treatment of type 2 diabetes. Troglitazone administration for 12 weeks elevated plasma adiponectin levels in mildly obese subjects, and increased adiponectin levels of both plasma and adipose mRNA expression in addition to decreasing of TNF- $\alpha$  mRNA expression in mice [74]. The effects of troglitazone on adiponectin levels significantly increased uniformly in lean, obese, and type 2 diabetes [75]. Increase in adiponectin in patients with type 2 diabetes was also demonstrated by the administrations of rosiglitazone and pioglitazone [76,77]. Glimpiride, a third-generation of sulfonylurea hypoglycemic agent, not only improved insulin resistance but also increased plasma adiponectin levels in elderly patients with type 2 diabetes [78]. In contrast, metformin, a class of biguanide that is effective in improving peripheral insulin resistance, was ineffective on plasma adiponectin levels and the adiponectin protein content of abdominal adipocyte, although the effects of glycemic control were similar in both the troglitazone and metformin groups in obese type 2 diabetic subjects [79]. These results suggest that the increasing levels of circulating adiponectin are not necessarily a simple consequence of improved insulin resistance in the clinical settings.

The renin–angiotensin system (RAS) acts on not only the systemic endocrine system but also local tissue. It is involved in the initiation and progression of atherosclerosis [19,80]. In particular, angiotensin II exerts numerous effects on the pathogenesis of atherogenesis: (1) vasoconstriction, (2) migration and proliferation of smooth muscle cells, (3) production of extracellular matrix and matrix metalloproteinase, and (4) synthesis of inflammatory and/or procoagulant mediators, such as IL-6, MCP-1, PDGF, and PAI-1 [19,80]. A recent study showed that treatment of angiotensin-converting enzyme

inhibitor, temocapril, or angiotensin II receptor antagonist, candesartan, increased plasma adiponectin levels in insulin-resistant patients with essential hypertension [81]. Several mechanisms by which RAS inhibition leads to an increase of adiponectin are proposed. The mechanisms are (1) enhanced insulin sensitivity, (2) recruitment and differentiation of preadipocytes, and (3) increased transcription and/or translation of adiponectin. Indeed, RAS blockades have been reported to enhance insulin sensitivity, suppression of expression and secretion of TNF- $\alpha$  in adipocytes. Moreover, during the differentiation of adipocyte, angiotensin II type 1 receptor (AT1) and type 2 receptor (AT2) is expressed in adipocyte [82].

### 5.2. Adiponectin therapy in atherosclerotic disease

A fragment of adiponectin, including the C-terminal globular domain, exists in human blood stream [83]. This globular domain adiponectin is pharmacologically active and regulates body weight and free acid oxidation in mice [83]. A recent elegant study demonstrated that the C-terminal globular domain of adiponectin protects against atherosclerosis [18]. Globular adiponectin transgenic apoE-KO mice ameliorated the progression of atherosclerosis despite similar lipid and glucose levels in control apoE-KO mice [18]. The expression of SR-A and TNF- $\alpha$  in globular adiponectin transgenic apoE-KO mice was decreased in the vascular wall [18]. These results suggest that globular adiponectin plays a protective role against atherosclerosis. Indeed, globular adiponectin ameliorated insulin resistance and increased fatty-acid oxidation more effectively than the full-length adiponectin [51,83]. The injection of recombinant adiponectin reduced basal glucose levels without increasing insulin levels. These effects derived from the suppression of glucose production in hepatocytes through increased ability of sub-physiological levels of insulin [84]. Moreover, adiponectin acts as a “pleiotropic cytokine” linked not only to body fat, but also to the various cell-to-cell interactions, such as inflammation, hematopoiesis, and the immune system. Therefore, it has been predicted that recombinant adiponectin may become beneficial in the treatment and prevention of cardiovascular disease.

