

# Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans

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**Bruun, Jens M., Aina S. Lihn, Camilla Verdich, Steen B. Pedersen, Søren Toubro, Arne Astrup, and Bjørn Richelsen.** Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab* 285: E527–E533, 2003. First published May 7, 2003; 10.1152/ajpendo.00110.2003.—Adiponectin is an adipose tissue-specific protein that is abundantly present in the circulation and suggested to be involved in insulin sensitivity and development of atherosclerosis. Because cytokines are suggested to regulate adiponectin, the aim of the present study was to investigate the interaction between adiponectin and three adipose tissue-derived cytokines (IL-6, IL-8, and TNF- $\alpha$ ). The study was divided into three substudies as follows: 1) plasma adiponectin and mRNA levels in adipose tissue biopsies from obese subjects [mean body mass index (BMI): 39.7 kg/m<sup>2</sup>,  $n = 6$ ] before and after weight loss; 2) plasma adiponectin in obese men (mean BMI: 38.7 kg/m<sup>2</sup>,  $n = 19$ ) compared with lean men (mean BMI: 23.4 kg/m<sup>2</sup>,  $n = 10$ ) before and after weight loss; and 3) in vitro direct effects of IL-6, IL-8, and TNF- $\alpha$  on adiponectin mRNA levels in adipose tissue cultures. The results were that 1) weight loss resulted in a 51% ( $P < 0.05$ ) increase in plasma adiponectin and a 45% ( $P < 0.05$ ) increase in adipose tissue mRNA levels; 2) plasma adiponectin was 53% ( $P < 0.01$ ) higher in lean compared with obese men, and plasma adiponectin was inversely correlated with adiposity, insulin sensitivity, and IL-6; and 3) TNF- $\alpha$  ( $P < 0.01$ ) and IL-6 plus its soluble receptor ( $P < 0.05$ ) decreased adiponectin mRNA levels in vitro. The inverse relationship between plasma adiponectin and cytokines in vivo and the cytokine-induced reduction in adiponectin mRNA in vitro suggests that endogenous cytokines may inhibit adiponectin. This could be of importance for the association between cytokines (e.g., IL-6) and insulin resistance and atherosclerosis.

interleukin-6; tumor necrosis factor- $\alpha$ ; interleukin-8; human adipose tissue

EXCESS ADIPOSE TISSUE is strongly associated with the development of insulin resistance and thereby the development of type 2 diabetes and cardiovascular disease (24). The mechanisms behind the development of these obesity-associated complications have still not been fully elucidated (18). However, evidence is accu-

mulating that the adipose tissue itself produces and releases a number of bioactive proteins, including proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ; see Ref. 20), interleukin-6 (IL-6; see Ref. 30), and interleukin-8 (IL-8; see Ref. 7), all of which have been reported to be affected by weight loss in obese subjects (2, 5, 11) and therefore could be of importance as part of the link between obesity and health complications (e.g., insulin resistance and premature atherosclerosis).

A newer candidate for the link between obesity and the development of insulin resistance and cardiovascular disease may be adiponectin, the gene product of the adipose tissue's most abundant gene transcript (26). In humans, high levels of adiponectin (range 2–20 mg/l) are found in the circulation (1). As opposed to other adipose-derived proteins, plasma levels of adiponectin have been found to be decreased in a number of deranged metabolic states, including obesity (1), dyslipidemia (28), type 2 diabetes, and coronary artery disease (21). Previous studies have demonstrated that the reduced circulating adiponectin levels could be reversed partially after induction of weight loss in obese and in insulin-resistant subjects (21, 40) as well as after treatment with insulin-sensitizing drugs such as thiazolidinediones (8, 27). The physiological role of adiponectin in relation to diseases associated with the metabolic syndrome remains to be determined; however, adiponectin has been demonstrated to improve insulin sensitivity in animal models of insulin resistance in vivo (3, 39). Besides the involvement in insulin sensitivity, adiponectin has been reported to exhibit antiatherosclerotic activity (31, 32) through an inhibition of TNF- $\alpha$ -induced activation of the nuclear transcription factor- $\kappa$ B (NF- $\kappa$ B; see Ref. 33). In addition, TNF- $\alpha$  has been demonstrated to decrease adiponectin gene expression in human preadipocytes (22) and 3T3-L1 adipocytes (13), suggesting a relationship between TNF- $\alpha$  and adiponectin.

To obtain additional information on the regulation of adiponectin, we investigated the effect of weight loss on

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adiponectin production and insulin sensitivity in relation to concomitant changes in adipose tissue-derived cytokines (IL-6, IL-8, and TNF- $\alpha$ ). Because we found that serum levels of adiponectin were negatively correlated with IL-6 and TNF- $\alpha$  in obese subjects, we hypothesized that changes in adiponectin levels might be mediated by changes in the levels of cytokines. Thus, in in vitro studies, we investigated the direct effect of these cytokines on adiponectin production in human adipose tissue.

