Available online at www.sciencedirect.com

SCIENCE DIRECT®

Metabolism Clinical and Experimental 54 (2005) 439–444

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

The reciprocal association of adipocytokines with insulin resistance and C-reactive protein in clinically healthy men☆

Carl Johan Behre[a,b,*], Björn Fagerberg[a,b], Lillemor Mattsson Hultén[b], Johannes Hulthe[b,c]

[a]Institute of Internal Medicine, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden
[b]Wallenberg Laboratory for Cardiovascular Research, Sahlgrenska University Hospital, Göteborg University, S-413 45 Göteborg, Sweden
[c]AstraZeneca R&D, S-431 83 Mölndal, Sweden

Received 10 February 2004; accepted 30 October 2004

Abstract

In experimental models, adiponectin improves and tumor necrosis factor α (TNF-α) impairs insulin action, and the expression of these adipocytokines seems to have a reciprocal regulation. The aim was to examine whether in a cross-sectional study, associations supporting this concept may be found in 58-year-old clinically healthy men, and also the relation to C-reactive protein (CRP).

In 102 men, euglycemic hyperinsulinemic clamp was used to assess glucose infusion rate (GIR). Total body fat (dual-energy x-ray absorptiometry), plasma adiponectin (radioimmunoassay), TNF-α, and CRP (enzyme-linked immunosorbent assay) were measured.

Adiponectin correlated positively to GIR (r = 0.33, P < .001) and negatively to total fat mass (r = −0.29, P = .004), whereas TNF-α showed reverse associations (r = −0.31, P < .01, and r = 0.31, P < .01). Adiponectin and TNF-α were negatively correlated (−0.28, P = .006). An interaction term (TNF-α/adiponectin ratio) and body fat together explained 31.3% (P < .001) in GIR variability. The odds ratio for having insulin resistance was 9.3 (95% CI, 2.2-38.9) in subjects with TNF-α values above and adiponectin levels below the median, as compared to subjects with TNF-α values below and adiponectin levels above the median. Total fat and TNF-α, but not adiponectin, were significantly associated with log CRP (R^2 = 20%, P < .001).

In conclusion, this study in man showed that plasma adiponectin and TNF-α were independently and reversely associated with insulin resistance. C-reactive protein levels were related to TNF-α and obesity.

1. Introduction

Increased levels of high-sensitive C-reactive protein (CRP), an acute phase reactant synthesized in the liver, has been shown to predict cardiovascular morbidity and mortality in large number of studies [1-4]. High-sensitive CRP has also been shown to be associated with the metabolic syndrome [5,6] and adiposity [7-12], but the mechanisms remain unclear. Further, dysregulation of a number of cytokines produced in the adipose tissue, conceptualized as adipocytokines [13], has been shown to be involved in the pathogenesis of the metabolic syndrome and cardiovascular disease [14-16]. An adipovascular axis [13] has been suggested with adipocytokines as the mechanistic link between fat and artery [17-20].

One of these adipocytokines, the 244–amino acid protein adiponectin, is in human being and mice exclusively secreted from the adipose tissue [21]. Paradoxically, the more obese a subject, the lower plasma levels of adiponectin [22]. This could be explained by an inhibitory influence of other adipocytokines, such as tumor necrosis factor α (TNF-α) [22,23]. Experimental studies in rodents and different tissue models have shown that adiponectin and TNF-α have multiple and opposite effects on insulin sensitivity and glucose metabolism. Hence, in myocytes, adiponectin has favorable effects on insulin and glucose metabolism by activating insulin receptor substrate-1–associated phosphoinositide 3 kinase and glucose uptake, whereas TNF-α has reverse effects [23]. Similarly, adiponectin accelerates free fatty acid clearance by enhancing fatty-acid transport protein 1 (FATP-1) messenger RNA (mRNA), and TNF-α has

☆ This work was supported by grants from the Swedish Heart-Lung Foundation, the Swedish Medical Research Council (12270,10880), AstraZeneca Mölndal, Sweden.

* Corresponding author. Institute of Internal Medicine, Sahlgrenska University Hospital, SE-413 45 Göteborg, Sweden. Tel.: +46 31 342 10 00; fax: +46 31 827270.

E-mail address: carljohan.behre@wlab.gu.se (C.J. Behre).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2004.10.010

reverse effects [23]. Adiponectin also enhances free fatty acid oxidation in muscle [24,25], and recombinant adiponectin suppresses hepatic gluconeogenesis and glucose-6-phosphatase [26]. Adiponectin improves insulin sensitivity and seems to be antiatherosclerotic, whereas TNF-α has opposite properties and is a proinflammatory cytokine. Tumor necrosis factor α may also be involved in the regulation of CRP production in the liver [27,28].

