Diet/Exercise Versus Pioglitazone: Effects of Insulin Sensitization with Decreasing or Increasing Fat Mass on Adipokines and Inflammatory Markers

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Background: Plasma adipokine concentrations are variably related to fatness/insulin resistance and may act via endocrine mechanisms. We assessed the relationship among plasma adipokine concentrations and their relationship with insulin sensitivity and body composition in obese adults before and after insulin sensitization accomplished using diet/exercise or pioglitazone.

Methods: Plasma adipokine concentrations, insulin sensitivity, and body composition were assessed in 39 upper-body obese insulin-resistant, nondiabetic adults before and after 19 wk of diet/exercise or 30 mg/d pioglitazone.

Results: Diet/exercise reduced body fat and visceral fat and improved insulin sensitivity parameters; pioglitazone improved insulin sensitivity to a similar degree but increased body fat. Adiponectin increased more after pioglitazone (4770 ± 487 vs. 8551 ± 693.6 ng/ml, P < 0.001) than after diet/exercise (4704 ± 367 to 5426 ± 325.3 ng/ml, P < 0.01), whereas TNFα, IL-6, and resistin did not change. C-reactive protein decreased with diet/exercise. Adipokine concentrations were not correlated with each other at baseline or after insulin sensitization, except TNFα and IL-6 (r = 0.43, P < 0.05); IL-6 was inversely correlated with resistin. Only adiponectin was correlated (P < 0.05) with indices of insulin sensitivity. Adiponectin concentrations were inversely correlated with visceral fat and with sc fat depots in men but positively correlated with sc fat in women.

Conclusion: Plasma adipokine concentrations were not consistently interrelated, and only adiponectin displayed the expected relationship with insulin sensitivity and sensitization. These findings do not support an endocrine role for resistin, TNFα, and IL-6 in mediating changes in insulin resistance after diet/exercise or pioglitazone.

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In addition to triglyceride storage, adipose tissue produces several hormones (leptin, resistin, adiponectin) and cytokines (IL-6, TNFα). The production of these so-called adipokines mostly parallels fat mass; only adiponectin concentrations are inversely correlated with fatness. Some adipokines have been linked to insulin resistance, whereas others, such as TNFα and the ILs (1, 2), stimulate acute-phase proteins such as C-reactive protein (CRP).

There remain uncertainties surrounding the exact role of adipokines in the pathophysiology of insulin resistance and obesity. Are they epiphenomena of obesity and insulin resistance or do they act to modulate insulin action via endocrine mechanisms? Are they primarily related to fatness, body fat distribution, and/or insulin resistance? If these molecules act through an endocrine role, then developing approaches to enhance or block their production or action would be a therapeutic goal. In addition, understanding the relationship of these adipokines and inflammatory markers to insulin resistance and adiposity will help determine whether assaying their blood concentrations offers important clinical information.

We conducted this study to assess the suitability of several adipokines/cytokines as possible markers for insulin resistance and changes in insulin resistance; changes in insulin resistance were induced by either diet and exercise or pioglitazone. Strong associations might support an endocrine function, whereas the absence of associations would argue against an endocrine role. We also wished to understand the interrelationships between the adipokines/cytokines to determine whether there are a few limited measures that can serve as surrogates for the remaining adipokines.

Subjects and Methods

These subjects are the same as those reported in a previous publication (3). Written, informed consent was obtained from 39 sedentary, nondiabetic upper body-obese men and premenopausal women [body mass index (BMI) 28–36 kg/m²]. Except for one Hispanic, all participants were Caucasian. Subjects were weight stable up to at least 6 months before entering the research program. Volunteers were excluded if they had a history of coronary heart disease, atherosclerosis, and systemic illness or biochemical evidence of renal or liver failure, blood pressure above 160/90 mm Hg, use of medication that could not safely be discontinued 2 wk before the study, smoking, pregnancy, and breast-feeding.

