The usefulness of circulating adipokine levels for the assessment of obesity-related health problems

Hidekuni Inadera

Department of Public Health, Faculty of Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan.

Correspondence to: Hidekuni Inadera, MD and PhD, Department of Public Health, Faculty of Medicine, University of Toyama, 2630 Sugitani, Toyama 930-0194, Japan. Tel: +81 76 434 7275; Fax: +81 76 434 5023; E-mail: inadera@med.u-toyama.ac.jp

Received: 2008.07.06; Accepted: 2008.08.27; Published: 2008.08.29

Because the prevalence of obesity has increased dramatically in recent years, one of the key targets of public health is obesity and its associated pathological conditions. Obesity occurs as a result of white adipose tissue enlargement, caused by adipocyte hyperplasia and/or hypertrophy. Recently, endocrine aspects of adipose tissue have become an active research area and these adipose tissue-derived factors are referred to as adipokines. These adipokines interact with a range of processes in many different organ systems and influence a various systemic phenomena. Therefore, dysregulated production of adipokines has been found to participate in the development of metabolic and vascular diseases related to obesity. The obese state is also known to be associated with increased local and systemic inflammation. Adipokines influence not only systemic insulin resistance and have pathophysiological roles in the metabolic syndrome and cardiovascular disease, but also contribute toward an increase in local and systemic inflammation. Thus, circulating levels of adipokines can be used as high-throughput biomarkers to assess the obesity-related health problems, including low grade inflammation. This review focuses on the usefulness of measuring circulating adipokine levels for the assessment of obesity-related health problems.

Key words: Adipokine, biomarker, insulin resistance, metabolic syndrome, obesity.

1. Introduction

The prevalence of obesity has increased dramatically as a result of our modern lifestyle and is one of the most important targets of public health programs [1]. Accumulating evidence derived from both clinical and experimental studies highlight the association of obesity with a number of chronic diseases such as type II diabetes mellitus (T2DM), atherosclerosis and cardiovascular disease (CVD). T2DM is a problem not only in developed countries but is also becoming an urgent problem in developing countries owing to the worldwide increase in obesity [2]. Therefore, there is considerable effort to understand the underlying biology of these disease states and to identify the contributing risk factors.

The clustering of CVD risk factors, most notably the simultaneous presence of obesity, T2DM, dyslipidemia, and hypertension was recognized as an important pathophysiological state [3-5]. The coexistence of these diseases has been termed the metabolic syndrome (MS). Insulin resistance (IR) is well known to be a key feature of MS, and is strongly associated with excess adiposity, especially in the intra-abdominal region. Individuals with MS are at increased risk for the development of CVD and other diseases related to plaque formation in artery walls, resulting in stroke and peripheral vascular disease. Because the prevalence of these diseases is increasing, high throughput assessment of disease states accompanied with obesity or MS are important issues from the public health point of view.

Excess white adipose tissue (WAT) is linked to obesity-related health problems. It is also recognized that obesity is accompanied by chronic, low-level inflammation of WAT [6, 7]. Inflammation has been considered to be associated with the development of IR and MS [8]. Recently, WAT has been recognized as an important endocrine organ that secretes a wide variety of biologically active adipokines [9-11]. Since some of these adipokines greatly influence insulin sensitivity, glucose metabolism, inflammation and atherosclerosis, they may provide a molecular link between increased adiposity and the development of T2DM, MS and CVD. The signals from WAT are thought to directly connect with IR and inflammation.
It is expected, therefore, that circulating levels of adipokines may be useful as biomarkers to evaluate the risk of other disease states associated with obesity.

This review describes the usefulness and clinical significance of circulating adipokine levels. First, I focused on three representative adipokines associated with IR, namely adiponectin, retinol binding protein 4 (RBP4) and resistin. Next, I discuss the inflammation-related markers such as tumor necrosis factor (TNF) \( \alpha \), interleukin (IL)-6 and C-reactive protein (CRP). Because leptin has not been recognized directly to be related with IR and inflammation, description of this adipokine was excluded. Finally, I have summarized the significance of other molecules, followed by a brief discussion for future research.

2. Adipose tissue as a secretory organ

In 1993, it was discovered that TNF\( \alpha \) expression was up-regulated in WAT of obese mice [12]. The role of WAT as a hormone-producing organ became well recognized in 1994 with the discovery of leptin as an adipocyte-secreted protein [13]. Systemic analysis of the active genes in WAT, by constructing a 3’-directed complementary DNA library, revealed a high frequency of genes encoding secretory proteins. Of the gene group classified by function, approximately 20–30% of all genes in WAT encode secretory proteins [14].

In adults, most organ systems have reached their final size and are programmed to be maintained at steady state. However, WAT is unique because of its almost unlimited expansion potential. Thus, WAT can become one of the largest organs in the body, and the total amount of an adipokine secreted from WAT may affect whole-body homeostasis. WAT contains various types of cells that include preadipocytes, adipocytes and stromal vascular cells. Moreover, bone marrow-derived macrophages home to WAT in obesity [6, 7]. The massive increase in fat mass leads to a dysregulation of circulating adipokine levels that may have pathogenic effects associated with obesity. Thus, dysregulated secretion of adipokines, not only from adipocytes but also from macrophages in WAT, will contribute to the pathogenesis of obesity by triggering IR and systemic inflammation (Fig. 1). It is expected, therefore, that circulating levels of adipokines can be used as a high-throughput biomarker to assess obesity-related health problems.

3. Adiponectin

Adiponectin is the most abundantly expressed adipokine in WAT [14]. The average levels of adiponectin in human plasma are 5–10 \( \mu \text{g/ml} \) [15]. Adiponectin is a multifunctional protein that exerts pleiotropic insulin-sensitizing effects. It lowers hepatic glucose production [16] and increases glucose uptake and fatty acid oxidation in skeletal muscle [17]. Moreover, adiponectin may possess anti-atherogenic properties by inhibiting the expression of adhesion molecules and smooth muscle cell proliferation, as well as suppressing the conversion of macrophages to foam cells [18, 19]. An anti-inflammatory role of adiponectin has also been reported [20].

A number of studies reported the significance of circulating levels of adiponectin (Table 1). Unlike most adipokines, adiponectin mRNA in WAT and serum levels are decreased in obesity [21]. Adiponectin is the only adipokine that is known to be down-regulated in obesity. Plasma concentrations are negatively correlated with body mass index (BMI) [15]. A longitudinal study in primates suggests that adiponectin decreases with weight gain as animals become obese [22]. In contrast, weight loss results in significant increases in circulating adiponectin levels [23, 24]. In addition to the association with whole-body fat mass, adiponectin levels differ with the distribution of body fat. Plasma levels of adiponectin exhibit strong negative correlations with intra-abdominal fat mass [25]. Visceral, but not subcutaneous abdominal fat, was reported to be inversely associated with plasma adiponectin levels in healthy women [26]. A low waist to hip ratio has been reported to be associated with high levels of plasma adiponectin independent of the body fat percentage [27].
Plasma adiponectin concentrations are lower in people with T2DM than in BMI-matched controls [28]. The plasma concentrations have been shown to correlate strongly with insulin sensitivity, which suggests that low plasma concentrations are associated with IR [29]. In a study of Pima Indians, a population that has one of the highest prevalence of obesity, IR and T2DM, individuals with high adiponectin levels were less likely to develop T2DM than those with low concentrations [30]. The high adiponectin concentration was, therefore, a predictive marker for the development of T2DM. Plasma concentrations of adiponectin are also reported to be associated with components of MS. High plasma concentrations of adiponectin were found to be related to an advantageous blood lipid profile [31, 32]. Plasma adiponectin levels are decreased in hypertensive humans, irrespective of the presence of IR [33]. Endothelium-dependent vasoreactivity is impaired in people with hypoadiponectinemia [34], which might be one of the mechanisms involved in hypertension in visceral obesity. A reciprocal association between CRP and adiponectin mRNA levels was reported in human WAT, suggesting that hypoadiponectinemia appears to contribute to low-grade

