Adiponectin is stimulated by adrenalectomy in ob/ob mice and is highly correlated with resistin mRNA

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Makimura, Hideo, Tooru M. Mizuno, Hugo Bergen, and Charles V. Mobbs. Adiponectin is stimulated by adrenalectomy in ob/ob mice and is highly correlated with resistin mRNA. Am J Physiol Endocrinol Metab 283: E1266–E1271, 2002—Plasma levels of the adipocyte product adiponectin, a putative insulin-sensing agent, are reduced in obesity, whereas plasma levels of resistin, an agent that some believe to confer insulin resistance, are thought to increase with obesity. Because adrenalectomy can increase insulin sensitivity, we hypothesized that adrenalectomy would increase expression of adiponectin and decrease expression of resistin. Therefore, we measured adiponectin mRNA, adiponectin peptide, and resistin mRNA in adrenalectomized ob/ob mice. Adrenalectomy restored adiponectin expression in ob/ob mice to wild-type levels and stimulated adiponectin peptide to above wild-type levels. Surprisingly, expression of adiponectin and resistin was highly positively correlated even after statistical removal of effects of insulin, glucose, and adiposity. In addition, adiponectin and resistin expression were also highly correlated in diet-induced obese mice. The data support a role for adiponectin in mediating some effects of adrenalectomy on insulin sensitivity.

Adiponectin expression is increased in models of dietary obesity (23) and adrenalectomy (23). Resistin peptide levels are reported to increase after a glucose load (3). More recently, Haluzik et al. (12) have demonstrated that adrenalectomy increases insulin sensitivity in gold-thio-glucose-induced obese mice as measured by insulin and glucose levels at both baseline and after a glucose load (3). We hypothesized that adrenalectomy increases insulin sensitivity of obesity, and Janke et al. (14) have reported that there is no correlation between resistin expression and insulin resistance in humans.

Adrenalectomy reverses various metabolic defects, including hyperglycemia and hyperinsulinemia in many animal models of obesity (4, 10, 16). Adrenalectomy has also been shown to increase insulin sensitivity in gold-thio-glucose-induced obese mice as measured by insulin and glucose levels at both baseline and after a glucose load (3). These results suggest that adrenalectomy may not generally be associated with insulin resistance as first reported. Way et al. (24) have reported that resistin expression is reduced in various rodent models of obesity, and Janke et al. (14) have reported that there is no correlation between resistin expression and insulin resistance in humans.

MATERIALS AND METHODS

Animals. The appropriate Animal Institutional Review Board had approved all studies.

SEVERAL GROUPS INDEPENDENTLY identified the same adipocyte-derived protein and variably named it AdipoQ (13), Acrp30 (22), adiponectin (15), or GBP28 (20). Adiponectin expression is reduced in both human obesity (13) and models of genetic obesity in mice (13). In addition, the peptide levels of adiponectin are reduced in obese humans (1). Furthermore, this reduction in adiponectin expression has been associated with insulin resistance (26, 27), suggesting that adiponectin functions to increase insulin sensitivity. Resistin, another adipocyte-derived peptide, was recently discovered (23). Resistin peptide levels are reported to increase in models of diet-induced obesity (23) and genetic models of murine obesity such as ob/ob and db/db mice (23). Insulin sensitivity of diet-induced obese mice increased when treated with antibodies to resistin (23), suggesting that resistin plays an opposing role to adiponectin, that of decreasing insulin sensitivity. However, there is growing evidence that resistin may not generally be associated with insulin resistance as first reported. Way et al. (24) have reported that resistin expression is reduced in various rodent models of obesity, and Janke et al. (14) have reported that there is no correlation between resistin expression and insulin resistance in humans.
To assess gene expression in genetic obesity and effects of adrenalectomy, 2-mo-old male ob/ob mice and their wild-type littermates were adrenalectomized or sham adrenalectomized as reported (16). This resulted in the following four groups: wild-type sham operated (n = 4), wild-type adrenalectomized (n = 7), ob/ob sham operated (n = 6), and ob/ob adrenalectomized (n = 5). Ad libitum-fed mice were killed 2 wk after surgery between 1700 and 1900, just before lights out, adipose tissue was taken from the epididymal fat pad, and RNA was extracted as described previously (18).