The volunteers underwent blood testing (plasma adipokine concentrations, complete blood count, chemistry panel, lipid profile), an iv glucose tolerance test [Bergman’s minimal model (4, 5)], computed tomography (CT) measures of visceral fat area (L2–3 level), and dual-energy x-ray absorptiometry (DEXA; DPX-IQ; Lunar Radiation, Madison, WI) (6). Fat biopsies were taken from femoral and abdominal sc depots.

Afterward, subjects were randomized to receive 30 mg pioglitazone (PIO) daily (10 men, 10 women) or a diet/exercise program (10 men, nine

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Abbreviations: BMI, Body mass index; CRP, C-reactive protein; CT, computed tomography; CV, coefficient of variation; DEXA, dual-energy x-ray absorptiometry; PCA, principal component analysis; PIO, pioglitazone; SI, insulin sensitivity index.

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women) for 18–20 wk, after which baseline studies were repeated. The PIO-treated volunteers were monitored every 4 wk for weight, liver function tests, and pill counts. The latter consisted of a daily 500-kcal deficit diet, combined with an exercise and bimonthly behavior modification program (3).

Assessment of fat compartment volume and fat cell size

CT images combined with data from DEXA were analyzed as previously described (6) to calculate the following fat compartments: total body fat, lower-body fat, upper-body nonvisceral fat, and visceral fat (3). Subcutaneous fat was aspirated using local anesthesia and prepared for cell sizing as previously described (3).

Assays

Adipokines. These were measured in plasma using the following assays: adiponectin, human adiponectin double antibody RIA kit (Linco Research, Inc., St. Charles, MO); intraassay coefficients of variation (CVs): 17.4, 47, and 7.3% at 5.1, 29.9, and 119 ng/ml, respectively; high-sensitivity CRP (Hitachi 912 chemistry analyzer by a latex particle-enhanced immunoturbidimetric assay; Kamiya Biomedical Corp., Seattle, WA); interassay CVs: 8.0, 2.0, and 1.0% at 0.05, 0.30, and 1.86 ng/dl, respectively; high-sensitivity IL-6, quantitative two-site enzyme immunoassay (R & D Systems, Minneapolis, MN); intraassay CVs: 8.0, 6.2, and 4.6% at 0.61, 1.02, and 2.83 pg/ml, respectively; interassay CVs: 16.0, 16.5, and 9.9% at 0.56, 3.58, and 8.16 pg/ml, respectively; resistin (quantitative two-site enzyme immunoassay; Linco Research; intraassay CVs: 1.1, 3.2, and 1.7% at 5.6, 10.8, and 15.1 ng/ml, respectively); high-sensitivity TNFα, quantitative two-site enzyme immunoassay (R & D Systems, intraassay CVs: 8.8, 5.9, and 5.3% at 2.6, 7.2, and 14.0 pg/ml, respectively; interassay CVs: 16.7, 12.6, and 10.8% at 2.4, 6.7, and 13.5 pg/ml, respectively); glucose, Beckman glucose analyzer (Beckman Instruments, Fullerton, CA); insulin, chemiluminescent sandwich assays (Sanofi Diagnostics, Chaska, MN); C-peptide, direct, double-antibody sequential RIA (Linco Research).

Statistics

Statistical analyses were done using SPSS 11.5: paired t tests for comparisons between before and after treatment, and 2 × 2 repeated-measures ANOVA with factors for group (diet/exercise vs. PIO) and time (the repeated measure — before and after intervention) for statistical comparisons of the two groups (PIO vs diet/exercise) and their responses to the interventions. Values are expressed as mean ± se of the mean; P < 0.05 was considered statistically significant. Because performing multiple correlations on large amounts of data increases the likelihood of drawing erroneous conclusions, we performed a principal component analysis (PCA) on the baseline data with a varimax rotation and Kaiser normalization. The goal was to identify which factors were sex dependent and which correlated with insulin sensitivity and/or fat deposits. This type of factor analysis is used for data reduction and works by uncovering the latent structure (dimensions) of a set of variables. It does not assume a specified dependent variable but simply examines which data cluster together by seeking the best fit.

Bivariate Pearson’s correlations were used to assess whether changes in plasma adipokine concentrations were associated with treatment-induced changes in other parameters. If parameters were identified as sex dependent in the PCA, we either performed partial correlations (correcting for sex) or presented bivariate correlations for both genders separately.