| Subjects | Major findings | References |
|----------|----------------|------------|
| Obese subjects | Decreased in obese subjects | [25] |
| Patients with CVD | Decreased in patients with CVD | [21] |
| Non-diabetic and T2DM subjects | Decreased in T2DM patients | [20] |
| Obese subjects | Increased after weight loss | [21, 22] |
| Caucasians and Pima Indians | Associated with IR | [153] |
| Pima Indians | Low plasma concentration precedes a decrease in insulin sensitivity | [29] |
| Pima Indians | Decreased in T2DM patients | [30] |
| Pima Indians | Inversely related to adiposity | [154] |
| Non-diabetic Japanese women | Negative correlation with serum triglyceride | [155] |
| Obese subjects | Increased after weight loss | [24] |
| Middle-aged population | Associated with intra-abdominal fat | [25] |
| Non-diabetic white volunteers | Positive correlation with HDL-cholesterol | [31] |
| Hypertensive patients | Correlation with vasodilator response | [34] |
| Japanese men | Decreased in patients with CVD | [38] |
| Japanese subjects | Associated with endothelial dysfunction | [156] |
| Japanese subjects | Decreased in patients with T2DM | [157] |
| Apparently healthy individuals | Associated with the risk of T2DM | [138] |
| Asian Indians with obesity | Low adiponectin was a strong predictor of T2DM | [159] |
| Non-obese and obese subjects | Correlation with advantageous lipid profile | [32] |
| Japanese men | Decreased in hypertensive men | [33] |
| Male participants | High adiponectin was associated with lower risk of myocardial infarction | [39] |
| Whites and African Americans | Higher adiponectin was associated with a lower incidence of T2DM | [40] |
| Patients with CVD | Decreased in patients with CVD | | |
| Pregnant women | Decreased in patients with gestational DM | [42] |
| Non-diabetic subjects | Obesity-independent association of IR with adiponectin levels | [165] |
| Obese individuals | Decreased in subjects with MS | [164] |
| Healthy premenopausal women | Associated with visceral fat mass | [26] |
| Obese juveniles | An inverse relation with the intima media thickness of common carotid arteries | [41] |
| Patients with chronic heart failure | High adiponectin was a predictor of mortality | [42] |
| British women | No association with CVD risk | [43] |
| American Indian | No association with later development of CVD | [44] |
| Hispanic children | Inversely associated with IR | [52] |
| Patients with CVD | Decreased in patients with CVD | [53] |
| Middle-aged men | Positive association with lower fat mass | [166] |
| Obese children | Low adiponectin was associated with components of MS | [36] |
| Older Black Americans | High adiponectin was associated with higher risk of CVD | [45] |
| Patients with CVD | High adiponectin was a predictor of mortality | [46] |
| Patients with CVD | High adiponectin was a predictor of mortality | [50] |
| Pregnant women | Elevated with preeclampsia | [51] |
| Patients with congestive heart failure | Positive correlation with disease severity | [52] |
| Caucasian | High adiponectin increased the risk of death from all causes | [48] |
| Aged men | High adiponectin increased the risk of death from all causes | [49] |
| Patients with incident CVD | No association with the prognostic outcome | [41] |
| General Dutch population | High levels of adiponectin predict mortality | [51] |
systemic chronic inflammation [35]. All these mechanisms may underlie the protective effects against the progression of atherosclerosis of adiponectin. A recent study revealed that adiponectin may function as a biomarker for MS, even in childhood obesity [36]. Collectively, adiponectin has been recognized as a key molecule in MS and has the potential to become a clinically relevant parameter to be measured routinely at general medical check-ups.

Plasma concentrations of adiponectin are also known to be lower in people with CVD than in controls, even after matching for BMI and age [37]. A case-control study performed in Japan revealed that the people with hypoadiponectinemia with the plasma levels less than 4 μg/ml had increased risk of CVD and multiple metabolic risk factors, indicating that hypoadiponectinemia is a key factor in MS [38]. Retrospective case-control studies have demonstrated that patients with the highest levels of adiponectin have a dramatically reduced 6-year risk of myocardial infarction compared with case controls with the lowest adiponectin levels, and this relationship persists even after controlling for family history, BMI, alcohol, history of diabetes and hypertension, hemoglobin A1c, CRP, and lipoprotein levels [39]. An inverse relationship between serum adiponectin levels and the intima media thickness of common carotid arteries was also reported [40]. These clinical studies clearly indicate that hypoadiponectinemia is a strong risk factor for CVD.

Although the above studies support the notion that adiponectin would protect against vascular diseases, recent epidemiological studies have failed to support this notion [41-51]. A recent prospective study reported adiponectin levels were not significantly associated with future secondary CVD events [41]. Thus, measurement of adiponectin may add no significant value to risk stratifications in patients with incident CVD, and effects of adiponectin may be more of importance in the early phases of atherosclerosis. Kistorp et al. reported that adiponectin was positively related to increased mortality in patients with chronic heart failure [42]. These authors suspect that the high adiponectin concentrations may reflect a wasting process in subjects with increased risk of death. Pilz et al. reported that high adiponectin levels predict all-cause, cardiovascular and noncardiovascular mortality [50]. A recent study also reported that a high adiponectin level was a significant predictor of all-cause and CVD mortality [51]. These authors hypothesized that a counter-regulatory increase in adiponectin occurs, which represents a defense mechanism of the body against cardiovascular alterations and a pro-inflammatory state associated with CVD. Thus, yet-unknown mechanisms may underlie the association between adiponectin and the risk of death, the prognostic value of adiponectin remains unresolved. Further prospective studies will be required to provide conclusive results about the association of adiponectin and mortality. It is also necessary to understand the underlying molecular mechanisms of elevated adiponectin concentrations in these disease states.

It must be highlighted that several physiological factors affect the circulating levels of adiponectin. First, aging, gender and puberty have effects on circulating adiponectin levels [52, 53]. An age-associated elevation of plasma adiponectin levels has been reported [51, 54]. Plasma adiponectin levels were significantly higher in female subjects, indicative of a sex hormone affect on circulating adiponectin levels [51, 55]. Adiponectin levels tend to decrease throughout puberty, which parallels the development of IR [36, 56]. Second, the glomerular filtration rate has been recognized as a strong inverse predictor of adiponectin. The clearance of adiponectin by the kidney may have a strong influence on its concentration [57]. Hence, high adiponectin levels may reflect impaired renal function. Last but not least, an increased adiponectin level has been suggested to act as a compensatory mechanism to dampen inflammation. Indeed, elevated plasma adiponectin concentrations are observed in several diseases associated with inflammation: arthritis [58], preeclampsia [59], and end-stage renal disease [60]. All of these factors must be considered when evaluating the clinical significance of circulating adiponectin levels in MS or vascular diseases related to obesity.

Circulating adiponectin forms several different complexes in the adipocyte before being secreted into the blood [61]. Commercial assays measure the total plasma concentration of adiponectin. Thus, the vast majority of clinical studies published to date have evaluated correlations between total adiponectin levels and various markers of MS. The most basic form of adiponectin secreted is the trimer. Adiponectin forms two higher-ordered structures through the noncovalent binding of two trimers (hexamers) and six trimers (18mers). The native protein circulates in serum as low molecular weight (LMW) hexamers and as larger multimeric structures of high molecular weight (HMW). Of these higher-ordered structures, the 18mer (HMW) form is assumed to act beneficial against IR; the function of the hexamer (LMW) form is suggested to play a pro-inflammatory role [55, 62]. Thus, the HMW form is more strongly associated with insulin sensitivity than is total adiponectin [63-65]. Overall, these results suggest that the assessment of total adiponectin may be insufficient and that the analysis of the levels of the
multimeric forms should be favorable to assess the significance of adiponectin.