To assess effects of diet-induced obesity, mature male C57Bl/6J mice were fed either a high-fat, high-simple-carbohydrate diet (diet no. F2685; Bioserve, Frenchtown, NJ) or remained on normal rodent chow as previously described (2). The high-fat diet consisted of 35.5% fat, 35.0% simple carbohydrate, 20.0% protein, 0.1% fiber, and 3.7% ash (5.4 kcal/g), whereas the control rodent chow consisted of 5.0% fat, 55% complex carbohydrates, 22.0% protein, 5.0% fiber, and 6.0% ash (3.5 kcal/g). After 14 wk on the diet, mice were either fed ad libitum or fasted for 48 h and killed, adipose tissue was taken, and RNA was prepared as described above. This also resulted in the following four groups of mice: chow fed (n = 6), chow fasted (n = 3), high-fat diet fed (n = 6), and high-fat diet fasted (n = 5).

**Peripheral hormones.** Blood glucose was measured by a Lifespan One-Touch II glucose meter (Johnson & Johnson, Mountain View, CA). Serum insulin and leptin were assayed by an ELISA with commercial kits (Crystal Chemical, Chicago, IL), and serum corticosterone and adiponectin were assayed by RIA with commercial kits (ICN Biomedicals, Costa Mesa CA and Linco Research, St. Charles, MO, respectively).

**Northern blot analysis.** Total RNA was extracted from white adipose tissue with TRIZol (GIBCO-BRL, Gaithersburg, MD), as described previously (18, 19). Templates for the production of both adiponectin and resistin probes were produced from 1 μg of mouse total adipose tissue RNA by RT-PCR, as described previously (18, 19). Primers for both adiponectin and resistin were designed using the MacVector program (Oxford Molecular Group) on a Macintosh platform. The standard criteria recommended by MacVector were used in choosing primers: 18–25 nt in length, 45–55% G + C content and melting temperature of 55–80°C. For adiponectin, the NH₂-terminal primer used was 5'-GCAAGCTTCTCGTGTTCCCTTAATC-3', and the COOH-terminal primer used was 5'-TGACATCTTCTTCTGCCCTCT-3'. For resistin, the NH₂-terminal primer used was 5'-CCCTCTCTTTCCTG -3', and the COOH-terminal primer used was 5'-TTTCTTCCAAGATCGTCCCAG-3'. Northern blot analysis was performed using single-stranded, internally labeled DNA probes created from the above templates for adiponectin and resistin. Blots and preparation of the leptin probe were carried out as described previously (18, 19). The membranes were reprobed with 18S rRNA to verify uniform RNA loading. The total integrated densities of hybridization signals were determined by a Phospholmager (STORM 860; Molecular Dynamics, Sunnyvale, CA).

**Statistics.** The total integrated density for each Northern blot band was analyzed, and the mean and SE for each experimental group were calculated and presented as a percentage of either the mean wild-type sham group (study 1) or chow ad libitum-fed groups (study 2). Statistical analysis entailed a two-way ANOVA followed, when indicated by appropriate P values (P < 0.05), by the Tukey-Kramer post hoc test. Regression and multiple-regression analysis were used to examine the shared variance of each gene product with other products and hormones.