Table 1. Effect of insulin sensitization on anthropometric characteristics and adipokine concentrations

<table>
<thead>
<tr>
<th>Table 1. Effect of insulin sensitization on anthropometric characteristics and adipokine concentrations</th>
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<tbody>
<tr>
<td><strong>Adipokine</strong></td>
</tr>
<tr>
<td><strong>Adiponectin (ng/ml)</strong></td>
</tr>
<tr>
<td><strong>CRP (ng/dl)</strong></td>
</tr>
<tr>
<td><strong>TNFα (pg/ml)</strong></td>
</tr>
<tr>
<td><strong>IL-6 (pg/ml)</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Parameter</strong></th>
<th><strong>Before intervention</strong></th>
<th><strong>After intervention</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</strong></td>
<td>32.1 ± 0.7</td>
<td>27.7 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Fat (%)</strong></td>
<td>40 ± 1.4</td>
<td>32 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Resistin (ng/ml)</strong></td>
<td>9.4 ± 1.2</td>
<td>7.7 ± 0.9</td>
</tr>
<tr>
<td><strong>IL-6 (pg/ml)</strong></td>
<td>1.2 ± 0.1</td>
<td>2.5 ± 0.7</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.001 before vs. after intervention.
<sup>b</sup> P < 0.05 before vs. after intervention.
<sup>c</sup> P < 0.001 effect of diet/exercise vs. pioglitazone.
<sup>d</sup> P < 0.01 before vs. after intervention.

Results

Baseline characteristics and adipokine relationships

As reported (3), the diet/exercise and PIO groups were well matched for age, BMI, body composition, and insulin resistance parameters (Table 1). The baseline insulin sensitivity index (Si) values ranged from 0.71 to 19.9 (milliunits per liter)−1·min−1, fasting glucose from 97 to 117 mg/dl, fasting insulin from 2.6 to 23.0 μU/ml, and fasting C-peptide from 0.35 to 1.09 nmol/liter. The groups also had similar preintervention adipokine concentrations (Table 1).

PCA identified adiponectin and CRP as sex-dependent variables (Table 2); adiponectin and CRP concentrations were greater in women than men: 5736 ± 471 vs. 3838 ± 280 ng/ml (P = 0.001) and 0.37 ± 0.09 vs. 0.17 ± 0.03 ng/ml (P = 0.04), respectively. Adiponectin was the only adipokine that clustered statistically with fasting plasma insulin, fasting C-peptide, and Si (component 1). The factor (component) loadings are the correlation coefficients between the variables (rows) and factors (columns) and are the basis for imputing a label to the different factors. Loadings above 0.6 are usually considered high and those below 0.4 are low. If sex was eliminated as a factor in the model CRP, TNFα and IL-6 clustered together (component 2), but they did not cluster with insulin sensitivity parameters.

Baseline correlations (Table 3)

Adiponectin. For men and women combined, baseline adiponectin concentrations were positively correlated with Si.
and inversely correlated with fasting plasma insulin and C-peptide concentrations (Table 3). As expected, there was a positive correlation between adiponectin and Si and a negative correlation with visceral fat for men and women considered together; however, body fat compartments were correlated positively with adiponectin in women and negatively with adiponectin in men (Fig. 1).

Resistin. Plasma resistin concentrations did not correlate with percent body fat, fat compartments, insulin sensitivity parameters, or the other adipokines (Table 3). To the extent that there were trends for associations between resistin and other parameters, the correlations were negative with IL-6 and positive with Si ($P = 0.09$).

CRP. The correlation coefficients between plasma CRP and other adipokines are given in Table 3. Given the small number of men and women in each group, the statistical power is limited and it is difficult to draw conclusions regarding the lack of certain associations. We did find that CRP concentrations were inversely correlated with adiponectin in men and that CRP and TNFα were positively correlated in women.

IL-6 and TNFα. Baseline IL-6 correlated with TNFα and percent body fat but not with other adipokines, insulin sensitivity parameters, or regional fat compartments. IL-6 correlated with CRP only if the values from the person with the highest CRP concentrations were excluded or if log transformation was performed ($r = 0.52, P = 0.001$).