4. Retinol binding protein 4 (RBP4)

RBP4 is a protein that is the specific carrier for retinol in the blood. It is one of a large number of proteins that solubilize and stabilize the hydrophobic and labile metabolites of retinoids in aqueous spaces in both extra- and intracellular spaces. Its physiological function appears to be to bind retinol and prevent its loss through the kidneys. RBP4, although largely produced in liver, is also made by adipocytes, with increased levels in obesity contributing to impaired insulin action [66]. Studies in transgenic rodent models showed overexpression of human RBP4 or injection of recombinant RBP4 induced IR in mice, whereas RBP4 knockout mice showed enhanced insulin sensitivity [66]. The same authors reported that high plasma RBP4 levels are associated with IR states in humans and suggested that RBP4 is an adipokine responsible for obesity-induced IR and, thus, a potential therapeutic target in T2DM [66, 67]. Since then, a number of clinical studies have been conducted to assess the significance of circulating levels of RBP4 (Table 2).

### Table 2 Clinical studies of circulating RBP4 levels

| Subjects | Major findings | References |
|----------|----------------|------------|
| Obese and T2DM subjects | Elevated in subjects with T2DM | Yang et al., (2005) [66] |
| IGT and T2DM subjects | Correlation with the magnitude of IR | Graham et al., (2006) [67] |
| IGT and T2DM subjects | Elevated in subjects with IGT or T2DM than normal glucose tolerance | Cho et al., (2006) [68] |
| Caucasian menopausal women | No correlation with adiposity | Janke et al., (2006) [72] |
| Japanese subjects | No correlation with BMI | Takashima et al., (2006) [168] |
| IGT and T2DM subjects | No correlation with IR | Erikstrup et al., (2006) [169] |
| Chinese subjects | Correlation with the components of MS | Qi et al., (2007) [69] |
| Healthy women | Associated with visceral fat | Lee et al., (2007) [70] |
| Chinese subjects | Correlation with visceral adiposity | Jia et al., (2007) [71] |
| Non diabetic person | No correlation with IR | Yao-Borengasser et al., (2007) [73] |
| Subjects with BMI from 18 to 30 | Negative correlation with insulin sensitivity | Gavi et al., (2007) [74] |
| Caucasian without T2DM | Associated with liver fat | Stefan et al., (2007) [76] |
| Nondiabetic individuals | Reflected ectopic fat accumulation | Perseghin et al., (2007) [77] |
| Obese children | Associated positively with CRP | Balagopal et al., (2007) [170] |
| Subjects with morbid obesity | Reduction after weight loss | Haider et al., (2007) [171] |
| Obese women | Reduction after weight loss | Vittkova et al., (2007) [172] |
| Patients with T2DM | Associated with IR | Takebayashi et al., (2007) [173] |
| Women with polycystic ovary syndrome | Elevated than BMI-matched subjects | Tan et al., (2007) [174] |
| Nondiabetic men | Negatively associated with insulin secretion | Broch et al., (2007) [175] |
| Patients with chronic liver disease | Decreased compared with control subjects | Yagmur et al., (2007) [176] |
| Patients with T2DM or CVD | Associated with pro-atherogenic lipoprotein levels | von Eynatten et al., (2007) [177] |

Cho et al. reported that plasma concentrations of RBP4 were higher in people with impaired glucose tolerance (IGT) or T2DM than in people with normal glucose tolerance [68]. A recent cross-sectional study of 3289 middle-aged population showed that plasma RBP4 levels increased gradually with increasing numbers of MS components [69]. Similar to other adipokines, circulating levels of RBP4 is associated with body fat distribution rather than body weight per se. RBP4 was reported to be more highly correlated with waist-to-hip ratio or visceral fat areas than with BMI [67, 70, 71]. However, Janke et al. reported that, in human abdominal subcutaneous (sc) adipose tissue, RBP4 mRNA is down-regulated in obese women, whereas circulating RBP4 concentrations were similar in lean, overweight, and obese women [72]. Yao-Borengasser et al. also reported that neither sc adipose tissue RBP4 mRNA expression nor circulating RBP4 levels show any correlation with BMI [73]. It is not clear why such differences are present among similarly conducted human studies. These inconsistencies most likely result from differences in age, ethnicity, sample size, and assay methods used. For example, sex and age were found to be independent determinants of plasma RBP4 concentrations [68, 74]. A recent study suggested that the sandwich ELISA kit commercially available for the assessment of RBP4 may overestimate the circulating levels [75]. Those authors also claimed that competitive EIAs may underestimate serum RBP4 levels in the setting of IR owing to assay saturation. Thus, it is probable that the reported RBP4 associations would become clearer if more reliable assays were employed.

Two recent studies have indicated that high circulating RBP4 is associated with elevated liver fat and, presumably, hepatic insulin resistance [76, 77]. In ro-
Int. J. Med. Sci. 2008, 5

...dents, only 20% of systemic RBP4 is produced by adipocytes, and RBP4 gene expression in adipocytes was 20% compared with expression in the liver [78]. Thus, it is possible that the increase in systemic RBP4 concentrations is not explained by increased RBP4 production in WAT. RBP4 is a transporter for retinol, which serves as a precursor for the synthesis of ligands for nuclear hormone receptors such as retinoid X receptor and retinoic acid receptor. Thus, circulating RBP4 can modulate metabolic pathways via these nuclear hormone receptors. Certainly, future prospective studies are needed to clarify whether a high RBP4 level plays a causal role in the development of MS, T2DM, and eventually for the development of CVD.

5. Resistin

After the identification of resistin as an adipokine in 2001 [79], several studies have been conducted to investigate the role and significance of this molecule. Resistin was discovered as a result of a hypothesis that WAT secretes a hormone that mediates IR and that insulin sensitizing drug thiazolidinediones act by suppressing the production of this hormone. Resistin is secreted by mature adipocytes in proportion to the level of obesity and acts on insulin-sensitive cells to antagonize insulin-mediated glucose uptake and utilization in mice. Treatment of wild-type mice with recombinant resistin resulted in IR, whereas administration of an anti-resistin antibody increased insulin sensitivity in obese and insulin-resistant animals [79]. However, human resistin is 59% homologous at the amino acid level to the mouse molecule, a relatively low degree of sequence conservation. Moreover, in contrast to mice, human resistin is expressed at lower levels in adipocytes but at higher levels in circulating blood monocytes [80]. As a result, there is still uncertainty about possible relationships between serum concentrations of resistin and markers of IR (Table 3).

Table 3 Clinical studies of circulating resistin levels

| Subjects | Major findings | References |
|----------|----------------|------------|
| Healthy Greek students | Correlation with body fat mass | Yannakoulia et al., (2003) [81] |
| Non-diabetic subjects | Correlation with IR | Silha et al., (2005) [82] |
| Patients with essential hypertension | Elevated in T2DM patients | Zhang et al., (2003) [83] |
| Patients with inflammatory diseases | Correlation with inflammatory markers | Stejskal et al., (2003) [91] |
| Obese subjects | Correlation with BMI | Azuma et al., (2003) [178] |
| Lean and obese subjects | Increase in obese subjects | Degawa-Yamauchi et al., (2003) [179] |
| Women | No relation with fat mass or IR | Lee et al., (2003) [180] |
| Patients with T2DM | No correlation with IR | Pfutzner et al., (2003) [181] |
| Obese subjects | Not changed after weight loss | Monzillo et al., (2003) [182] |
| Diabetic subjects | Correlation with CRP | Shetty et al., (2004) [87] |
| Obese Caucasian subjects | Correlation with HOMA-R | Silha et al., (2004) [183] |
| Non obese subjects | Correlation with insulin sensitivity | Heilbronn et al., (2004) [184] |
| Pima Indians | Correlation with fat mass but not IR | Vozarova de Courten et al., (2004) [185] |
| Diabetic subjects | Elevated in T2DM patients | Youn et al., (2004) [186] |
| Japanese subjects | Elevated in T2DM patients | Fujinami et al., (2004) [187] |
| Patients with T2DM | Correlation with hepatic fat content | Bajaj et al., (2004) [188] |
| Women | Associated with the presence of CVD | Pischon et al., (2005) [88] |
| Subjects who had a family history of premature coronary artery disease | Correlation with the levels of inflammatory markers | Reilly et al., (2005) [90] |
| Japanese subjects | Associated with the presence and severity of CVD | Ohmori et al., (2005) [189] |
| Men | Correlation with CRP | Bo et al., (2005) [190] |
| Patients with rheumatoid arthritis | Elevated than the patients with osteoarthritis | Senolt et al., (2007) [89] |

The role of resistin in the pathophysiology of obesity and IR in humans is controversial. Several studies have shown positive correlations of circulating resistin levels with body fat mass [80, 81] or IR [82, 83]. However, the other studies found no relationship between resistin gene expression and body weight or insulin sensitivity [84-86]. These conflicting data may reflect variations in the study design and the lack of adjustment for potential confounding factors. It also seems possible that resistin is a marker for, or contributes to, IR in a specific population. The predominantly paracrine role of resistin might explain the weakness of the correlations between circulating resistin levels and some of the metabolic variables.