### 5.3. Adiponectin receptors

Until recently, numerous researchers have focused on the discovery of the adiponectin receptor. In June 2003, the cloning of complementary DNAs encoding AdipoR1 and AdipoR2 was reported [16]. AdipoR1 is located at chromosome 1p36.13-q41, and AdipoR2 is located at chromosome 12p13.31. AdipoR1 and AdipoR2 are expressed ubiquitously in most organs, especially skeletal muscle in AdipoR1, and liver in AdipoR2. AdipoR1 and AdipoR2 contain seven transmembrane domains and activate signaling molecules, such as PPAR- $\alpha$ , AMPK, and MAPK [16]. This and the work of other groups have demonstrated the molecular structure and mode of multimerization of adiponectin [10,11,13,85,86]. Active forms of adiponectin are mainly distinguished by the globular domain and the full-length structure (Fig. 1B) [10,86]. It was reported recently that trimers of the globular domain and full-length adiponectin might be important in the stimulatory effect of adiponectin on the AMPK pathway in myocytes; moreover, molecular weight multimer, hexamer, and trimer of full-length adiponectin are the main stimulants in hepatocytes through AMPK pathway [86]. These results, including various and heterogeneous molecular structures and two receptors of adiponectin, point to a new direction of future basic and clinical research on the molecular structure–receptor relationship.

### 6. Conclusions

The epidemic level of overweight and sedentary lifestyle throughout the world is leading to a dramatic increase in the prevalence of metabolic syndrome. It has been established that cardiovascular and overall mortality increase in patients with metabolic syndrome, which is closely associated with obesity, and type 2 diabetes. Prevention, early identification, and adequate treatment of metabolic syndrome are required. Various *in vitro*, *in vivo*, and human studies so far have shown that adipocyte derived abundant plasma protein, adiponectin, has anti-diabetic, anti-inflammatory, and anti-atherogenic properties. Adiponectin could become a promising target for future investigations in reducing the morbidity and mortality of atherosclerotic disease.

### References

- [1] Linton MF, Fazio S. A practical approach to risk assessment to prevent coronary artery disease and its complications. *Am J Cardiol* 2003;92:19i–26i.
- [2] Scott CL. Diagnosis, prevention, and intervention for the metabolic syndrome. *Am J Cardiol* 2003;92:35i–42i.
- [3] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications: Part 1. diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 1998;15:539–53.
- [4] Expert panel on detection evaluation and treatment of high blood cholesterol in adults. Executive summary of the third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–97.
- [5] Isomaa B, Almgren P, Tuomi T, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001;24:683–9.
- [6] Lakka HM, Laaksonen DE, Lakka TA, Niskanen L, Kumpusalo EK, Tuomilehto J, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* 2002;288:2709–16.
- [7] Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation stimulating protein, and adiponectin. *Curr Opin Lipidol* 2002;13:51–9.
- [8] Funahashi T, Nakamura T, Shimomura I, et al. Role of adipocytokines on the pathogenesis of atherosclerosis in visceral obesity. *Intern Med* 1999;38:202–6.
- [9] Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 1996;221:286–9.
- [10] 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;120:803–12.
- [11] 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;270:26746–9.
- [12] Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* 1996;271:10697–703.
- [13] 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.
- [14] Saito K, Tobe T, Minoshima S, et al. Organization of the gene for gelatin-binding protein (GBP28). *Gene* 1999;229:67–73.
- [15] Takahashi M, Arita Y, Yamagata K, et al. Genomic structure and mutations in adipose-specific gene, adiponectin. *Int J Obes Relat Metab Disord* 2000;24:861–8.
- [16] Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003;423:762–9.