Plasma TNFα concentrations did not correlate with insulin sensitivity parameters or other adipokines at baseline, except IL-6 (Table 3). The correlations between plasma TNFα concentrations and percent body fat and leg fat did not reach statistical significance ($P = 0.07$ and 0.08, respectively). Plasma TNFα concentrations were positively correlated with abdominal but not femoral fat cell size (Fig. 2). Log transformation of the data did not alter these outcomes; none of other adipokine concentrations were correlated with fat cell size.

Response to interventions

Volunteers in the diet/exercise group lost $11.8 \pm 1.1$ kg of weight, $2.5 \pm 0.3$ kg of visceral fat, $3.4 \pm 0.7$ kg of upper-body nonvisceral fat, and $2.9 \pm 0.3$ kg of leg fat ($P < 0.001$) but a nonsignificant amount of fat-free mass ($-0.5 \pm 0.6$ kg). The PIO group gained weight ($2.7 \pm 0.7$ kg) and fat ($1.3 \pm 0.4$ kg, both $P < 0.01$), predominantly in the leg depot ($1.0 \pm 0.2$ kg, $P < 0.001$); visceral fat was unchanged (3).

Both interventions significantly improved all insulin sensitivity parameters, and there were no significant differences between the effects of PIO and diet/exercise (3). Diet/exercise decreased abdominal fat cell size more than femoral fat cell size, whereas PIO, oppositely, decreased femoral more than abdominal adipocyte size (3).

Plasma concentrations of resistin, TNFα, and IL-6 were not significantly altered by either intervention (Table 1). The increase in adiponectin concentrations was greater ($P < 0.001$) after PIO than diet/exercise.

TABLE 3. Baseline bivariate adipokine correlations

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Resistin</th>
<th>TNFα</th>
<th>IL-6</th>
<th>Adiponectin</th>
<th>CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All subjects</td>
<td>All</td>
<td>All</td>
<td>All</td>
<td>All</td>
</tr>
<tr>
<td>Resistin</td>
<td>1</td>
<td>$-0.05$</td>
<td>$-0.39^a$</td>
<td>$-0.06$</td>
<td>$-0.20$</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>$-0.02$</td>
<td>0.05</td>
<td>$0.34^a$,b</td>
<td>1</td>
<td>$0.28$</td>
</tr>
<tr>
<td>CRP</td>
<td>$0.34^a$,b</td>
<td>0.31</td>
<td>0.32,b</td>
<td>0.26</td>
<td>0.33</td>
</tr>
<tr>
<td>TNFα</td>
<td>1</td>
<td>0.43a</td>
<td>$0.39^a$</td>
<td>0.37</td>
<td>$0.16$</td>
</tr>
<tr>
<td>Si</td>
<td>$0.28^a$</td>
<td>$-0.02$</td>
<td>$-0.10$</td>
<td>$0.33^a$</td>
<td>$0.39$</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>$-0.14$</td>
<td>$-0.21$</td>
<td>$-0.08$</td>
<td>$-0.26$</td>
<td>$-0.33$</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>$-0.16$</td>
<td>0.01</td>
<td>0.27</td>
<td>$-0.38^a$</td>
<td>$-0.15$</td>
</tr>
<tr>
<td>Fasting C-peptide</td>
<td>$-0.10$</td>
<td>$-0.04$</td>
<td>0.23</td>
<td>$-0.36^a$</td>
<td>$-0.26$</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>0.08</td>
<td>0.31</td>
<td>0.32b</td>
<td>$0.39^a$</td>
<td>$0.29$</td>
</tr>
<tr>
<td>Total fat (kg)</td>
<td>0.05</td>
<td>0.15</td>
<td>0.20</td>
<td>$0.16$</td>
<td>$0.55^a$</td>
</tr>
<tr>
<td>UBNV fat (kg)</td>
<td>0.08</td>
<td>0.07</td>
<td>0.19</td>
<td>0.20</td>
<td>0.15</td>
</tr>
<tr>
<td>Leg fat (kg)</td>
<td>0.06</td>
<td>0.30</td>
<td>0.27</td>
<td>$0.36^a$</td>
<td>$0.39$</td>
</tr>
<tr>
<td>Visceral fat (kg)</td>
<td>$-0.08$</td>
<td>0.07</td>
<td>$-0.11$</td>
<td>$-0.40^a$</td>
<td>$0.54^a$</td>
</tr>
</tbody>
</table>

UBNV, Upper body nonvisceral.