In human beings, cross-sectional studies give evidence that adiponectin is positively associated with insulin sensitivity [29-34]. There are some data from human beings supporting the results from animal studies regarding the effect of adiponectin on oxidative metabolism of glucose [29] and insulin signaling [31]. The adipocytokine TNF-α, implicated in the decrease of insulin sensitivity seen in obesity and type 2 diabetes, is in these states inversely expressed compared to adiponectin [35], suggesting opposite roles in the metabolic syndrome. However, several studies in human beings have failed to find any increase in TNF-α in insulin-resistant subjects [36-38]. Further, the insulin-sensitizing thiazolidinediones reduce TNF-α levels [39,40] but increase adiponectin levels [39,40]. In a previous small study consisting of obese subjects, an inverse relationship was seen between TNF-α and adiponectin, both before and after weight reduction [41]. However, to our knowledge, no previous population-based study has described the suggested reciprocal influence of the high-sensitive CRP and TNF-α proinflammatory activity and the antiinflammatory cytokine adiponectin on insulin sensitivity assessed with the clamp method.

Accordingly, the aim of the present study was to examine whether the opposite associations of adiponectin and TNF-α vs insulin sensitivity, as observed in experimental studies, also are observed in a population-based sample of clinically healthy 58-year-old men (n = 102). The aim was also to explore the relationship of these adipocytokines to circulating CRP.

2. Subjects and methods

2.1. Study population

The subjects were included in a previously described study that had the primary objective to examine the association between insulin resistance and clinically silent atherosclerosis [42,43]. The inclusion criteria were male sex, aged 58 years, and Swedish ancestry. Exclusion criteria were established cardiovascular disease clinical diabetes mellitus or other established disease, treatment with cardiovascular drugs, or unwillingness to participate.

The design was a cross-sectional study on the basis of a stratified sampling of randomly selected and screened men as previously described [42-45]. One hundred four men with varying degrees of obesity and insulin sensitivity underwent a euglycemic hyperinsulinemic clamp [42]. Of these, 102 subjects participated in the present study and the subjects had the following characteristics, as previously described: body mass index, 26.0 ± 4.2 kg/m^2; body fat, 21.3 ± 0.07 kg; systolic and diastolic blood pressure, 138 ± 21/83 ± 11 mm Hg; total serum cholesterol, 6.03 ± 1.12 mmol/L; high-density lipoprotein cholesterol, 1.26 ± 0.36 mmol/L; serum triglycerides, 1.56 ± 1.10 mmol/L (median, 1.36); and blood glucose, 4.7 ± 0.58 mmol/L.

The subjects received both written and oral information before they gave their consent to participate. The study was approved by the Ethics Committee at Sahlgrenska University Hospital.

2.2. Measurements

Body weight was measured on a balance scale with the subject dressed in underwear. Body mass index was calculated as weight (in kilograms) divided by height squared (m^2). Waist, hip circumferences, and sagittal abdominal diameter were measured. The sagittal diameter is the measure from the examination table to the highest level of the abdomen [46]. Systolic and diastolic blood pressures were measured in duplicate after 5 minutes of supine rest. The mean values were used in the analysis. Heart rate was counted from the radial pulse. Whole blood glucose was measured with the glucose oxidase technique. Blood samples were drawn, and serum and plasma were frozen in aliquots at −70°C within 4 hours.

A euglycemic hyperinsulinemic clamp examination ad modum de Fronzo was performed slightly modified, as previously published [47]. After the clamp examination, fat-free mass and total body fat mass were measured using the dual-energy x-ray absorptiometry (DXA) body composition model (Lunar DPX-L, Lunar Corp, Madison, Wis). Insulin sensitivity was calculated as the glucose infusion rate (GIR) per minute adjusted for fat-free mass during the final hour of the examination [47]. Insulin resistance was defined as GIR below 6.85 mg/kg fat-free mass per minute. This definition was calculated as the mean value of the 25th percentile of GIR from 2 clamp examinations performed with 3 weeks interval in 32 men from the background population of the present study [47].