## MATERIALS AND METHODS

**Subjects.** The present study was divided into three sub-studies as follows: two clinical studies and one in vitro study using human adipose tissue fragments.

The first study was a 20-wk intervention study where six (4 males and 2 females) obese subjects [mean body mass index (BMI):  $39.7 \pm 0.6$  kg/m<sup>2</sup>] received a very low calorie diet (3.4 MJ/day) for 8 wk followed by an additional 12 wk on a weight-stabilizing diet. Fasting blood samples and subcutaneous abdominal adipose tissue biopsies were obtained at baseline and at the end of the study. None of the subjects received any medication. The subjects were fasted overnight, and the adipose tissue was removed using a sterile technique, as previously described (7, 34). Plasma samples were frozen at  $-20^{\circ}\text{C}$ , and the adipose tissue was snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for later RNA extraction.

In the second study (Table 1), nineteen abdominal obese men (waist circumference  $>102$  cm) with a mean BMI of  $38.7 \pm 0.7$  kg/m<sup>2</sup> (range: 34.1–43.8 kg/m<sup>2</sup>) were compared at baseline with 10 lean men with a mean BMI of  $23.4 \pm 0.4$  kg/m<sup>2</sup> (range: 21.1–24.7 kg/m<sup>2</sup>). All subjects were nondiabetic, and none of them used any medication. The obese subjects underwent a 24-wk intervention where weight loss was induced by a 4.2 MJ/day, low-calorie diet for 8 wk. This was followed by 8 wk on energy restriction providing 6.2 MJ/day and an additional 8 wk on a calculated weight maintenance diet. Fasting blood samples were obtained at baseline and, for the obese subjects, after 24 wk. Plasma samples for determination of fasting insulin and fasting glucose were analyzed at the local clinical biochemical laboratory, and measures for insulin resistance were obtained using the

homeostasis model assessment [HOMA = (fasting insulin  $\times$  fasting glucose)/22.5], as previously described (12, 19). Body composition was assessed by dual-energy X-ray absorptiometry scan using a Lunar DPX-IQ Image Densitometer (DPX; Lunar Radiation, Madison, WI).

In the in vitro study, subcutaneous abdominal adipose tissue from six healthy women (mean BMI:  $25.0 \pm 0.7$  kg/m<sup>2</sup>; range: 23.5–27.1 kg/m<sup>2</sup>) undergoing liposuction at a plastic surgical clinic were used. Adipose tissue was minced into fragments and placed in organ culture, as previously described (7). The adipose tissue was preincubated for 24 h. Thereafter, the medium was replaced; IL-6 (50  $\mu\text{g/l}$ ), IL-6 (50  $\mu\text{g/l}$ ) plus the IL-6 soluble receptor (IL-6sR; 100  $\mu\text{g/l}$ ), IL-8 (1 mg/l), or TNF- $\alpha$  (10  $\mu\text{g/l}$ ) was added; and the incubation continued for up to 48 h. The concentrations of the cytokines above were chosen, since these concentrations are suggested to elicit maximal biological effects as found in adipose tissue (7, 16, 17) or other in vitro cell systems (36). Separate incubations using either IL-6 or IL-6 plus IL-6sR were chosen since previous reports have demonstrated that no gene expression of IL-6sR was observed in human adipose stromal cells (10) and that induction of aromatase activity in human adipose stromal cells was absent when incubated with IL-6 alone but present after addition of IL-6 plus IL-6sR (41). Adipose tissue incubations were performed as duplicate incubations. Adipose tissue was snap-frozen in liquid nitrogen and kept at  $-80^{\circ}\text{C}$  for later RNA extraction.

**Adiponectin and cytokine protein measurements.** Plasma levels of adiponectin were measured using RIA (Linco Research). The assay had an intra-assay coefficient of variation of 5.0% ( $n = 12$ ). Cytokine protein levels were measured in the plasma samples by using a specific, highly sensitive human ELISA method. IL-8 (US-H-IL-8; Biosource Technologies Europe) had an intra-assay coefficient of variation of 6.0% ( $n = 3$ ). IL-6 (Quantikine HS600; R&D Systems Europe) had an intra-assay coefficient of variation of 5.5% ( $n = 12$ ). TNF- $\alpha$  (Quantikine HSTA00C; R&D Systems Europe) had an intra-assay coefficient of variation of 3.5% ( $n = 12$ ).