$^a P < 0.05$.

$^b r = 0.27 (P = 0.11)$ and 0.28 ($P = 0.12$) for TNFα and IL-6, respectively, when corrected for gender.

$^c P < 0.01$. 
Correlations after insulin sensitization

Resistin. The change in resistin concentrations after diet/exercise correlated with the change in insulin ($r = 0.61$, $P < 0.01$) and adiponectin (men only, $r = 0.80$, $P = 0.005$) but in directions opposite to the expectation. The change in resistin concentrations correlated with the change in adiponectin concentration ($r = -0.69, P < 0.01$) for the PIO group data; all other resistin associations were either in the opposite direction (total body and leg fat: $r = -0.53$ and $-0.59$, respectively, both $P < 0.05$) or absent (changes in insulin sensitivity parameters, body composition, and other adipokines).

Adiponectin. In the diet/exercise group, there were no statistically significant correlations between changes in adiponectin and changes in insulin sensitivity parameters in men or women. In contrast, the changes in adiponectin concentrations were inversely correlated with changes in total fat in men ($P = 0.07$) and visceral fat ($P = 0.06$) did not reach statistical significance. In women, the correlations between adiponectin concentrations and changes in body fat were positive (Fig. 3) but weak and nonsignificant ($r = 0.18, 0.32$, and $0.42$ for total, leg, and visceral fat, respectively).

For the PIO-treated group, the adiponectin concentration changes were not significantly correlated with changes in insulin sensitivity or body composition in men, women, or the combined group.

CRP, IL-6, and TNFα. The change in CRP concentrations correlated significantly with the change in total body fat in the diet/exercise group ($r = 0.53$, $P = 0.03$). Changes in IL-6 and TNFα after either diet/exercise or PIO were not correlated with changes in other inflammatory markers, adipokine concentrations, insulin sensitivity parameters, or body composition, with the exception of a postpioglitazone correlation between the change in IL-6 and leg fat ($r = 0.48, P < 0.05$).

**Fig. 1.** Association of adiponectin with insulin sensitivity and body fat. Closed circles, women; open diamonds, men; Si [milliunits per liter]$^{-1} \cdot \text{min}^{-1}$. Adiponectin correlates with Si in the complete group ($r = 0.33, P < 0.05$), but this is just short of reaching statistical significance in men ($r = 0.37, P = 0.11$) and women ($r = 0.39, P = 0.11$) separately. Adiponectin correlations with the various fat compartments are inverse (as expected) in men but positive in women. In the latter, visceral fat is not correlated with adiponectin ($r = 0.15, P = \text{NS}$). *, $P < 0.05$. 

$\text{Si (mU/l)}^{-1} \cdot \text{min}^{-1}$

**Visceral fat (kg)**

**Leg fat (kg)**

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Adipokines and Insulin Sensitization

We recently compared the effects of a diet/exercise weight loss program with PIO on body fat compartments and insulin sensitivity in upper body-obese adults (3). There has subsequently been considerable interest in the possible endocrine role of the adipokines/inflammatory markers as they relate to insulin sensitivity and body fat.

Because weight loss and pioglitazone increased insulin sensitivity to a comparable degree but had opposite effects on adipose tissue mass, we took advantage of samples collected during the course of this study (3) to assess the relationship among adipokines, insulin resistance, and body fat characteristics and try to distinguish fat mass effects from insulin-sensitizing effects. We also assessed whether changes in insulin resistance and body fat were related to changes in plasma adipokine concentrations. Strong correlations between circulating adipokine concentrations and insulin sensitivity would circumstantially support an endocrine role for these molecules in mediating insulin resistance. On the other hand, if there were no association between adipokine/inflammatory marker concentrations and insulin sensitivity, this would argue against a significant endocrine role for these molecules.