2.3. Laboratory procedures

High-sensitive enzyme-linked immunosorbent assay kits were used to measure TNF-α (R&D System Europe Ltd, Abingdon, UK) and CRP (Medix Biochemica, Kauniainen, Finland). Plasma levels of adiponectin were determined by a radioimmunoassay kit (LINCO Research Inc, St Charles, Mo) that uses ^{125}I-labeled murine adiponectin and a multispecies adiponectin rabbit antiserum. Human recombinant adiponectin was used as a standard. Inter- and intraassay coefficient of variation was 5.2% and 3.6%, respectively. No significant difference was obtained when plasma was compared to serum (n = 20). All analyses were performed at the Wallenberg Laboratory, Göteborg, Sweden.

Cholesterol and triglyceride levels were determined by fully enzymatic techniques [48,49]. High-density lipoprotein was determined after precipitation of apolipoprotein B–containing lipoproteins with manganese chloride and dextran sulfate.

2.4. Statistical analysis

All statistics were analyzed using SPSS for Windows 11.0 (Chicago, Ill). The results are presented as mean values, standard deviations and numbers (%). Skewed variables are presented as mean, median (minimum and maximum value), and were log transformed before parametric analyses. Nonparametric Spearman's rank correlations coefficients were used to illustrate the relationship between the variables under study. Multiple regression was used in the analyses examining the associations between the studied variables. Two-sided $P < .05$ was considered statistically significant.

3. Results

The characteristics of the subjects regarding plasma adiponectin, TNF-α, CRP, total body fat, and insulin sensitivity are presented in Table 1.

3.1. Adiponectin, TNF-α, and insulin sensitivity

Adiponectin correlated positively to GIR (Table 2) and negatively to total fat mass ($r = -0.29$, $P = .004$). The association between adiponectin and GIR remained after adjustment for total fat mass (Table 2). Tumor necrosis factor α, on the other hand, showed inverse associations and correlated negatively to GIR (Table 1) and positively to total fat ($r = 0.31$, $P = .002$). However, the association between TNF-α and GIR did not remain after adjustment for total fat mass (Table 2). Adiponectin and TNF-α were negatively correlated (−0.28, $P = .006$).

In a multiple regression analysis, log adiponectin (β coefficient, 4.1; SE, 1.57; $P = .01$) and log TNF-α (β coefficient, −4.6; SE, 2.08; $P = .03$) contributed independently and reversely to the variability in GIR. These opposite and independent effects of adiponectin and TNF-α on GIR are also described in Fig. 1.

We further explored the relationship among plasma TNF-α (over and below median), plasma adiponectin (over and below median, respectively), and GIR by calculating the odds ratio (OR) for having insulin resistance. The OR for having insulin resistance was 9.3 (95% CI, 2.2-38.9) in subjects with TNF-α values above and adiponectin levels below the median, as compared to subjects with TNF-α values below and adiponectin levels above the median.

In a multiple regression model, adiponectin and TNF-α showed independent associations with GIR. This relationship disappeared after further adjustment for total fat (data not shown). However, if an interaction term was used (log ratio of TNF-α and adiponectin), this interaction term was associated with GIR (β coefficient, −2.5; SE, 1.0; $P = .014$) independent of total fat (β coefficient, $-1.6\ 10^{-4}$; SE, 0.0, $P < .001$), and R^2 was 31.3% ($P < .001$). Furthermore, a plot of the residuals against the fitted values did not disclose any specific pattern.

3.2. CRP and insulin sensitivity

C-reactive protein correlated negatively to GIR (Table 2) and positively to total fat ($r = 0.38$, $P < .001$). However, the association between CRP and GIR did not remain after adjustment for total fat mass (Table 2). C-reactive protein

Table 2
Correlation coefficients between GIR and adipocytokines and CRP with and without adjustment for total body fat (n = 102)

	Unadjusted	Adjusted for total fat mass (partial correlation coefficient)
Log adiponectin	0.33**	0.23*
Log TNF-α	−0.31**	−0.17
Log CRP	−0.26**	−0.10

* $P < .05$.
** $P < .01$.