### RESULTS

**Effects of adrenalectomy and leptin deficiency.** Consistent with previous findings, the body weights for sham-operated leptin-deficient ob/ob mice were significantly higher than sham-operated wild-type mice (Table 1), and adrenalectomy reduced body weight in the ob/ob mice but not wild-type mice (Table 1). Similarly, adipose weights for sham-operated ob/ob mice were significantly higher than sham-operated wild-type mice (Table 1), and adrenalectomy reduced adipose weight in the ob/ob mice but not in wild-type mice (Table 1). The blood glucose levels for sham-operated ob/ob mice were also significantly elevated compared with sham-operated wild-type mice (Table 1), and adrenalectomy once again significantly reduced blood glucose in the ob/ob mice but not in wild-type mice (Table 1). Adrenalectomy of ob/ob mice dramatically increased insulin sensitivity, as indicated by a >10-fold reduction of insulin in adrenalectomized ob/ob compared with sham-operated ob/ob mice (Table 1). In addition, serum corticosterone was elevated in the sham-operated ob/ob mice compared with the sham-operated wild-type mice (Table 1), and adrenalectomy reduced the levels of corticosterone in both wild-type and ob/ob mice, as expected (Table 1). Consistent with our previous finding after adrenalectomy, levels of corticosterone were at low physiological levels (16). The leptin peptide levels for the wild-type sham-operated and adrenalectomized mice were unchanged, and ob/ob mice did not exhibit any assayable leptin peptide (Table 1).

Adiponectin mRNA was reduced by ~70% in sham-operated ob/ob mice compared with sham-operated wild-type mice (Fig. 1, A and B), precisely agreeing with previously reported results (13). However, the peptide levels between the two groups were similar.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Food, g</th>
<th>Adipose Weight, g</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
<th>Corticosterone, ng/ml</th>
<th>Leptin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type sham operated</td>
<td>4</td>
<td>27.69 ± 0.3*</td>
<td>3.7 ± 0.4†</td>
<td>0.4 ± 0.07*</td>
<td>162.5 ± 21.7*</td>
<td>1.7 ± 0.5*</td>
<td>84.0 ± 49.5*</td>
<td>0.82 ± 0.3*</td>
</tr>
<tr>
<td>Wild type adrenalectomized</td>
<td>7</td>
<td>27.03 ± 1.0*</td>
<td>4.8 ± 0.3*</td>
<td>0.3 ± 0.04*</td>
<td>142.8 ± 9.0*</td>
<td>1.1 ± 0.2†</td>
<td>43.2 ± 12.9*</td>
<td>0.82 ± 0.3*</td>
</tr>
<tr>
<td>ob/ob Sham operated</td>
<td>6</td>
<td>46.33 ± 1.3†</td>
<td>6.0 ± 0.9†</td>
<td>4.0 ± 0.81†</td>
<td>212.4 ± 32.7†</td>
<td>107.3 ± 30.1†</td>
<td>228 ± 37.2†</td>
<td>0 ± 0†</td>
</tr>
<tr>
<td>ob/ob Adrenalectomized</td>
<td>5</td>
<td>40.05 ± 2.0‡</td>
<td>4.3 ± 1.0‡</td>
<td>2.7 ± 0.12‡</td>
<td>118.0 ± 13.2‡</td>
<td>8.6 ± 3.3‡</td>
<td>28.4 ± 9.7*</td>
<td>0 ± 0†</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; n, no. of experiments. Groups with different symbols are statistically different (P < 0.05) by ANOVA followed by the Tukey-Kramer post hoc test.

**Table 1. Effects of adrenalectomy and leptin deficiency on body weight, mean overnight food intake, adipose weight, blood glucose, serum insulin, serum corticosterone, and serum leptin levels**
type and ob/ob mice such that leptin mRNA in adrenalectomized ob/ob mice was statistically equivalent to leptin mRNA in sham-operated wild-type mice (reduction of 100 ± 14 to 60 ± 10.22% in wild-type mice and reductions of 365 ± 45.18 to 175 ± 49.57% in ob/ob mice).

Regression analysis indicated that resistin and adiponectin mRNA were highly and positively correlated (Fig. 2; \( r^2 = 0.69; P < 0.0001 \)); these two variables were significantly correlated even after statistical removal of the effects of genotype, adrenalectomy, body weight, insulin, and blood glucose. In addition, both mRNAs were negatively correlated with leptin mRNA (\( P < 0.05 \)), but the correlations with leptin mRNA were no longer significant after removal of any other main variable (genotype, adrenalectomy, body weight, insulin, or blood glucose). Adiponectin was also correlated negatively with insulin, but this correlation was very weak (\( r^2 = -0.14; P < 0.0158 \)), whereas resistin was not correlated with insulin at all.