**Adiponectin mRNA level.** RNA was isolated using TRIzol reagents. For the real-time RT-PCR, cDNA was made with random hexamer primers, as described elsewhere (GeneAmp PCR kit; Perkin-Elmer Cetus, Norwalk, CT). PCR-mastermix containing the specific primers, Hot Star *Taq* DNA polymerase, and SYBR-Green PCR buffer were then added. Adiponectin sense primer CATGACCAGGAAACCACGACT and anti-sense primer TGAATGCTGAGCGGTAT spanned a product of 301 bp. As previously described (6), real-time quantification of adiponectin mRNA to  $\beta$ -actin mRNA was performed with a SYBR-Green real-time PCR assay using an ICycler PCR machine from Bio-Rad. Adiponectin and  $\beta$ -actin mRNA were amplified in separate tubes, and the increase in fluorescence was measured in real time. Threshold cycle ( $C_T$ ) was defined as the fractional cycle number at which the fluorescence reached 10 times the SD of the baseline. Samples were amplified in duplicate, and relative gene expression of  $\beta$ -actin to adiponectin was calculated as  $1/2(C_T \text{ target} - C_T \beta\text{-actin})$ .

**Statistical analysis.** The SPSS statistical packet (SPSS/8.0; SPSS) was used for the calculations. In obese subjects, a paired *t*-test was used for comparison of anthropometric data, body composition, various parameters of insulin sensitivity, circulating levels of adiponectin and cytokines, as well as adiponectin mRNA levels in adipose tissue biopsies before and after weight loss. When comparing plasma levels of adiponectin, cytokines, anthropometric data, body composition, and various parameters of insulin sensitivity in lean and obese subjects, a nonpaired *t*-test was used. For compar-

Table 1. Characteristics of the patients in study 2

	Baseline		Week 24 Reduced Obese ( $n = 19$ )
	Lean ( $n = 10$ )	Obese ( $n = 19$ )	
Body weight, kg	$75.7 \pm 1.8$	$127.6 \pm 3.2^*$	$108.9 \pm 3.8\ddagger$
BMI, kg/m <sup>2</sup>	$23.4 \pm 0.4$	$38.7 \pm 0.7^*$	$33.0 \pm 1.0\ddagger$
Fat mass, kg	$14.4 \pm 1.7$	$50.3 \pm 2.3^*$	$35.5 \pm 3.0\ddagger$
Trunk fat mass, kg	$3.2 \pm 0.6$	$21.5 \pm 1.2^*$	$12.4 \pm 1.6\ddagger$
Waist, cm	$83.1 \pm 1.2$	$124.9 \pm 1.9^*$	$105.6 \pm 2.8\ddagger$
Insulin, pmol/l	$33.8 \pm 3.5$	$110.2 \pm 11.1^*$	$77.9 \pm 15.8\ddagger$
Glucose, mmol/l	$4.9 \pm 0.1$	$5.3 \pm 0.1\ddagger$	$5.0 \pm 0.1$
HOMA	$7.1 \pm 0.8$	$26.0 \pm 2.9^*$	$14.5 \pm 2.1\ddagger$
IL-6, ng/l	$2.5 \pm 0.6$	$4.1 \pm 0.4\ddagger$	$3.1 \pm 0.4\ddagger$
IL-8, ng/l	$3.2 \pm 0.5$	$4.4 \pm 0.3\ddagger$	$5.7 \pm 0.3\ddagger$
TNF- $\alpha$ , ng/l	$4.6 \pm 0.5$	$4.8 \pm 0.5$	$3.4 \pm 0.4\ddagger$

Data represent mean values  $\pm$  SE; n, no. of subjects. HOMA, homeostasis model assessment. Shown are baseline characteristics of the 19 obese men [body mass index (BMI) range: 33.9–43.8 kg/m<sup>2</sup>] and 10 lean men (BMI range: 21.1–24.7 kg/m<sup>2</sup>) included in the study, as well as characteristics of the 19 obese subjects after weight loss (at week 24).  $\ddagger P < 0.05$ ,  $*P < 0.001$  and (lean vs. obese).  $\ddagger P < 0.01$  and  $\ddagger P < 0.001$ , obese baseline vs. obese week 24.

ison between the various adipose tissue incubations, an ANOVA with a Dunnett's test for post hoc multiple comparison was used. To determine the relationship between plasma levels of cytokines, various metabolic or anthropometric parameters, and adiponectin at baseline as well as after weight loss, a bivariate correlation with a Pearson correlation coefficient ( $r_p$ ) was used. Multiple regression analysis was undertaken to address the association between adiponectin and the above-mentioned cytokines and metabolic and anthropometric parameters, taking into account the possible association between these. Values are presented as means  $\pm$  SE. Threshold for significance was set at  $P < 0.05$ .