Two studies have shown that among the blood markers, the most significant association of the circulating resistin level was with plasma CRP [87, 88]. Thus, higher resistin levels may be a marker of systemic inflammation. Indeed, the circulating level of resistin is up-regulated in patients with rheumatoid arthritis [89]. The circulating resistin level is also reported to be an inflammatory marker of atherosclerosis...
Considering that the resistin concentration is elevated in the patients with severe inflammatory disease [91], hyperresistinemia may be a biomarker and/or a mediator of inflammatory states in humans. Overall, the resistin levels in humans are thought to correlate more closely with inflammation than with IR.

6. Inflammation-related molecules

Obesity is associated with a state of chronic, low-grade inflammation characterized by abnormal cytokine production and the activation of inflammatory signaling pathways in WAT [92]. Obese hypertrophic adipocytes and stromal cells within WAT directly augment systemic inflammation. Although WAT is usually populated with 5-10% macrophages, diet-induced weight gain causes a significant macrophage infiltration, with macrophages comprising up to 60% of all cells found in WAT in a rodent model [6]. Thus, several adipokines implicated in inflammation are cytokines which are produced by macrophages. The accumulation of WAT resident macrophages and elaboration of inflammatory cytokines have been implicated in the development of obesity-related IR. Indeed, increases in inflammatory cytokine expression by WAT are associated with a parallel increase in WAT macrophage content [6, 7, 93]. Thus, obesity leads to increased production of several inflammatory cytokines, which play a critical role in obesity-related inflammation and metabolic pathologies.

A number of studies have reported that several humoral markers of inflammation are elevated in people with obesity and T2DM [94, 95] (Table 4). Pfeiffer et al. showed that men with T2DM had higher TNFα concentrations compared with nondiabetic subjects [96]. However, several studies reported no association between circulating levels of TNFα and insulin sensitivity [97, 98]. Since there was no arteriovenous difference with TNFα [99], TNFα is considered to work mainly in an autocrine or paracrine manner, where the local concentrations would be more likely to exert its metabolic effects [99, 100]. Moreover, circulating TNFα has been reported to be associated with a soluble receptor that inhibits its biological activity [101], suggesting that the action of TNFα is primarily a local one. Therefore, it seems unlikely that the circulating levels of TNFα would be a good biomarker to reflect the IR state of the whole body.

Table 4 Clinical studies of circulating inflammatory markers

| Subjects                                | Major findings                                                                 | References                                  |
|-----------------------------------------|-------------------------------------------------------------------------------|--------------------------------------------|
| **TNFα**                                |                                                                               |                                            |
| Nondiabetic offsprings of T2DM patients | Not major contributing factor for obesity induced IR                          | Kellerer et al., (1996) [97]               |
| Adult males                             | Elevated in patients with T2DM                                               | Pfeiffer et al., (1997) [96]               |
| Obese patients with T2DM                | Correlation with the visceral fat area                                        | Katsuki et al., (1998) [191]               |
| T2DM subjects                           | Elevated in T2DM as compared to control                                      | Winkler et al., (1998) [192]               |
| Aged men                                | Correlation with BMI                                                         | Nilsson et al., (1998) [193]               |
| Canadian population                     | Positive correlation with IR                                                 | Zimmann et al., (1999) [194]               |
| Obese subjects                          | Elevated in obese subjects than in controls                                  | Corica et al., (1999) [195]                |
| Normotensive obese patients             | Elevated in patients with android obesity than gynoid obesity                | Winkler et al., (1999) [196]               |
| Obese subjects                          | No relationship with BMI                                                     | Kern et al., (2001) [98]                   |
| Premenopausal obese women               | Reduced after weight loss                                                    | Ziccardi et al., (2002) [197]              |
| Nondiabetic obese women                 | Associated with fat amount                                                   | Maachi et al., (2004) [198]                |
| Premenopausal obese women               | Reduced after weight loss                                                    | Marfella et al., (2004) [199]              |
| **IL-6**                                |                                                                               |                                            |
| White nondiabetic subjects              | Correlation with BMI                                                         | Yudkin et al., (1999) [100]                |
| Healthy middle-aged women               | Associated with BMI                                                          | Hak et al., (1999) [200]                   |
| Obese nondiabetic women                 | Reduced after weight loss                                                    | Bastard et al., (2000) [103]               |
| Obese subjects                          | Correlation with obesity and IR                                              | Kern et al., (2001) [98]                   |
| Pima Indians                            | Correlation with IR                                                          | Vozarova et al., (2001) [102]             |
| Premenopausal obese women               | Reduced after weight loss                                                    | Ziccardi et al., (2002) [197]              |
| Premenopausal obese women               | Reduced after weight loss                                                    | Esposito et al., (2003) [104]              |
| Obese patients                          | Reduced after weight loss                                                    | Kopp et al., (2003) [105]                  |
| Obese subjects                          | Reduced after weight loss                                                    | Monzillo et al., (2003) [182]              |
| Premenopausal obese women               | Reduced after weight loss                                                    | Giugliano et al., (2004) [106]             |
| Nondiabetic offspring of patients with  | Not associated with the components of MS                                     | Salimenniemi et al., (2004) [110]          |
| T2DM                                    |                                                                               |                                            |
| Premenopausal obese women               | Reduced after weight loss                                                    | Marfella et al., (2004) [199]              |
| Japanese men                            | Not associated with the components of MS                                     | Matsushita et al., (2006) [111]            |
| T2DM subjects                           | Associated with IR                                                           | Natauli et al., (2006) [201]               |
| Adolescents                             | Positive correlation with BMI                                                | Herder et al., (2007) [135]                |
CRP
White nondiabetic subjects Positive correlation with BMI Yudkin et al., (1999) [100]
Healthy middle-aged women Associated with BMI Hak et al., (1999) [200]
Young adults Elevated in obese person Visser et al., (1999) [202]
Adult men Correlation with body fat mass Lemieux et al., (2001) [115]
Obese women Reduced after weight loss Heilbronn et al., (2001) [117]
Middle-aged men Predictor of T2DM development Freeman et al., (2002) [116]
Obese postmenopausal women Reduced after weight loss Tchernof et al., (2002) [118]
Premenopausal obese women Reduced after weight loss Ziccardi et al., (2002) [197]
Healthy obese women Correlation with IR independent of obesity McLaughlin et al., (2002) [203]
Premenopausal obese women Reduced after weight loss Esposito et al., (2003) [104]
Healthy American women Prognostic marker to the MS Ridker et al., (2003) [114]
Premenopausal obese women Obesity is the major determinant of elevated CRP levels Escobar-Morreale et al., (2003) [204]
Premenopausal obese women Reduced after weight loss Marfella et al., (2004) [199]
Obese subjects Correlation with serum TNFα levels Shadid et al., (2006) [86]
T2DM subjects Associated with IR Natali et al., (2006) [201]
Overweight women Reduced after weight loss Moran et al., (2007) [205]

A considerable proportion of circulating IL-6 is derived from WAT, and WAT is estimated to produce about 25% of the systemic IL-6 in vivo [99]. Fasting plasma IL-6 concentrations were negatively correlated with the rate of insulin-stimulated glucose disposal in Pima Indians [102]. Bastard et al. reported that the IL-6 values were more strongly correlated with obesity and IR parameters than TNFα, and a very low-calorie diet induced significant decreases in circulating IL-6 levels in obese women [103]. Other studies have also showed that weight loss results in decreased circulating levels of IL-6 [104-106]. Although several reports have indicated that IL-6 plays a role in the development of IR [95, 107], some investigators have insisted that IL-6 prevents IR [108, 109]. Some of these discrepancies may be explained by the widely different characteristics of the study populations regarding age, sex, glucose tolerance status, and degree of obesity. Overall, the association of IL-6 and IR seems complex and IL-6 alone might not be an appropriate marker of IR or MS [110, 111].