**Effects of diet and fasting.** Mice fed a high-fat, high-simple-carbohydrate diet had a significantly higher body weight compared with mice fed a normal rodent chow diet (Table 2), and fasting decreased body weight in both chow and high-fat diet groups (Table 2). Similarly blood glucose was elevated in the mice fed the high-fat diet compared with the chow-fed mice (Table 2), and fasting reduced glucose in both chow and diet groups of mice (Table 2). Serum insulin was also elevated in the mice fed a high-fat diet (Table 2), and fasting decreased insulin in both chow and diet groups (Table 2).

Consistent with the reduction of adiponectin mRNA in leptin-deficient obese mice, diet-induced obesity was also associated with a reduction of adiponectin mRNA (Fig. 3, A and B). Interestingly, fasting also reduced adiponectin mRNA in mice on both diets (Fig. 3, A and B). The regulation of resistin mRNA was highly similar to the regulation of adiponectin; both mRNAs were reduced by the obesity-inducing diet and by fasting (Fig. 3C). As observed in leptin-deficient obese mice, diet-induced obesity was associated with elevated leptin mRNA (100 ± 18.6 to 173.7 ± 1.9%), and fasting significantly reduced leptin mRNA in mice on both

![Fig. 1.](image1.png)  
**A:** representative Northern blot bands for adiponectin mRNA in study 1. Effects of adrenalectomy (ADX) and leptin deficiency on adiponectin mRNA (B), serum adiponectin peptide (C), and resistin mRNA (D). Data are expressed as means ± SE of wild-type sham-operated levels. Groups with different letters are statistically different (\( P < 0.05 \)) by ANOVA followed by the Tukey-Kramer post hoc test.
Table 2. Effects of diet and fasting on body weight, blood glucose, and serum insulin levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Body Weight, g</th>
<th>Glucose, mg/dl</th>
<th>Insulin, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow ad libitum fed</td>
<td>6</td>
<td>29.5 ± 0.7⁎</td>
<td>220.2 ± 29.6⁎</td>
<td>2.4 ± 0.7⁎</td>
</tr>
<tr>
<td>Chow fasted</td>
<td>3</td>
<td>22.6 ± 1.2†</td>
<td>100 ± 3.1†</td>
<td>0.5 ± 0.0⁎</td>
</tr>
<tr>
<td>Diet ad libitum fed</td>
<td>6</td>
<td>48.7 ± 2.3‡</td>
<td>303.6 ± 37.4‡</td>
<td>28.2 ± 6.6‡</td>
</tr>
<tr>
<td>Diet fasted</td>
<td>5</td>
<td>39 ± 3.2‡</td>
<td>106.6 ± 5.8‡</td>
<td>7.7 ± 3.7⁎</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE; n, no. of experiments. Groups with different letters are statistically different (P < 0.05) by ANOVA followed by the Tukey-Kramer post hoc test.

diets (reduction of 100 ± 18.6 to 9.3 ± 3.3% in chow group and reduction of 173.7 ± 1.9 to 122.7 ± 20.1% in diet group). Consistent with these results, and with the results in ob/ob mice, resistin mRNA and adiponectin mRNA were highly correlated (Fig. 4: \( r^2 = 0.92; P < 0.0001 \)); the correlation between these mRNAs was significant even after statistical removal of the effects of all other primary variables (diet, fasting, body weight, insulin, glucose, and plasma leptin). As with ob/ob mice, resistin and adiponectin mRNAs were also negatively correlated with leptin mRNA, but these correlations were no longer significant after statistical removal of any other primary variables. In this study, neither adiponectin nor resistin mRNAs were correlated with insulin.