**Ethics.** Informed, written consent was obtained from all subjects. The studies were approved by the Ethics Committees of Aarhus, Copenhagen, Frederiksberg, and Zealand in accordance with the Helsinki II Declaration.

## RESULTS

**Study 1: effects of weight loss on adiponectin in plasma and adipose tissue.** In the 20-wk weight loss intervention study, six obese subjects reduced their body weight by an average of 20 kg ( $122.0 \pm 2.1$  vs.  $102.3 \pm 5.0$  kg;  $P < 0.05$ ). The male subjects had a higher reduction in body weight compared with the two women, but there was no difference in the percentage of weight loss ( $14.0 \pm 2.3$  vs.  $15.0 \pm 1.0\%$ ). After the 20-wk weight loss period, circulating levels of adiponectin were increased by 51% ( $2.3 \pm 0.6$  vs.  $3.4 \pm 0.8$  mg/l;  $P < 0.05$ ; Fig. 1). This was paralleled by findings in the adipose tissue biopsies, where adiponectin mRNA levels were increased by 45% ( $P < 0.05$ ; Fig 1). The weight loss-induced increments in both circulating adiponectin levels and mRNA levels in the adipose tissue samples were correlated, although insignificantly ( $r_p = 0.59$ ,  $P = 0.22$ ), probably because of a type 2 error because of the limited number of subjects in the study.

**Study 2: baseline comparison between obese and lean subjects and effect of weight loss.** As shown in Table 1, obese subjects compared with lean subjects were found to have significantly higher amounts of total body fat and more visceral adipose tissue, as estimated by trunk fat mass and waist circumference. In addition, obese subjects were more insulin resistant, as assessed by fasting insulin levels and HOMA (Table 1). Plasma levels of adiponectin were 53% higher in lean compared with obese subjects ( $4.6 \pm 0.4$  vs.  $3.0 \pm 0.3$  mg/l;  $P < 0.01$ ; Fig. 2). In contrast, plasma levels of IL-6 (Fig. 2) and IL-8 were 64% ( $P < 0.05$ ) and 38% ( $P < 0.05$ ) higher in obese compared with lean subjects, respectively. No difference was observed in the plasma levels of TNF- $\alpha$  between the two groups (Table 1).

In lean subjects at baseline, plasma levels of adiponectin were found to be inversely correlated with BMI ( $r_p = -0.65$ ;  $P < 0.05$ ), but no association was found between adiponectin and other parameters of adiposity, insulin sensitivity, or circulating levels of IL-8, IL-6, or TNF- $\alpha$  (data not shown). In obese subjects at baseline, plasma levels of adiponectin were found to be inversely correlated with measures of insulin sensitivity, such as HOMA ( $P < 0.05$ ) and fasting

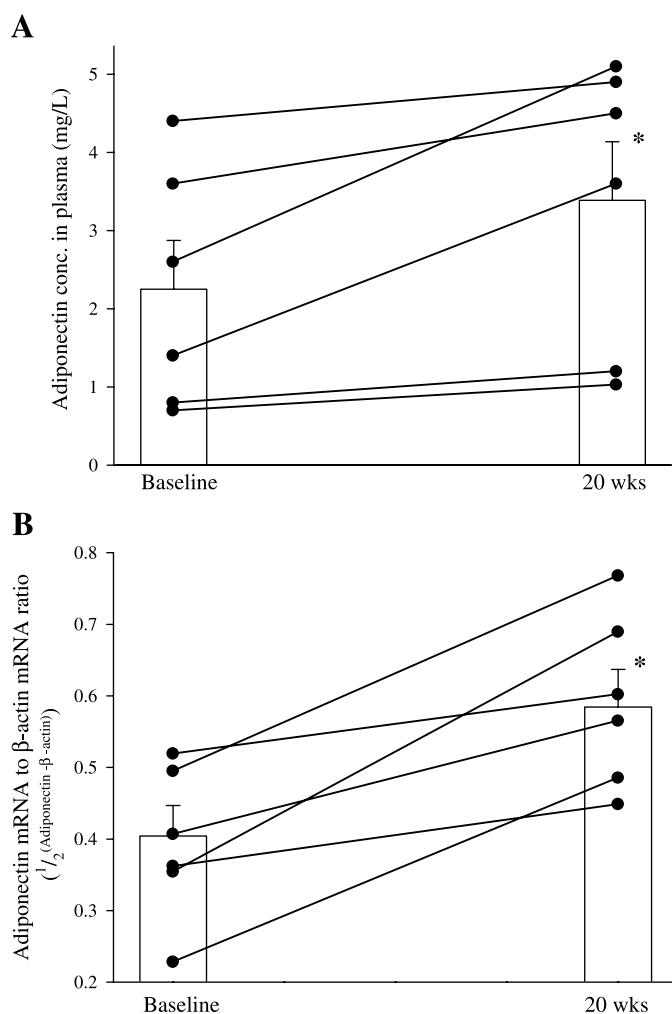


Fig. 1. Adiponectin in plasma and adipose tissue in association with weight loss. **Study 1:** circulating levels of adiponectin in plasma (A) and adiponectin mRNA levels (B) in adipose tissue biopsies assessed at baseline and after a 20-wk diet-induced weight loss ( $19.7 \pm 4.7$  kg) in 6 obese individuals. Solid lines represent each of the 6 individuals, and bars represent mean values  $\pm$  SE ( $n = 6$  subjects). \* $P < 0.05$  compared with baseline.