IL-6 derived from visceral adipose tissue draining directly into the portal system and causes the obesity-associated rise of liver CRP production [112]. Although CRP was traditionally thought to be produced exclusively by the liver in response to inflammatory cytokines, emerging data indicate that CRP can also be produced by nonhepatic tissues. Adipocytes isolated from human WAT produced CRP in response to inflammatory cytokines [113]. Adiponectin has been suggested to play a role in modulating CRP levels. In fact, adiponectin knockout mice showed higher CRP mRNA levels in WAT compared with the wild-type mice [35]. Therefore, hypoadiponectinemia also appears to be responsible for a low-grade systemic chronic inflammatory state, which is closely related to high CRP levels.

Several studies have shown that CRP is more strongly associated with IR than either TNFα or IL-6 [110, 111, 114]. CRP has been reported to be associated with body fat and other inflammatory markers [86, 115]. Abundant evidence has accumulated to show that CRP is associated with MS and predicts T2DM and CVD events independently of traditional risk factors [114, 116]. Thus, elevated CRP levels in obesity, and the decreases associated with weight loss indicate a link between CRP and obesity-associated risks for CVD [104, 117, 118].

7. Chemokines: monocyte chemoattractant protein-1 and IL-8

Monocyte chemoattractant protein-1 (MCP-1) is a chemokine, which plays a pivotal role in the recruitment of monocytes and T lymphocytes to the sites of inflammation. MCP-1 is expressed in adipocytes and considered to be an adipokine [119, 120]. MCP-1 mediates the infiltration of macrophages into WAT in obesity and may play an important role in establishing and maintaining a proinflammatory state that predisposes to the development of IR and MS [121]. Macrophage infiltration into WAT is increased by the secretion of MCP-1, which is expressed by adipocytes, as well as by macrophages and other cell types, especially in obese, insulin-resistant subjects [122]. A number of studies have reported significantly higher circulating MCP-1 levels in obese [122, 123] or T2DM patients [124, 125]. Conversely, obese patients who lost weight showed decreased levels of MCP-1 [122, 126]. However, a recent study indicated that there was no difference in circulating MCP-1 levels between nonobese and obese subjects, when either abdominal venous or arterialized blood was analyzed [127]. Previous studies showed that plasma MCP-1 levels were influenced by numerous factors, including aging [128], hypertension [129], hypercholesterolemia [130], vascular disease
IL-8 is responsible for the recruitment of neutrophils and T lymphocytes into the subendothelial space and considered to be an atherogenic factor that leads to intimal thickening. IL-8 is produced and secreted by human adipocytes [133]. Plasma IL-8 levels are increased in obese subjects, linking obesity with increased cardiovascular risk [134]. The circulating IL-8 level is associated with obesity-related parameters such as BMI, waist circumference and CRP [123]. However, Herder et al. reported that, among the seven immunological mediators (IL-6, IL-18, TNFα, IL-8, MCP-1, IP-10, and adiponectin) expressed and secreted by WAT, high BMI was significantly associated with elevated circulating levels of IL-6, IL-18, and IP-10 as well as lower levels of adiponectin [135]. Thus, the clinical relevance of circulating levels of MCP-1 and IL-8 to predict obesity-related disease conditions is still unresolved.

8. Other molecules

Plasminogen activator inhibitor-1 (PAI-1) is an important endogenous inhibitor of tissue plasminogen activator and is a main determinant of fibrinolytic activity. PAI-1 contributes to the pathogenesis of atherothrombosis and CVD. Experimental data indicate that WAT has a capacity to produce PAI-1 [136]. Much of the elevation of circulating levels of PAI-1 in obesity is attributable to upregulated production from WAT [136-138]. The increased plasma PAI-1 levels in obesity and positive correlations with visceral fat depots are reported in several studies [139-142]. Conversely, weight loss is associated with reduced PAI-1 activity in obese subjects [143]. Hyperinsulinemia caused by IR may increase both adipocyte and hepatic synthesis of PAI, which could play a role in the development of the vascular complications [144, 145].

Obesity is associated with expansion of the capillary bed in regional fat depots. Adipocytes or other cell types present in WAT secrete angiogenic factors such as vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF), which act in an autocrine or paracrine manner within WAT but may have endocrine effects throughout the body. Serum VEGF levels were found to positively correlate with BMI [146, 147]. HGF has also been reported to be elevated in obese subjects [148] and elevated serum HGF in obese subjects is reduced with weight loss [149]. These angiogenic factors may be involved in the development of obesity-related metabolic disorders such as inflammation and CVD.

Cathepsin S was recently identified as a novel adipokine [150]. Cathepsin S is a cysteine protease that has the ability to degrade many extracellular elements and is involved in the pathogenesis of atherosclerosis [151]. Cathepsin S is secreted by adipocytes and its circulating levels are increased in obese subjects than in nonobese subjects [152]. Conversely, weight loss is associated with a decrease in circulating cathepsin S levels as well as WAT cathepsin S content [152]. Thus, cathepsin S could constitute a novel biomarker of adiposity that may be linked with enlarged WAT and may also play a role in vascular pathogenesis in obesity.

9. Conclusions

Obesity is recognized as a worldwide public health problem that contributes to a wide range of disease conditions. The development of a method for convenient prediction of obesity-related health problems represents a major challenge for public policy makers facing the epidemic of obesity. WAT is an endocrine organ that communicates with other tissues via secretion of adipokines. Adipokines, which integrate metabolic and inflammatory signals are attractive candidates for predicting the risk of CVD. With obesity, the production of most adipokines is enhanced, except for the anti-inflammatory and insulin-sensitizing effector, adiponectin. Enlarged adipocytes and macrophages embedded within WAT produce more RBP4, resistin and proinflammatory cytokines, such as TNFα and IL-6. Markers of inflammation including CRP have been proposed for use in clinical practice to aid in the identification of asymptomatic patients at high risk for CVD. Thus, measurement of adiponectin and inflammatory markers could be used to assess the risk of developing CVD.

It is important to note, however, that only a limited number of adipokines are released into the bloodstream at levels that are detectable with current assays, resulting in increased circulating levels in the obese state. Some adipokines acting in a paracrine or autocrine manner may play an important role; thus, circulating levels of the adipokines may represent only spillover from WAT and may not be associated with the disease condition. Moreover, except for adiponectin, many of the adipokines are not expressed exclusively in WAT. Thus, there remains uncertainty as to the most appropriate and optimal marker for use in clinical practice. Since various WAT in different regions may have unique characteristics related to differential expression of adipokines, different types of
fat distribution may offer the explanations for the discrepancies observed between different studies. Further epidemiological studies with solid clinical end points are needed to determine which combination of adipokines can be a reliable risk marker for CVD and may provide an improved method for identifying persons at risk for future cardiovascular events. Elucidation of the significance of circulating adipokines may provide a therapeutic target for adipokine-based pharmacological and/or interventional therapies in obesity and related complications.

**Abbreviations**

- BMI: body mass index; CRP: C-reactive protein; CVD: cardiovascular disease; IL: interleukin; IR: insulin resistance; MCP-1: monocyte chemoattractant protein-1; MS: metabolic syndrome; RBP4: retinol binding protein 4; T2DM: type 2 diabetes mellitus; TNF: tumor necrosis factor; WAT: white adipose tissue.