- [17] Shapiro L, Scherer PE. The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. *Curr Biol* 1998;8:335–8.
- [18] 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;278:2461–8.
- [19] Ross R. Atherosclerosis—an inflammatory disease. *N Engl J Med* 1999;340:115–26.
- [20] Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999;100:2473–6.
- [21] Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF- $\kappa$ B signaling through a cAMP-dependent pathway. *Circulation* 2000;102:1296–301.
- [22] 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;103:1057–63.
- [23] 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;96:1723–32.
- [24] Arita Y, Kihara S, Ouchi N, et al. Adipocyte-derived plasma protein adiponectin acts as a platelet-derived growth factor-BB-binding protein and regulates growth factor-induced common postreceptor signal in vascular smooth muscle cell. *Circulation* 2002;105:2893–8.
- [25] Matsuda M, Shimomura I, Sata M, et al. Role of adiponectin in preventing vascular stenosis. The missing link of adipovascular axis. *J Biol Chem* 2002;277:37487–91.
- [26] Comuzzie AG, Funahashi T, Sonnenberg G, et al. The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. *J Clin Endocrinol Metab* 2001;86:4321–5.
- [27] Chen H, Montagnani M, Funahashi T, Shimomura I, Quon MJ. Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003;278:45021–6.
- [28] 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.
- [29] Kubota N, Terauchi Y, Yamauchi T, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. *J Biol Chem* 2002;277:25863–6.
- [30] Okamoto Y, Kihara S, Ouchi N, et al. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2002;106:2767–70.
- [31] Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T. Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001;104:2673–8.
- [32] Shimabukuro M, Higa N, Asahi T, et al. Hypoadiponectinemia is closely linked to endothelial dysfunction in man. *J Clin Endocrinol Metab* 2003;88:3236–40.
- [33] Mannami T, Konishi M, Baba S, Nishi N, Terao A. Prevalence of asymptomatic carotid atherosclerotic lesions detected by high-resolution ultrasonography and its relation to cardiovascular risk factors in the general population of a Japanese city: the Suita study. *Stroke* 1997;28:518–25.
- [34] O’Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson Jr SK. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 1999;340:14–22.
- [35] Jansson PA, Pellme F, Hammarstedt A, et al. A novel cellular marker of insulin resistance and early atherosclerosis in humans is related to impaired fat cell differentiation and low adiponectin. *FASEB J* 2003;17:1434–40.
- [36] 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;20:1595–9.
- [37] Kumada M, Kihara S, Sumitsuji S, et al. Association of hypo-adiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 2003;23:85–9.
- [38] de Winter RJ. C-reactive protein and cardiac troponin for early risk stratification in patients with acute coronary syndromes. *Clin Chim Acta* 2001;311:53–6.
- [39] Biasucci LM. C-reactive protein and secondary prevention of coronary events. *Clin Chim Acta* 2001;311:49–52.
- [40] Liuzzo G, Rizzello V. C-reactive protein and primary prevention of ischemic heart disease. *Clin Chim Acta* 2001;311:45–8.
- [41] Blake GJ, Ridker PM. C-reactive protein and other inflammatory risk markers in acute coronary syndromes. *J Am Coll Cardiol* 2003;41:37S–42S.
- [42] Ouchi N, Kihara S, Funahashi T, et al. Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* 2003;107:671–4.
- [43] Kojima S, Funahashi T, Sakamoto T, et al. The variation of plasma concentrations of a novel, adipocyte derived protein, adiponectin, in patients with acute myocardial infarction. *Heart* 2003;89:667–8.
- [44] Yasojima K, Schwab C, McGeer EG, McGeer PL. Generation of C-reactive protein and complement components in atherosclerotic plaques. *Am J Pathol* 2001;158:1039–51.
- [45] Shimada K, Miyauchi K, Mokuno H, et al. Predictive value of the adipocyte-derived plasma protein adiponectin for restenosis after elective coronary stenting. *Jpn Heart J* 2002;43:85–91.
- [46] Kilaru S, Frangos SG, Chen AH, et al. Nicotine: a review of its role in atherosclerosis. *J Am Coll Surg* 2001;193:538–46.
- [47] Miyazaki T, Shimada K, Mokuno H, Daida H. Adipocyte-derived plasma protein, adiponectin, is associated with smoking status in patients with coronary artery disease. *Heart* 2003;89:663–4.
- [48] Delporte ML, Funahashi T, Takahashi M, Matsuzawa Y, Briard SM. Pre- and post-translational negative effect of beta-adrenoceptor agonists on adiponectin secretion: in vitro and in vivo studies. *Biochem J* 2002;367:677–85.
- [49] Zoccali C, Mallamaci F, Tripepi G, et al. Adiponectin, metabolic risk factors, and cardiovascular events among patients with end-stage renal disease. *J Am Soc Nephrol* 2002;13:134–41.