insulin ( $P < 0.05$ ), as well as with measures of adiposity [BMI ( $P < 0.05$ ) and waist circumference ( $P < 0.05$ )], but only marginally with total fat mass ( $P = 0.07$ ). In addition, plasma levels of adiponectin were found to be inversely correlated with plasma levels of IL-6 ( $P < 0.01$ ) and TNF- $\alpha$  ( $P < 0.05$ ) but only marginally with plasma levels of IL-8 ( $P = 0.06$ ). In obese subjects, multiple regression analysis displayed IL-6 as the main predictor ( $P < 0.05$ ) of circulating adiponectin levels and BMI ( $P = 0.07$ ) and waist circumference ( $P = 0.11$ ) as marginally significant predictors (data not shown). When the two groups were combined, plasma adiponectin was found to be significantly correlated with measures of insulin sensitivity and with measures of adiposity (Table 2). Plasma adiponectin was correlated with IL-6, but not TNF- $\alpha$  or IL-8 (Table 2). Multiple regression analysis showed that BMI was the main predictor ( $P < 0.01$ ) of circulating adiponectin levels.

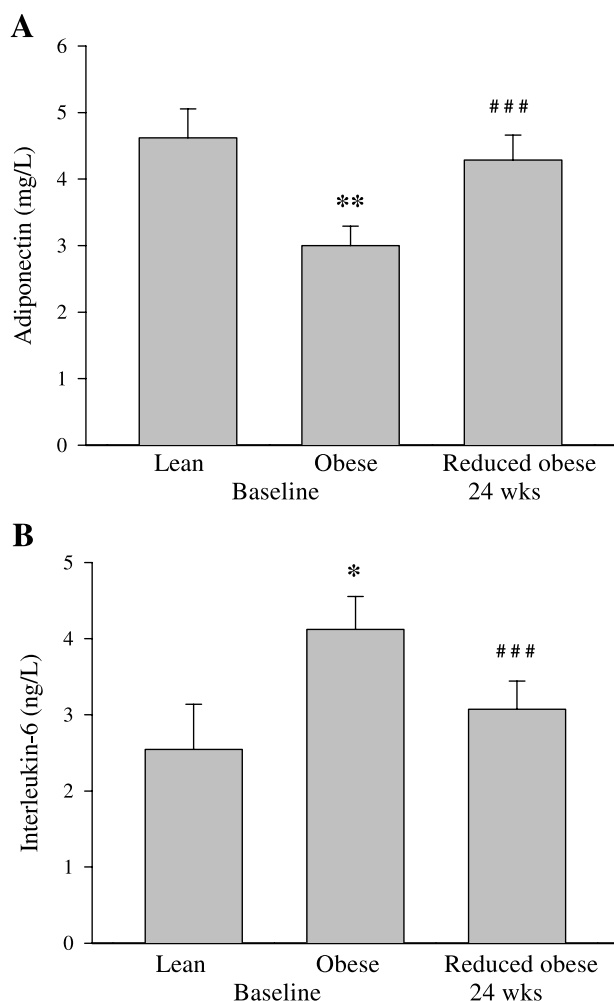


Fig. 2. Adiponectin and interleukin (IL)-6 in plasma in lean and obese subjects. *Study 2*: circulating levels of adiponectin (A) and IL-6 (B) in 10 lean and 19 obese subjects before and after a 24-wk diet-induced weight loss ( $19.2 \pm 2.0$  kg). Data represent mean values  $\pm$  SE. \* $P < 0.05$  and \*\* $P < 0.01$  compared with lean subjects. \*\*\* $P < 0.001$  compared with obese subjects at baseline.