Table 1
Plasma concentrations of adiponectin, TNF-α, CRP, and total body fat by tertiles of GIR (geometric mean for adiponectin, TNF-α, CRP and mean for total body fat, SD)

	Tertiles of GIR			
	1 (n = 34)	2 (n = 34)	3 (n = 34)	All (n = 102)
GIR (mg/kg fat-free mass per min)	4.68 ± 1.32	8.65 ± 0.90	11.79 ± 1.84	8.87 ± 3.09
Plasma adiponectin (μg/mL)	10.53 ± 6.19	14.39 ± 6.27	14.37 ± 6.05	12.95 ± 6.35
Plasma TNF-α (pg/mL)	2.43 ± 0.66	2.11 ± 1.13	1.92 ± 0.64	2.14 ± 0.85
CRP (mg/L)	1.64 ± 2.51	0.9 ± 1.89	0.87 ± 2.28	1.09 ± 2.26
Total fat (kg)	28.09 ± 6.59	18.40 ± 7.60	17.22 ± 6.70	21.57 ± 8.37

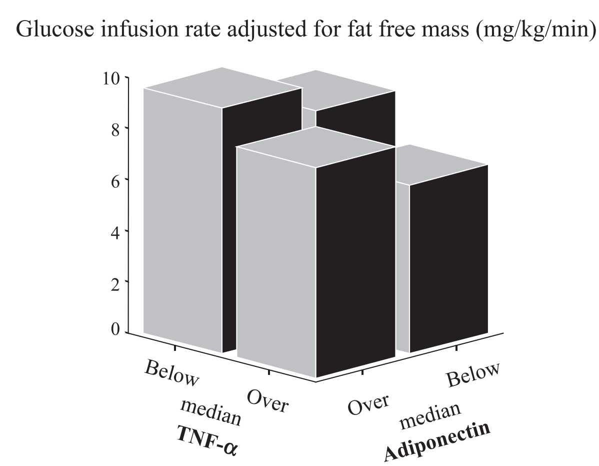

Fig. 1. Glucose infusion rate adjusted for fat-free mass in relation to plasma concentrations of adiponectin and TNF-α in 102 clinically healthy men.

was also associated with adiponectin ($r = -0.30$, $P = .003$) and TNF-α ($r = 0.34$, $P = .001$). In a multiple regression analyses, total fat (beta-coefficient $-1.2\ 10^{-5}$, SE 0.0, $P <$ .03) and TNF-α (β coefficient 0.91, SE 0.30, $P = .003$), but not adiponectin, were significantly associated with log CRP ($R^2 = 20\%$, $P < .001$).

4. Discussion

The results of the present study showed that plasma concentrations of adiponectin and TNF-α were associated in an opposite manner to insulin sensitivity, suggesting opposite effects. Adiponectin that was negatively correlated to TNF-α showed a positive association with insulin sensitivity that was independent of total body fat. Tumor necrosis factor α correlated inversely to insulin sensitivity, however, not independently of body fat. There seems to be an interaction between high adiponectin and low TNF-α levels on insulin sensitivity. Thus, an interaction term consisting of the ratio of TNF-α and adiponectin and total body fat explained together almost one third of the variability in insulin sensitivity. Furthermore, subjects with high TNF-α (above median) and concomitant low adiponectin levels(below median) had an increased risk of having impaired insulin sensitivity as compared with subjects with low TNF-α and concomitant high adiponectin levels (OR = 9.3). These findings were observed in middle-aged, clinically healthy men with varying degrees of obesity recruited from the general population.

Taken together, the inverse relationship observed in the present paper raises the question whether the interaction between TNF-α and adiponectin, rather than the individual cytokines per se, is of importance for the development of insulin resistance. This suggestion is supported by the observation that the interaction term consisting of the ratio of TNF-α and adiponectin, but not TNF-α and adiponectin separately, showed associations with GIR that were independent of total fat. However, the present study has too limited power to clarify this issue in further detail.

Yokota et al [50] have shown that adiponectin lowers TNF-α production in macrophages, supporting the hypothesis that the negative correlation between TNF-α activity and adiponectin could be explained by a reciprocal inhibitory influence on the level of gene expression. Because adiponectin is uniquely expressed in the adipose tissue in human beings, the most important site for this interaction would be the adipose tissue. However, there is also evidence from in vitro studies supporting the concept that TNF-α may act as an inhibitor of adiponectin expression in adipose tissue. In these studies, TNF-α was demonstrated to decrease adiponectin gene expression in human preadipocytes [51] 3T3-L1 adipocytes [52] and in whole adipose tissue [41]. In a recent study of adiponectin knockout mice, there were high levels of TNF-α mRNA in adipose tissue and high plasma TNF-α concentrations indicating the reciprocal effect between the 2 adipocytokines [23]. These knockout mice exhibited impaired free fatty acid clearance and severe diet-induced insulin resistance with impaired insulin signaling in muscle. All these dysregulations, including increased TNF-α expression, were reversed after viral mediated adiponectin expression [23]. The mechanism underlying for the opposite physiological effects of adiponectin and TNF-α may relate to the structural resemblance of adiponectin and TNF-α [53].