Plasma adipokine concentrations (adiponectin and resistin) and inflammatory markers (TNFα, IL-6, and CRP) were not strongly correlated with each other at baseline or after insulin sensitization. Baseline concentrations of the inflammatory markers CRP, IL-6, and TNFα were interrelated, provided that sex was not included in the analysis. These interrelationships were not detected using the posttreatment data. TNFα and IL-6 were not correlated with parameters of insulin sensitivity and only weakly with body fatness. These findings suggest that, although inflammatory markers may be interrelated, they are not uniformly linked with indices of insulin resistance. The intercorrelation of IL-6, TNFα, and CRP is consistent with our current understanding of the physiology of mild inflammation and has also been found by some (7, 8) but not all (9, 10) other investigators.

The lack of consistent response of inflammatory markers after insulin sensitization suggests that insulin sensitivity per se might not be the overwhelming determinant of inflammation. We acknowledge that most (7, 8, 11) but not all (9–12) investigators report correlations between IL-6 concentrations and insulin sensitivity as well as with CRP (8), BMI (8, 12, 13), TNFα (7), and fatness. Plasma TNFα concentrations seem to be more strongly related to anthropometric indices of obesity (7) and fat cell size (14) but not necessarily with insulin sensitivity (7, 9, 11, 15).

We found that baseline and posttreatment plasma concentrations of the adipokine resistin were not statistically related to fatness, insulin resistance or inflammation, which is consistent with recent reports (16–20). Although considered an adipokine, it appears that human adipose tissue resistin (hFIZZ3) production is primarily by macrophages and monocytes and that its actions are to stimulate lipolysis and modulate preadipocyte biology (21).

In contrast, the adipokine adiponectin was more reliably associated with insulin sensitivity markers: its baseline concentrations were correlated with insulin sensitivity parameters, and after insulin sensitization using diet/exercise, this association was sustained in men. Pioglitazone treatment altered the relationship between insulin sensitivity and adiponectin, consistent with the effects of other thiazolidinediones (22).

Our findings further suggest that adiponectin, insulin sensitivity, and body fat interact differently in men and women and that the increases in adiponectin with thiazolidinediones do not predict the improvement in insulin sensitivity seen with these agents. This is consistent with the observation that thiazolidinediones, but not metformin (23), raise adiponectin levels, even in diabetic nonresponders (24) and lean, insulin-sensitive subjects, in the absence of insulin sensitization (25). Thiazolidinediones may affect adiponectin concentrations directly, perhaps by influencing the high to low molecular weight ratio (26). Mouse (26) and, more recently, human studies (26, 27) have suggested that not the absolute amounts but the ratio of high molecular to low molecular weight oligomeric forms of adiponectin determine insulin sensitivity. Thiazolidinediones appear to impact the high to low molecular weight ratio of circulating adiponectin (26); this could explain why pioglitazone increased adiponectin concentrations much more than diet/exercise and more than could be accounted for by improvements in insulin action. Abbasi et al. (28) confirmed that rosiglitazone administration, but not caloric restriction, influenced this ratio. It remains to be seen whether and to what extent adiponectin directly affects insulin action in humans; this will likely require administration of adiponectin agonists or antagonists.

FIG. 2. TNFα concentrations are correlated with abdominal fat cell size.
Baseline adiponectin and CRP concentrations were greater in women than men despite comparable Si values \[3.0 \pm 0.4 \text{ (women)} vs. 4.4 \pm 1.0 \text{ (men)}, P = \text{NS}\], which is consistent with previous reports \([29, 29, 30]\). We would have expected that greater adiponectin concentrations (associated with insulin sensitivity) would have been linked with lesser, not greater, CRP concentrations in women. If women had less of the more biologically active high-molecular-weight isoforms of adiponectin \([26]\), this could explain this finding. Unfortunately, we did not measure the high-molecular-weight isoform, and in any case testosterone is reported to selectively inhibit the high-weight isoform \([31]\), so women would be expected to have greater rather than lesser concentrations of this form, compared with men. We are unaware of previous reports describing sex differences in the relationship between body fat and adiponectin \([31]\) or reports of the association between changes in body fat and changes in adiponectin in response to treatment \([31]\). Future studies will be needed to confirm our findings.