**Conflict of Interest**

The authors have declared that no conflict of interest exists.

**References**

1. Mello MM, Studdert DM, Brennan TA. Obesity- the new frontier of public health law. N Engl J Med 2006; 354: 2601-10.
2. Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world- a growing challenge. N Engl J Med 2007; 356: 213-5.
3. Wingard DL, Barrett-Connor E, Criqui MH, Suarez L. Clustering of heart disease risk factors in diabetic compared to nondiabetic adults. Am J Epidemiol 1983; 117: 19-26.
4. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 1988; 37: 1595-607.
5. Ferrannini E, Haffner SM, Mitchell BD, Stern MP. Hyperinsulinemia: the key feature of a cardiovascular and metabolic syndrome. Diabetologia 1991; 34: 416-22.
6. Weissberg SP, McCann D, Desai M, et al. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-808.
7. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003; 112: 1821-30.
8. Garg R, Tripathy D, Dandona P. Insulin resistance as a proinflammatory state: mechanisms, mediators, and therapeutic interventions. Curr Drug Targets 2003; 4: 487-92.
9. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89: 2548-56.
10. Berg AH, Combs TP, Du X, et al. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001; 7: 947-53.
11. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002; 8: 1288-95.
12. Shimada K, Miyazaki T, Daida H. Adiponectin and atherosclerotic disease. Clin Chim Acta 2004; 344: 1-12.
13. Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.
14. Maeda K, Okubo K, Shimomura I, et al. cDNA cloning and expression of a novel adipose specific collagen-like factor, apMI (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 1996; 221: 286-9.
15. 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.
16. Berg AH, Combs TP, Du X, et al. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001; 7: 947-53.
17. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002; 8: 1288-95.
18. Okamoto Y, Kihara S, Ouchi N, et al. Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. Circulation 2002; 106: 2767-70.
19. Zmijewski PE, Teoh H, Stewart DL, Verma S. Adiponectin and cardiovascular disease. Am J Physiol Heart Circ Physiol 2007; 292: H1655-63.
20. Hu L, Pories WJ, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem 1996; 271: 10697-703.
21. 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.
22. 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.
23. Bruun JM, Lihn AS, Verdich C, et al. Regulation of adiponectin by adipose tissue-derived cytokines in vivo and in vitro investigations in humans. Am J Physiol Endocrinol Metab 2003; 285: E527-33.
24. Cnop M, Havel PJ, Utschneider 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: 459-69.
25. Kwon K, Jung SH, Choi C, Park SH. Reciprocal association between visceral obesity and adiponectin: in healthy premenopausal women. Int J Cardiol 2006; 108: 239-43.
26. Staiger H, Tschritter O, Machann J, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. J Clin Endocrinol Metab 2004; 89: 2665-71.
27. Klein S, Vozarova B, Funahashi T, et al. Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. Diabetes 2002; 51: 1884-8.
28. Tsirigotis O, Fritsche A, Thamer C, et al. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 2003; 52: 239-43.
29. Baratta R, Amato S, Degano C, et al. Adiponectin relationships with lipid metabolism is independent of body fat mass: evidence from both cross-sectional and intervention studies. J Clin Endocrinol Metab 2004; 89: 2665-71.
30. Iwashima Y, Katsuya T, Ishikawa K, et al. Hypoadiponectinemia is an independent risk factor for hypertension. Hypertension 2004; 43: 1318-23.
31. Ouchi N, Ohishi M, Kihara S, et al. Association of hypoadiponectinemia with impaired vasoreactivity. Hypertension 2003; 42: 231-4.
35. 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.
36. Winer JC, Zern TL, Takasaki SE, et al. Adiponectin in childhood and adolescent obesity and its association with inflammatory markers and components of the metabolic syndrome. J Clin Endocrinol Metab 2006; 91: 4145-23.
37. Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999; 100: 2473-6.
38. Kumada M, Kihara S, Sumitsuji S, et al. Association of hypoadiponectinemia with coronary artery disease in men. Arterioscler Thromb Vasc Biol 2003; 23: 85-9.
39. Pischon T, Girman CJ, Hotamisligil GS, et al. Plasma adiponectin levels and risk of myocardial infarction in men. JAMA 2004; 291: 1730-7.
40. Pilz S, Horejsi R, Moller R, et al. Early atherosclerosis in obese juveniles is associated with low serum levels of adiponectin. J Clin Endocrinol Metab 2005; 90: 4792-6.
41. von Eynatten M, Hamann A, Twardella D, et al. Atherogenic dyslipidaemia but not total- and high-molecular weight adiponectin are associated with the prognostic outcome in patients with coronary heart disease. Eur Heart J 2008; 29: 1307-15.
42. Kistorp C, Faber J, Galatius S, et al. Plasma adiponectin, body mass index, and mortality in patients with chronic heart failure. Circulation 2005; 112: 1756-62.
43. Lawlor DA, Davey Smith G, Ebrahim S, et al. Plasma adiponectin levels are associated with insulin resistance, but do not predict future risk of coronary heart disease in women. J Clin Endocrinol Metabol 2005; 90: 5677-83.
44. Lindsay RS, Resnick HE, Zhu J, et al. Adiponectin and coronary heart disease: the Strong Heart Study. Arterioscler Thromb Vasc Biol 2005; 25: e15-6.
45. Kanaya AM, Wassel Fyr C, Vittinghoff E, et al. Serum adiponectin and coronary heart disease risk in older Black and White Americans. J Clin Endocrinol Metabol 2006; 91: 5044-50.
46. Cavusoğlu E, Ruwende C, Chopra V, et al. Adiponectin is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction in patients presenting with chest pain. Eur Heart J 2006; 27: 2300-9.
47. Pilz S, Maerz W, Weihrauch G, et al. Adiponectin serum concentrations in men with coronary artery disease: the Lüdwigs- shafen Risk and Cardiovascular Health (LURIC) study. Clin Chim Acta 2006; 364: 251-5.
48. Laughlin GA, Barrett-Connor E, May S, Langenberg C. Association of adiponectin with coronary heart disease and mortality: the Rancho Bernardo study. Am J Epidemiol 2007; 165: 164-74.
49. Wannamethee SG, Whincup PH, Lennon L, Sattar N. Circulating adiponectin levels and mortality in elderly men with and without cardiovascular disease and heart failure. Arch Intern Med 2007; 167: 1510-7.
50. Pilz S, Mangge H, Wellnitz B, et al. Adiponectin and mortality in patients undergoing coronary angiography. J Clin Endocrinol Metab 2006; 91: 4277-86.
51. Dekker JM, Funahashi T, Nijpels G, et al. Prognostic value of adiponectin for cardiovascular disease and mortality. J Clin Endocrinol Metab 2008; 93: 1489-96.
52. Butte NF, Comuzzie AG, Cai G, et al. Genetic and environmental factors influencing fasting serum adiponectin in Hispanic children. J Clin Endocrinol Metab 2005; 90: 4170-6.
53. Ong KK, Frystyk J, Flyvbjerg A, et al. Sex-discordant associations with adiponectin levels and lipid profiles in children. Diabetes 2006; 55: 1337-41.
54. Isole T, Saitoh S, Takagi S, et al. Influence of gender, age and renal function on plasma adiponectin level: the Tanno and Sobetsu study. Eur J Endocrinol 2005; 153: 91-8.
55. Aso Y, Yamamoto R, Wakabayashi S, et al. Comparison of serum high-molecular weight (HMW) adiponectin with total adiponectin concentrations in type 2 diabetic patients with coronary artery disease using a novel enzyme-linked immunosorbent assay to detect HMW adiponectin. Diabetes 2006; 55: 1954-60.
56. Bottner A, Kratzsch J, Muller G, et al. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J Clin Endocrinol Metab 2004; 89: 4053-61.
57. Tentolouris N, Doulgerakis D, Moyssakis I, et al. Plasma adiponectin concentrations in patients with chronic renal failure: relationship with metabolic risk factors and ischemic heart disease. Horm Metab Res 2004; 36: 721-7.
58. Senolt L, Pavelka K, Housa D, Haluzik M. Increased adiponectin is negatively linked to the local inflammatory process in patients with rheumatoid arthritis. Cytokine 2006; 35: 247-52.
59. Haugen F, Ranheim T, Harsem NK, et al. Increased plasma levels of adipokines in preeclampsia: relationship to placenta and adipose tissue gene expression. Am J Physiol Endocrinol Metabol 2006; 290: E326-33.
60. Shoji T, Shinozaki H, Hatsuda S, et al. Altered relationship between body fat and plasma adiponectin in end-stage renal disease. Metabolism 2005; 54: 330-4.
61. Pajvani UB, Du X, 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: 9073-85.
62. Mandle H, Almer G, Haj-Yahya S, et al. Nuchal thickness of subcutaneous adipose tissue is tightly associated with an increased LMW/total adiponectin ratio in obese juveniles. Atherosclerosis 2008; [Epub ahead of print].
63. Fisher FF, Trujillo ME, Hanif W, et al. Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. Diabetologia 2005; 48: 1084-7.
64. Hara K, Horikoshi M, Yamauchi T, et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2006; 29: 1357-62.
65. Liu Y, Retnakaran R, Hanley A, et al. Total and high molecular weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in Thai subjects. J Clin Endocrinol Metabol 2007; 92: 4313-8.
66. Yang Q, Graham TE, Mody N, et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005; 436: 356-62.
67. Graham TE, Yang Q, Bluhm M, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006; 354: 2552-63.
68. Cho YM, Youn BS, Lee H, et al. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 2006; 29: 2457-61.
69. Qi Q, Yu Z, Ye X, et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. J Clin Endocrinol Metab 2007; 92: 4324-9.
70. Lee JH, Im JA, Lee HR, et al. Visceral adiposity is associated with serum retinol binding protein-4 levels in healthy women. Obesity 2007; 15: 2225-32.
71. Jia W, Wu H, Bao Y, et al. Association of serum retinol-binding protein 4 and visceral adiposity in Chinese subjects with and without type 2 diabetes. J Clin Endocrinol Metab 2007; 92: 3224-9.
72. Janke J, Engeli S, Boschmann M, et al. Retinol binding protein 4 expression in humans: relationship to insulin resis-
tance, inflammation, and response to pioglitazone. J Clin Endocrinol Metab 2007; 92: 2590-7.
74. Gavi S, Stuart LM, Kelly P, et al. Retinol-binding protein 4 is associated with insulin resistance and body fat distribution in nonobese subjects without type 2 diabetes. J Clin Endocrinol Metab 2007; 92: 1886-90.
75. Graham TE, Wason CJ, Bluher M, Kahn BB. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetesologia 2007; 50: 314-23.
76. Stefan N, Hennige AM, Staiger H, et al. High circulating reti-