As expected, CRP was positively associated with total body fat and TNF-α supporting the concept that adipocyte-derived TNF-α may be one stimulatory factor in the production of CRP in the liver. In the present study, total body fat and TNF-α were independently associated with CRP, and together they explained 20% of the variability in CRP. Interleukin 6 (IL-6) is the adipocytokine that is most directly involved in the regulation of CRP synthesis [8,54]. Tumor necrosis factor α is also acting on CRP synthesis through IL-6 [27,28]. Adiponectin showed a negative association with CRP, but this did not remain after adjustment for total body fat. A previous study in Japanese women found that low-grade CRP elevation was associated with decreased adiponectin concentrations [55], whereas a study of young healthy men observed that fat mass and leptin, but not adiponectin, were associated with CRP [56].

The limitation of the present study is that only clinically healthy 58-year-old Caucasian men were studied. The rational was to examine a group that is at high risk for cardiovascular disease and to reduce a number of potentially confounding factors, for example, age, sex, concomitant disease, and accompanying medication. This was a cross-sectional study and no conclusions can be drawn about causality.

In summary, the contribution of the present study is that it extends the previously observed reciprocal action of TNF-α and adiponectin expression levels on insulin action, based on experimental studies, to show that in a population-based sample of clinically healthy middle-aged men without cardiovascular disease, but with a wide range of insulin resistance, low adiponectin and high TNF-α plasma concentrations were associated with a very high risk of insulin resistance. Circulating CRP concentrations were related to total body fat and TNF-α, but not adiponectin in a multivariate analysis.

Acknowledgments

We thank Dr Lena Bokemark for screening of subjects, and project assistant Marie-Louise Ekholm, research nurse Magdalena Göthberg, and laboratory technologists Christina Claesson, Carita Fagerlund, and Caroline Schmidt for excellent research assistance.

References

[1] Ridker PM, Buring JE, Cook NR, et al. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391 - 7.

[2] Tracy RP, Lemaitre RN, Psaty BM, et al. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. Arterioscler Thromb Vasc Biol 1997;17:1121-7.
[3] Koenig W, Sund M, Frohlich M, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999;99:237-42.
[4] Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000;321:199-204.
[5] Pradhan AD, Manson JE, Rifai N, et al. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 2001;286: 327-34.
[6] 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.
[7] Visser M, Bouter LM, McQuillan GM, et al. Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999;282:2131-5.
[8] Yudkin JS, Stehouwer CD, Emeis JJ, et al. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol 1999;19:972-8.
[9] Festa A, D'Agostino Jr R, Howard G, et al. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). Circulation 2000;102:42-7.
[10] Festa A, D'Agostino Jr R, Williams K, et al. The relation of body fat mass and distribution to markers of chronic inflammation. Int J Obes Relat Metab Disord 2001;25:1407-15.
[11] Forouhi NG, Sattar N, McKeigue PM. Relation of C-reactive protein to body fat distribution and features of the metabolic syndrome in Europeans and South Asians. Int J Obes Relat Metab Disord 2001;25:1327-31.
[12] Lemieux I, Pascot 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.
[13] Matsuda M, Shimomura I, Sata M, et al. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J Biol Chem 2002;277:37487-91.
[14] Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473-6.
[15] Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296-301.
[16] 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.
[17] Weyer C, Tataranni PA, Pratley RE. Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. Diabetes Care 2000;23:779-85.
[18] Alessi MC, Peiretti F, Morange P, et al. Production of plasminogen activator inhibitor 1 by human adipose tissue: possible link between visceral fat accumulation and vascular disease. Diabetes 1997;46: 860-7.
[19] Hotamisligil GS, Spiegelman BM. Tumor necrosis factor alpha: a key component of the obesity-diabetes link. Diabetes 1994;43:1271-8.
[20] Hotamisligil GS, Murray DL, Choy LN, et al. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. Proc Natl Acad Sci U S A 1994;91:4854-8.
[21] Maeda K, Okubo K, Shimomura I, et al. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun 1996;221:286-9.
[22] 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.
[23] Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8: 731-7.
[24] Yamauchi T, Kamon J, Waki H, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941-6.
[25] 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.
[26] Combs TP, Berg AH, Obici S, et al. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J Clin Invest 2001; 108:1875-81.
[27] Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1990;265:621-36.
[28] Stephens JM, Butts MD, Pekala PH. Regulation of transcription factor mRNA accumulation during 3T3-L1 preadipocyte differentiation by tumour necrosis factor-alpha. J Mol Endocrinol 1992;9:61-72.
[29] Tschritter O, Fritsche A, Thamer C, et al. Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 2003;52:239-43.
[30] Stefan N, Stumvoll M. Adiponectin—its role in metabolism and beyond. Horm Metab Res 2002;34:469-74.
[31] Stefan N, 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.
[32] 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.
[33] 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.
[34] 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.
[35] Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab 2002;13:18-23.
[36] Hauner H, Bender M, Haastert B, et al. Plasma concentrations of soluble TNF-alpha receptors in obese subjects. Int J Obes Relat Metab Disord 1998;22:1239-43.
[37] Bluher M, Kratzsche J, Pascke R. Plasma levels of tumor necrosis factor-alpha, angiotensin II, growth hormone, and IGF-1 are not elevated in insulin-resistant obese individuals with impaired glucose tolerance. Diabetes Care 2001;24:328-34.
[38] Bruun JM, Verdich C, Toubro S, et al. Association between measures of insulin sensitivity and circulating levels of interleukin-8, interleukin 6 and tumor necrosis factor-alpha. Effect of weight loss in obese men. Eur J Endocrinol 2003;148:535-42.
[39] Maeda N, Takahashi M, Funahashi T, et al. PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001;50:2094-9.
[40] Combs TP, Wagner JA, Berger J, et al. Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: a potential mechanism of insulin sensitization. Endocrinology 2002;143:998-1007.
[41] 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.