- [50] 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;50:1126–33.
- [51] Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipodystrophy and obesity. *Nat Med* 2001;7:941–6.
- [52] Lillioja S, Nyomba BL, Saad MF, et al. Exaggerated early insulin release and insulin resistance in a diabetes-prone population: a metabolic comparison of Pima Indians and Caucasians. *J Clin Endocrinol Metab* 1991;73:866–76.
- [53] 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;86:1930–5.
- [54] Lindsay RS, Funahashi T, Hanson RL, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2002;360:57–8.
- [55] Stefan N, Bunt JC, Salbe AD, Funahashi T, Matsuzawa Y, Tataranni PA. Plasma adiponectin concentrations in children: relationships with obesity and insulinemia. *J Clin Endocrinol Metab* 2002;87:4652–6.
- [56] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- $\alpha$ : direct role in obesity-linked insulin resistance. *Science* 1993;259:87–91.
- [57] 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;290:1084–9.
- [58] Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. *Nat Med* 2002;8:731–7.
- [59] Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R. Adiponectin gene expression is inhibited by beta-adrenergic stimulation via protein kinase A in 3T3-L1 adipocytes. *FEBS Lett* 2001;507:142–6.
- [60] 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 beta3-adrenergic agonists, retinoic acid, leptin and fasting. *Biochim Biophys Acta* 2002;1584:115–22.
- [61] 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;67:1470–80.
- [62] 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 U S A* 2000;97:14478–83.
- [63] Stumvoll M, Tschritter O, Fritsche A, et al. Association of the T–G polymorphism in adiponectin (exon 2) with obesity and insulin sensitivity: interaction with family history of type 2 diabetes. *Diabetes* 2002;51:37–41.
- [64] 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;51:536–40.
- [65] Schaffler A, Barth N, Palitzsch KD, Drobnik W, Scholmerich J, Schmitz G. Mutation analysis of the human adipocyte-specific apM-1 gene. *Eur J Clin Invest* 2000;30:879–87.
- [66] Ukkola O, Ravussin E, Jacobson P, Sjostrom L, Bouchard C. Mutations in the adiponectin gene in lean and obese subjects from the Swedish obese subjects cohort. *Metabolism* 2003;52:881–4.
- [67] 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;11:2607–14.
- [68] Menzaghi C, Ercolino T, Di Paola R, et al. A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes* 2002;51:2306–12.
- [69] 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;51:2325–8.
- [70] 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;86:3815–9.
- [71] Esposito K, Pontillo A, Di Palo C, et al. Effect of weight loss and lifestyle changes on vascular inflammatory markers in obese women: a randomized trial. *JAMA* 2003;289:1799–804.
- [72] Hulver MW, Zheng D, Tanner CJ, et al. Adiponectin is not altered with exercise training despite enhanced insulin action. *Am J Physiol Endocrinol Metab* 2002;283:E861–5.
- [73] Lee CH, Olson P, Evans RM. Minireview: lipid metabolism, metabolic diseases, and peroxisome proliferator-activated receptors. *Endocrinology* 2003;144:2201–7.
- [74] Maeda N, Takahashi M, Funahashi T, et al. PPAR $\gamma$  ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. *Diabetes* 2001;50:2094–9.
- [75] Yu JG, Javorschi S, Hevener AL, et al. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese, and type 2 diabetic subjects. *Diabetes* 2002;51:2968–74.
- [76] Yang WS, Jeng CY, Wu TJ, et al. Synthetic peroxisome proliferator-activated receptor- $\gamma$  agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. *Diabetes Care* 2002;25:376–80.
- [77] Hirose H, Kawai T, Yamamoto Y, et al. Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. *Metabolism* 2002;51:314–7.
- [78] Tsunekawa T, Hayashi T, Suzuki Y, et al. Plasma adiponectin plays an important role in improving insulin resistance with glimepiride in elderly type 2 diabetic subjects. *Diabetes Care* 2003;26:285–9.
- [79] Phillips SA, Ciaraldi TP, Kong AP, et al. Modulation of cir-

- culating and adipose tissue adiponectin levels by antidiabetic therapy. *Diabetes* 2003;52:667–74.
- [80] Dzau VJ, Bernstein K, Celermajer D, et al. The relevance of tissue angiotensin-converting enzyme: manifestations in mechanistic and endpoint data. *Am J Cardiol* 2001;88:1L–20L.
- [81] Furuhashi M, Ura N, Higashiura K, et al. Blockade of the renin–angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension* 2003;42:76–81.
- [82] Mallow H, Trindl A, Loffler G. Production of angiotensin II receptors type one (AT1) and type two (AT2) during the differentiation of 3T3-L1 preadipocytes. *Horm Metab Res* 2000;32:500–3.
- [83] 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 U S A* 2001;98:2005–10.
- [84] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001;7:947–53.
- [85] 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;277:29359–62.
- [86] 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;278:40352–63.