After the diet-induced weight loss (after 24 wk), the obese subjects had reduced their body weight with an average of 20 kg ( $P < 0.001$ ) and their total body fat mass with  $\sim 15$  kg ( $P < 0.001$ ; Table 1). The decreases

Table 2. Association between adiponectin and measures of adiposity, insulin sensitivity, or cytokines

	Adiponectin	P
BMI, kg/m <sup>2</sup>	-0.69	<0.001
Fat mass, kg	-0.42	<0.05
Waist circumference, cm	-0.44	<0.05
Fasting insulin, pmol/l	-0.64	<0.001
HOMA	-0.65	<0.001
IL-6, ng/l	-0.49	<0.01
IL-8, ng/l	-0.31	=0.10
TNF- $\alpha$ , ng/l	-0.34	=0.08

Baseline association between plasma levels of adiponectin and various measures of adiposity and insulin sensitivity as well as plasma levels of proinflammatory cytokines (IL-6, IL-8, and TNF- $\alpha$ ) in 29 subjects (BMI range: 21.1–43.8 kg/m<sup>2</sup>). Bivariate correlation with Pearson correlation coefficient.

in both total body fat mass and visceral fat mass were related to an improvement in insulin sensitivity, as demonstrated by a decrement in fasting insulin ( $110.2 \pm 11.1$  vs.  $77.9 \pm 15.8$  pmol/l;  $P < 0.01$ ) and HOMA ( $26.0 \pm 2.9$  vs.  $14.5 \pm 2.1$ ;  $P < 0.001$ ). Weight loss induced a 43% increase in circulating levels of adiponectin ( $3.0 \pm 0.3$  vs.  $4.3 \pm 0.4$  mg/l;  $P < 0.001$ ) and a 25% decrease in circulating levels of IL-6 ( $4.1 \pm 0.4$  vs.  $3.1 \pm 0.4$  ng/l;  $P < 0.001$ ), thereby approaching plasma levels found in lean subjects (Fig. 2).

In obese subjects, the difference in baseline and final concentration of adiponectin ( $\Delta$ adiponectin) was inversely correlated to the concomitant changes in measures of adiposity and insulin sensitivity (Table 3 and Fig. 3).  $\Delta$ Adiponectin was also found to be significantly correlated with  $\Delta$ IL-6 ( $r_p = -0.46$ ;  $P < 0.05$ ; Fig. 3), but not with  $\Delta$ TNF- $\alpha$  or  $\Delta$ IL-8 (data not shown). Changes in insulin ( $\Delta$ insulin) and BMI ( $\Delta$ BMI) were found by regression analysis to be the main predictors ( $P < 0.01$ ) for alterations in the circulating adiponectin levels, whereas  $\Delta$ IL-6 was not found to be independently associated with  $\Delta$ adiponectin ( $P = 0.18$ ).

*In vitro study: regulation of adiponectin by cytokines.* The adipose tissue fragments were incubated with cytokines in concentrations suggested to elicit maximal biological effects, as described in MATERIALS AND METHODS.

Adiponectin mRNA levels were reduced significantly after incubation of whole adipose tissue fragments with IL-6 plus IL-6sR ( $P < 0.05$ ) or TNF- $\alpha$  ( $P < 0.01$ ) for up to 48 h (Fig. 4). Neither incubation with IL-6 alone nor IL-8 had any effect on adiponectin mRNA levels (Fig 4).

## DISCUSSION

In the present paper, it was demonstrated that diet-induced weight loss increases adiponectin mRNA levels in subcutaneous abdominal adipose tissue biopsies, paralleled by an increase in plasma levels of adiponectin. Plasma levels of adiponectin at baseline were found to be inversely correlated with measures of insulin sensitivity (fasting insulin and HOMA) and adiposity (BMI and total fat mass), including measures of visceral adiposity, such as waist circumference. In agreement with these observations, the changes in

Table 3. Association between changes in plasma levels of adiponectin and changes in metabolic and anthropometric parameters

	$\Delta$ Adiponectin	P
$\Delta$ BMI, kg/m <sup>2</sup>	-0.59	<0.01
$\Delta$ Waist, cm	-0.53	<0.05
$\Delta$ Fat mass, kg	-0.40	=0.09
$\Delta$ Trunk fat mass, %	-0.39	=0.10
$\Delta$ Insulin, pmol/l	-0.55	<0.01
$\Delta$ HOMA	-0.48	<0.05

Correlation between the difference in baseline and final concentration of plasma levels of adiponectin and measures of adiposity and insulin sensitivity in the 19 obese subjects. Bivariate correlation with Pearson correlation coefficient.  $\Delta$ , Change.