[42] Bokemark L, Wikstrand J, Attvall S, et al. Insulin resistance and intima-media thickness in the carotid and femoral arteries of clinically healthy 58-year-old men. The Atherosclerosis and Insulin Resistance Study (AIR). J Intern Med 2001;249:59 - 67.
[43] Bokemark L, Wikstrand J, Wedel H, et al. Insulin, insulin propeptides and intima-media thickness in the carotid artery in 58-year-old clinically healthy men. The Atherosclerosis and Insulin Resistance study (AIR). Diabet Med 2002;19:144 - 51.
[44] Hulthe J, Bokemark L, Wikstrand J, et al. The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) study. Arterioscler Thromb Vasc Biol 2000;20:2140 - 7.
[45] Hulthe J, Wikstrand J, Fagerberg B. Relationship between C-reactive protein and intima-media thickness in the carotid and femoral arteries and to antibodies against oxidized low-density lipoprotein in healthy men: the Atherosclerosis and Insulin Resistance (AIR) study. Clin Sci (Lond) 2001;100:371 - 8.
[46] Sjöström L, Chowdhury B, et al. The sagittal diameter is a valid marker of the visceral adipose tissue volume. In: Angel A, Anderson AH, Bouchard C, Lau L, Leiter L, Mendelson R, editors. Progress in obesity research. London: John Libbey & Co; 1996. p. 309 - 19.
[47] Bokemark L, Froden A, Attvall S, et al. The euglycemic hyperinsulinemic clamp examination: variability and reproducibility. Scand J Clin Lab Invest 2000;60:27 - 36.
[48] Borner K, Klose S. Enzymatic determination of total cholesterol with the Greiner Selective Analyzer (GSA-II) (author's transl). J Clin Chem Clin Biochem 1977;15:121 - 30.
[49] Wahlefeld A. Triglycerides determination after enzymatic hydrolysis. In: Bergemeyer HU, editor. Methods of enzymatic analysis. 2nd ed, vol. 4. New York: Verlag Chemie, Weinheim Academic Press; 1974. p. 1831-40.
[50] 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.
[51] Kappes A, Loffler G. Influences of ionomycin, dibutyryl-cycloAMP and tumour necrosis factor-alpha on intracellular amount and secretion of apM1 in differentiating primary human preadipocytes. Horm Metab Res 2000;32:548 - 54.
[52] Fasshauer M, Kralisch S, Klier M, et al. Adiponectin gene expression and secretion is inhibited by interleukin-6 in 3T3-L1 adipocytes. Biochem Biophys Res Commun 2003;301:1045 - 50.
[53] 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.
[54] 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.
[55] Matsubara M, Katayose S. Decreased plasma adiponectin concentration in women with low grade C-reactive protein elevation. Eur J Endocrinol 2003;14:657 - 62.
[56] Kazumi T, Kawaguchi A, Hirano T, Yoshino G. C-reactive protein in young, apparently healthy men: association with serum leptin, QTc, and high-density lipoprotein-cholesterol. Metabolism 2000;52:1113 - 6.