To our knowledge, ours is one of the only two studies directly comparing diet/exercise and thiazolidinediones in nondiabetic volunteers. Rosiglitazone treatment resulted in similar effects on adiponectin concentrations relative to lifestyle intervention, compared with our results \([22]\). Nonetheless, lifestyle changes have been reported to affect adiponectin \([15, 19, 32]\), CRP \([19, 24, 32, 33]\), IL-6 \([15, 32, 33]\), and TNF\(\alpha\) \([7, 15]\) favorably in type 2 diabetic volunteers; effects on resistin are variable \([16, 19]\). Thiazolidinedione effects are generally absent on IL-6 \([8]\), variable on TNF\(\alpha\) and resistin \([15, 19, 34, 35]\), but consistently favorable on adiponectin \([22, 24, 25, 35]\).

The discrepancies of our findings with some other publications may result from both some limitations and strengths of our study. Our study sample was relatively small but included carefully selected, upper body-obese volunteers, who might be expected to have a narrower range of insulin sensitivity than a more diverse population. The statistical power of this relatively small sample size could be limited if
the biological variability in plasma adipokine concentrations is worse than the 16–24% variability of Si (5). Fortunately, most of the adipokines for which there are data available have less biological variability than Si, with the exception of IL-6 (36). It is possible that there are weaker relationships among adipokines or between adipokines and insulin sensitivity that we did not detect. This might also influence the statistical power of PCA to detect underlying structures in the relationships of interest. Although there is no consensus on whether absolute sample size or the ratio of subjects to items has the greatest influence hereon, we acknowledge that a sample of 39 subjects is small, and it is unclear how much of the true factor structure can be thus identified. We nevertheless chose to use PCA as an initial screen to limit the likelihood that we would report false-positive correlations (type 2 statistical error) as a result of performing numerous regression analyses.

Moreover, not all reported studies have included more participants (9, 13, 15, 19, 30, 34, 35) or included subjects with a greater range in Si. Some included lean (9, 11) and/or diabetic subjects (9, 15, 24, 34, 37). The latter is especially relevant because effects of improved glycemic control may confound direct insulin sensitization effects (38–40). This phenomenon is illustrated, among others, by the absence of the sex difference in adiponectin concentrations in type 2 diabetic subjects (41).

Although our method of determining insulin sensitivity (IV glucose tolerance test) is not considered as robust as the hyperinsulinemic, euglycemic clamp, it is superior to homeostasis model assessment analysis, fasting plasma glucose, and/or insulin concentrations. Our choice of body composition (DEXA and visceral fat by CT) is superior to methods such as bioelectric impedance analysis, DEXA only, skinfold thickness, or waist circumference measurements. Thus, offsets a relatively small number of participants, we used relatively accurate and precise measures of outcome, included equal numbers of men and women to allow us to test for sex-dependent effects, and used statistical approaches (PCA) that allow discernment of underlying relationships. This approach reduces the chance of reporting coincidental positive correlations and can therefore be useful at detecting complex interrelationships between adipokines and inflammatory markers, especially in large studies.

In summary, of the adipokine/inflammatory markers that we measured, only adiponectin was consistently related to insulin sensitivity and body fat distribution, but changes in adiponectin were not predicted by changes in insulin sensitivity. CRP was associated with body fat and the other inflammatory markers. To the extent that correlations between insulin action and circulating hormone concentrations are proposed to mediate insulin resistance, we found no evidence that circulating resistin, TNF-α, or IL-6 are linked to insulin resistance. We suggest that it is important to stratify by sex when testing for relationships between adiponectin (and CRP) concentrations and body fat and insulin sensitivity and that the use of PCA may provide a guide for further analysis of complex relationships between the numerous molecules that have become known as adipokines.

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