nol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. Diabetes Care 2007; 30: 1173-8.
77. Perseghin G, Lattuada G, De Cobelli F, et al. Serum reti-

nol-binding protein-4, leptin, and adiponectin concentrations are related to ectopic fat accumulation. J Clin Endocrinol Metab 2007; 92: 4883-8.
78. Tsutsucu M, Okuno M, Tannous L, et al. Retinoids and reti-

nol-binding protein expression in rat adipocytes. J Biol Chem 1992; 267: 1805-10.
79. Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. Nature 2001; 409: 307-12.
80. Savage DB, Sewter CP, Klenk ES, et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes 2001; 50: 2199-202.
81. Yannakoulia M, Yiannakouris N, Bluher S, et al. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metab 2003; 88: 1730-6.
82. Silha JV, Krsek M, Skrha JV, et al. Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. Eur J Endocrinol 2003; 149: 331-5.
83. Zhang JL, Qin YW, Zheng X, et al. Serum resistin level in es-

sential hypertension patients with different glucose tolerance. Diabetes Res 2002; 34: 671-3.
84. Cancedda R, Benussi F, Grattoni P, et al. Reduction of macrophage infiltration and chemotactant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 2005; 54: 2277-86.
85. Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 1998; 41: 1241-8.
86. Yudkin JS, Kumari M, Humphries SE, Mohamed-Ali V. In-

flammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 2000; 148: 209-14.
87. Pfeiffer A, Janott J, Mohlig M, et al. Circulating tumor necrosis factor alpha is elevated in male but not in female patients with type II diabetes mellitus. Horm Metab Res 1997; 29: 111-4.
88. Kellner M, Reit K, Renn W, et al. Circulating TNF-alpha and leptin levels in offspring of NIDDM patients do not correlate to individual insulin sensitivity. Horm Metab Res 1996; 28: 737-43.
89. Kern PA, Ranganathan S, Li C, et al. Adipose tissue tumor necrosis factor and intereleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 2001; 280: E745-51.
90. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 1997; 82: 4196-200.
91. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: association with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999; 19: 972-8.
92. Engelberts I, Stephane S, Francot GJ, et al. Evidence for different effects of soluble TNF-receptors on various TNF measurements in human biological fluids. Lancet 1991; 338: 515-6.
93. Vozzarova B, Weyer C, Hanson K, et al. Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. Obes Res 2001; 9: 414-7.
94. Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin-6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin Endocrinol Metab 2000; 85: 3338-42.
95. 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.
96. Kopp HP, Kopp CW, Festa A, et al. Impact of weight loss on inflammatory proteins and their association with the insulin re-

sistance syndrome in morbidly obese patients. Arterioscler Thromb Vasc Biol 2003; 23: 1042-7.
97. Giugliano G, Nicolletti G, Gnella E, et al. Effect of liposuction on insulin resistance and vascular inflammatory markers in obese women. Br J Plast Surg 2004; 57: 190-4.
98. Bermudez EA, Riffai N, Buring J, et al. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardio-

vascular risk factors in women. Arterioscler Thromb Vasc Biol 2002; 22: 1668-73.
99. Feibraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 2002; 16: 1335-47.
100. Carey AL, Bruce CR, Sacchetti M, et al. Interleukin-6 and tumor necrosis factor-alpha are not increased in patients with Type 2 diabetes: evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. Diabetologia 2004; 47: 1029-37.
101. Salmenniemi U, Ruotsalainen E, Pilhalajamaki J, et al. Multiple abnormalities in glucose and energy metabolism and coordi-

nated changes in levels of adiponectin, cytokines, and adhesion molecules in subjects with metabolic syndrome. Circulation 2004; 110: 3842-8.
102. Matsushita K, Yatsuya H, Tamakoshi K, et al. Comparison of circulating adiponectin and proinflammatory markers regarding
their association with metabolic syndrome in Japanese men. Arterioscler Thromb Vasc Biol 2006; 26: 871-6.

112.Mortensen RF. C-reactive protein, inflammation, and innate immunity. Immunol Res 2001; 24: 163-76.

113.Calabro P, Chang DW, Willerson JT, Yeh ET. Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: linking obesity to vascular inflammation. J Am Coll Cardiol 2005; 46: 1112-3.

114.Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14719 initially healthy American women. Circulation 2003; 107: 391-7.

115.Lemieux I, Pacot A, Prud’homme D, et al. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. Arterioscler Thromb Vasc Biol 2001; 21: 961-7.

116.Freeman DJ, Norrie J, Caslake MJ, et al. C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. Diabetes 2002; 51: 1596-600.

117.Heilbronn LK, Noakes M, Clifton PM. Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentrations in obese, healthy women. Arterioscler Thromb Vasc Biol 2001; 21: 968-70.

118.Tchernof A, Nolan A, Sites CK, et al. Weight loss reduces C-reactive protein levels in obese postmenopausal women. Circulation 2002; 105: 564-9.

119.Gerhardt CC, Romero IA, Cancelo R, et al. Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. Mol Cell Endocrinol 2001; 175: 81-92.