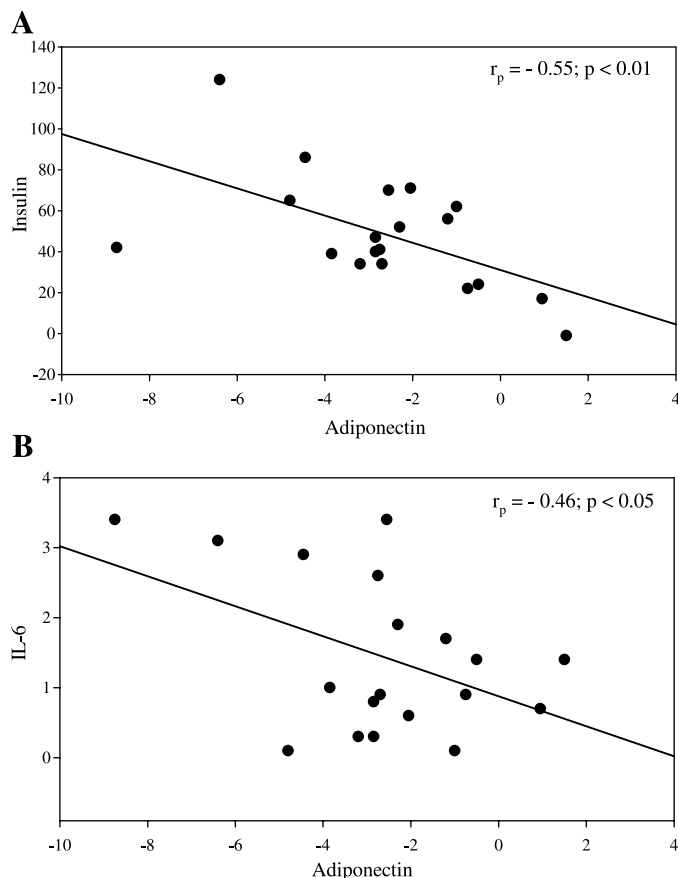


Fig. 3. Correlation between adiponectin and insulin or IL-6 in association with weight loss. Correlation between changes in plasma levels at baseline and after 24 wk weight loss for insulin ( $\Delta$ insulin) and adiponectin ( $\Delta$ adiponectin) (A) and IL-6 ( $\Delta$ IL-6) and adiponectin ( $\Delta$ adiponectin) (B) in 19 obese subjects. Bivariate correlation with Pearson correlation coefficient ( $r_p$ ).

measures of insulin sensitivity ( $\Delta$ insulin and  $\Delta$ HOMA) and adiposity ( $\Delta$ BMI and  $\Delta$ waist) before and after weight loss were also found to be inversely correlated with the concomitant changes in circulating adiponectin. Some discrepancy still exists on the relationship between adiponectin and insulin resistance, especially in rodents, where reports by Yamauchi et al. (39) and Berg et al. (3) found that treatment with adiponectin was able to reverse insulin resistance in various obesity-prone and diabetic mouse strains. In contrast, Ma and colleagues (25) found no impaired glucose tolerance or insulin resistance in adiponectin-deficient mice compared with the wild type. However, our present findings are in agreement with several recent human studies (1, 21, 38).

To our knowledge, this is the first description of a relationship between adiponectin and cytokines. In obese subjects at baseline, the plasma adiponectin levels were found to be inversely correlated with circulating levels of IL-6 and TNF- $\alpha$ , but only marginally with IL-8. Interestingly, the weight loss-induced increment in adiponectin was also found to be inversely correlated with the decrement in IL-6. In multiple regression analysis, IL-6 was found to be an independent predic-

tor of plasma adiponectin at baseline in obese subjects. However, after the two groups were combined and after weight loss, BMI and insulin were found to be the main predictors of circulating adiponectin levels. Because *in vivo* findings indicated that there could be a link between adiponectin and some of the adipose tissue-derived cytokines, inhibiting adiponectin production, we performed *in vitro* investigations on the direct effects of the three cytokines on adipose tissue adiponectin mRNA levels.

*In vitro* incubations were performed with adipose tissue from women. Even though circulating levels of adiponectin have been found to be higher in women compared with men, the association between adiponectin and measures of adiposity, type 2 diabetes, or cardiovascular disease displays no gender difference (1, 21). As described in MATERIALS AND METHODS, adipose tissue fragments were incubated with IL-6 alone as well as with IL-6 plus its soluble receptor. In this setting, we found that incubation of human adipose tissue fragments with IL-6 together with IL-6sR could decrease adiponectin mRNA levels almost to the same extent as TNF- $\alpha$ . No effect of IL-6 alone on adiponectin mRNA levels was observed in our *in vitro* study. Our findings are somewhat in contrast to the recent findings by Fasshauer et al. (14), who found that incubation with IL-6 alone could decrease adiponectin gene expression in the 3T3-L1 cell line. The discrepancy could be because 3T3-L1 cells have different characteristics compared with human adipose tissue (e.g., concerning expression of the IL-6sR) or because tissue incubations were performed differently. The direct inhibitory effect of TNF- $\alpha$  on adiponectin mRNA levels is