120.Dietze-Schroeder D, Sell H, Uhlig M, et al. Autocrine action of adiponectin on human fat cells prevents the release of insulin resistance-inducing factors. Diabetes 2005; 54: 2003-11.

121.Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006; 444: 875-80.

122.Christiansen T, Richelsen B, Bruun JM. Monocyte chemotactant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. Int J Obes 2005; 29: 146-50.

123.Kim CS, Park HS, Kawada T, et al. Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity-related parameters. Int J Obes 2006; 30: 1347-55.

124.Nomura S, Shouzu A, Omoto S, et al. Significance of chemokines and activated platelets in patients with diabetes. Clin Exp Immunol 2008; 121: 437-43.

125.Piemonti L, Calori G, Mercalli A, et al. Fasting plasma leptin, tumor necrosis factor-alpha receptor 2, and monocyte chemottracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. Diabetes Care 2003; 26: 2883-9.

126.Schernthaner GH, Kopp HP, Kriwanek S, et al. Effect of massive weight loss induced by bariatric surgery on serum levels of interleukin-18 and monocyte-chemoattractant-protein-1 in morbid obesity. Obes Surg 2006; 16: 709-15.

127.Dahlanen I, Kaaman M, Olsson T, et al. A unique role of monocyte chemotactant protein-1 among chemokines in adipose tissue of obese subjects. J Clin Endocrinol Metab 2005; 90: 5834-40.

128.Inadera H, Egashira K, Takemoto M, et al. Increase in circulating levels of monocyte chemotactant protein-1 with aging. J Interferon Cytokine Res 1999; 19: 1179-82.

129.Parissis JT, Venetsanou KF, Kalantzis MV, et al. Serum profiles of granulocyte-macrophage colony-stimulating factor and C-C chemokines in hypertensive patients with or without significant hyperlipidemia. Am J Cardiol 2000; 85: 777-9.
Erikstrup C, Mortensen OH, Pedersen BK. Retinol-binding protein 4 and insulin resistance. J Clin Endocrinol Metab 2007; 92: 1168-71.

Vitkova M, Klimcakova E, Kovacikova M, et al. Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. J Clin Endocrinol Metab 2007; 92: 2330-5.

Takebayashi K, Suet sugu M, Wakabayashi S, et al. Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. J Clin Endocrinol Metab 2007; 92: 2712-9.

Tan BK, Chen J, Lehner H, et al. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids. J Clin Endocrinol Metab 2007; 92: 2764-72.

Broch M, Vendrell J, Ricart W, et al. Circulating retinol-binding protein-4, insulin sensitivity, insulin secretion, and insulin disposition index in obese and nonobese subjects. Diabetes Care 2007; 30: 1802-6.

Yagmur E, Weiskirchen R, Gressner AM, et al. Insulin resistance in liver cirrhosis is not associated with circulating retinol-binding protein 4. Diabetes Care 2007; 30: 1168-72.

von Eynatten M, Lepper PM, Liu D, et al. Retinol-binding protein 4 is associated with components of the metabolic syndrome, but not with insulin resistance, in men with type 2 diabetes or coronary artery disease. Diabetologia 2007; 50: 1950-7.

Azuma K, Katsukawa F, Oguchi S, et al. Correlation between serum resistin level and adiposity in obese individuals. Obes Res 2003; 11: 997-1001.

Degawa-Yamauchi M, Bovenkerk JE, Julier BE, et al. Serum resistin (FIZZ3) protein is increased in obese humans. J Clin Endocrinol Metab 2003; 88: 5452-5.

Lee JH, Chan JL, Yiannakouris N, et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. J Clin Endocrinol Metab 2003; 88: 4848-56.

Plutzner A, Langenfeld M, Kunt T, et al. Evaluation of human resistin assays with serum from patients with type 2 diabetes and different degrees of insulin resistance. Clin Lab 2003; 49: 571-6.

Monzillo LU, Hamdy O, Horton ES, et al. Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance. Obes Res 2003; 11: 1048-54.

Silha J, Krsek M, Skrha J, et al. Plasma resistin, leptin and adiponectin levels in non-diabetic and diabetic obese subjects. Diabet Med 2004; 21: 497-9.

Heilbronn LK, Rood J, Janderova L, et al. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. J Clin Endocrinol Metab 2004; 89: 1844-8.
188. Bajaj M, Suraamornkul S, Hardies LJ, et al. Plasma resistin concentration, hepatic fat content, and hepatic and peripheral insulin resistance in pioglitazone-treated type II diabetic patients. Int J Obes Relat Metab Disord 2004; 28: 783-9.

189. Ohmori R, Momiyama Y, Kato R, et al. Associations between serum resistin levels and insulin resistance, inflammation, and coronary artery disease. J Am Coll Cardiol 2005; 46: 379-80.

190. Bo S, Gambino R, Pagani A, et al. Relationships between human serum resistin, inflammatory markers and insulin resistance. Int J Obes 2005; 29: 1315-20.

191. Katsu A, Sumida Y, Murashima S, et al. Serum levels of tumor necrosis factor-alpha are increased in obese patients with non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1998; 83: 859-62.

192. Winkler G, Salamon F, Salamon D, et al. Elevated serum tumour necrosis factor-alpha levels can contribute to the insulin resistance in Type II (non-insulin-dependent) diabetes and in obesity. Diabetologia 1998; 41: 860-1.

193. Nilsson J, Jovinge S, Niemann A, et al. Relation between plasma tumor necrosis factor-alpha and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1998; 18: 1199-202.

194. Zinman B, Hanley AJ, Harris SB, et al. Circulating tumor necrosis factor-alpha concentrations in a native Canadian population with high rates of type 2 diabetes mellitus. J Clin Endocrinol Metab 1999; 84: 272-8.

195. Corica F, Allegra A, Corsonello A, et al. Relationship between plasma leptin levels and the tumor necrosis factor-alpha system in obese subjects. Int J Obes Relat Metab Disord 1999; 23: 355-60.

196. Winkler G, Lakatos P, Salamon F, et al. Elevated serum TNF-alpha level as a link between endothelial dysfunction and insulin resistance in normotensive obese patients. Diabet Med 1999; 16: 207-11.

197. Ziccardi P, Nappo F, Giugliano G, et al. Reduction of inflammatory cytokine concentrations and improvement of endothelial functions in obese women after weight loss over one year. Circulation 2002; 105: 804-9.

198. Maachi M, Pieroni L, Bruckert E, et al. Systemic low-grade inflammation is related to both circulating and adipose tissue TNFalpha, leptin and IL-6 levels in obese women. Int J Obes Relat Metab Disord 2004; 28: 993-7.

199. Marfellia R, Esposito K, Siniscalchi M, et al. Effect of weight loss on cardiac synchronization and proinflammatory cytokines in premenopausal obese women. Diabetes Care 2004; 27: 47-52.

200. Hak AE, Stehouwer CD, Bots ML, et al. Associations of C-reactive protein with measures of obesity, insulin resistance, and subclinical atherosclerosis in healthy, middle-aged women. Arterioscler Thromb Vasc Biol 1999; 19: 1986-91.

201. Natali A, Toschi E, Baldeweg S, et al. Clustering of insulin resistance with vascular dysfunction and low-grade inflammation in type 2 diabetes. Diabetes 2006; 55: 1133-40.

202. Visser M, Bouter LM, McQuillan GM, et al. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999; 282: 2131-5.

203. McLaughlin T, Abbasi F, Lamendola C, et al. Differentiation between obesity and insulin resistance in the association with C-reactive protein. Circulation 2002; 106: 2908-12.

204. Escobar-Morreale HF, Villuendas G, Botella-Carretero JJ, et al. Obesity, and not insulin resistance, is the major determinant of serum inflammatory cardiovascular risk markers in pre-menopausal women. Diabetologia 2003; 46: 625-33.

205. Moran LJ, Noakes M, Clifton PM, et al. C-reactive protein before and after weight loss in overweight women with and without polycystic ovary syndrome. J Clin Endocrinol Metab 2007; 92: 2944-51.