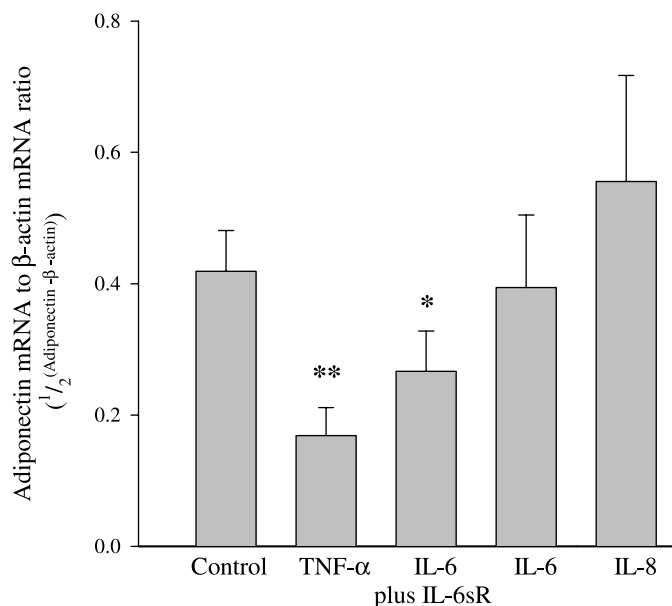


Fig. 4. *In vitro* effect of cytokines on adiponectin mRNA levels in human adipose tissue. Adipose tissue fragments were incubated as described in MATERIALS AND METHODS with either tumor necrosis factor (TNF)- $\alpha$  (10  $\mu$ g/l), IL-6 (50  $\mu$ g/l), IL-6 (50  $\mu$ g/l) plus IL-6 soluble receptor (IL-6sR; 100  $\mu$ g/l), or IL-8 (1 mg/l). Data represent mean values  $\pm$  SE ( $n = 6$ ). \* $P < 0.05$  and \*\* $P < 0.01$  compared with control incubations.

in accord with two recently published papers showing that TNF- $\alpha$  in 3T3-L1 adipocytes (13) and in human preadipocytes (22) was able to inhibit adiponectin mRNA levels. Circulating levels of IL-6 have been found to be correlated with different measures of cardiovascular disease (29, 35) and insulin resistance (23) and to be increased in the obese state (2). In addition, Fried and coworkers (15) demonstrated that IL-6 secretion was higher in omental compared with subcutaneous adipose tissue. Adiponectin compared with IL-6 has been reported to be inversely correlated with cardiovascular disease, insulin resistance, and obesity. The findings in the present study, indicating an inverse association between IL-6 and adiponectin in vivo and in vitro, suggest that these effects of IL-6 may be mediated partly by an IL-6-induced inhibition of adiponectin. Because both adiponectin and IL-6 are produced and released from the human adipose tissue, this interaction could be exerted in a paracrine or autocrine manner. Development of atherosclerotic disease has been suggested to be mediated through a long-term, low-grade inflammatory process involving the NF- $\kappa$ B pathway (4, 9, 37). This pathway is known to be activated by TNF- $\alpha$  and reported to be inhibited (attenuated) by adiponectin in vitro (33). In the present study, neither TNF- $\alpha$  nor IL-8 was found to be correlated with adiponectin after weight loss in vivo. This could be because of differences in the time span by which regulation of TNF- $\alpha$ , IL-8, and adiponectin production is achieved. In the in vitro incubations, we found profound effects of IL-6 plus IL-6sR and TNF- $\alpha$  on adiponectin mRNA levels. Although Mohamed-Ali et al. (30) found no net release of TNF- $\alpha$  from the abdominal subcutaneous adipose tissue depot in the basal (nonstimulated) situation, TNF- $\alpha$  is known to be produced in the adipose tissue, affecting adipose tissue metabolism through autocrine and paracrine pathways. Moreover, TNF- $\alpha$  is associated with measures of adiposity and insulin sensitivity and is of importance as a marker of general activation of the cytokine system (e.g., through activation of NF- $\kappa$ B; see Refs. 4 and 37). Thus the findings in vitro could suggest that local production of IL-6 and TNF- $\alpha$  in the adipose tissue may directly inhibit the local production of adiponectin. However, IL-8, which is also produced in the adipose tissue (7), does not seem to have any direct effect on adiponectin production.

In conclusion, an inverse relationship between plasma levels of adiponectin and adipose tissue-derived cytokines, particularly IL-6, was demonstrated in vivo. Furthermore, in vitro incubation of human adipose tissue fragments with IL-6 plus IL-6sR and TNF- $\alpha$  resulted in a decrement in adiponectin mRNA levels, suggesting that endogenous cytokines may inhibit adiponectin, which could be of importance for the association between cytokines (e.g., IL-6) and insulin resistance and atherosclerosis.

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## DISCLOSURES

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