DISCUSSION

A key observation of the present study is that adrenalectomy reversed the reduction in adiponectin mRNA associated with leptin-deficient obesity and in fact led to an elevation of plasma adiponectin to above wild-type levels. Surprisingly, resistin mRNA was reduced in diet-induced obesity, in contrast to the elevation of plasma levels of resistin reported in diet-induced obesity by Steppan et al. (23) but in accordance with the findings of decreased resistin expression in various obese models by Way et al. (24). Furthermore, the expression of resistin and adiponectin was highly correlated across all conditions, even after statistical removal of the effects of other major hormonal variables. Although some of these results are surprising, they may be explained by simple hypotheses, as described below.

Adrenalectomy increases insulin sensitivity in ob/ob mice, as evidenced by the dramatic decrease in plasma insulin and glucose (16). In the present study, we observed that adrenalectomy normalized expression of adiponectin in ob/ob mice (Fig. 1, A and B) and resulted in a twofold increase in the peptide levels (Fig. 1C). This is consistent with in vitro studies showing that dexamethasone inhibits adiponectin mRNA (9, 11). In addition, Masuzaki et al. (17) have shown that transgenic mice overexpressing the 11β-hydroxysteroid dehydrogenase type 1 gene exhibit elevated local levels of corticosterone in adipose tissue and subsequently have lower adiponectin expression (17). Because adiponectin has been postulated to increase insulin sensitivity, our finding is consistent with the hypotheses that adrenalectomy increases insulin sensitivity, perhaps through the stimulation of adiponectin.

Although surgical removal of the entire adrenal gland may not be refined enough to isolate the responsible agents, others have used similar (4) or more refined surgical procedures such as adrenal cortectomies to remove just the adrenal cortex (5) or pharmacological agents such as RU-486 to block glucocorticoid receptors (21) and achieved similar physiological effects. In addition, replacement of glucocorticoids after adrenalectomy famously returns phenotypes that have been reversed by adrenalectomy (7). Conversely, eleva-
tions in glucocorticoids are known to lead to insulin resistance (25). This convergence of evidence suggests that the principal effect of adrenalectomy to increase insulin sensitivity is in fact because of the removal of glucocorticoids and not because of the removal of other hormonal factors from other components of the adrenal gland or its associated pathways.

Adiponectin mRNA and peptide were positively correlated in the leptin-deficient obese mouse, with both mRNA and peptide levels showing an induction with adrenalectomy (Fig. 1, A–C). However, ob/ob mice showed lower adiponectin mRNA levels compared with wild-type mice (Fig. 1, A and B) despite the fact that the peptide levels of the two groups were similar (Fig. 1C), and adrenalectomized ob/ob mice showed higher plasma adiponectin compared with the wild-type sham-operated mice despite similar mRNA levels (Fig. 1, A–C). This apparent disparity may be explained by the significant difference in adiposity between the leptin-deficient obese mice and the wild-type mice (Table 1). In both cases, the plasma levels of adiponectin in ob/ob mice presumably reflect the increased adiposity of the leptin-deficient obese mice that persists even after adrenalectomy. A similar mechanism probably explains the seeming disparity between the decrease in resistin mRNA seen in diet-induced obesity and the reported elevation in resistin peptide in obesity (23). The elevated resistin peptide appears to reflect an increase in resistin-producing adipose tissue that quantitatively dominates the decrease in mRNA per microgram total RNA observed in the present study.

The degree of obesity and adipose tissue size may also explain the seemingly contradictory finding on adiponectin peptide levels with other forms of obesity. Although adiponectin peptide levels have been found to be decreased in both humans (1) and in diet-induced obesity in mice (27), we found in fact that leptin-deficient mice have similar peptide levels as their wild-type littermates (Fig. 1C). This may at first seem contradictory until closer examination of relative adipose tissue size within these obese models. The relatively much larger adiposity of the ob/ob mice, compared with humans and diet-induced obesity, may be sufficient to result in equal levels of the peptide with their wild-type littermates while in other forms of obesity this is not the case.

Adiponectin expression was reduced in the diet-induced obese mice compared with chow-fed mice (Fig. 3, A and B), confirming the findings of Yamauchi et. al. (27). Paradoxically, fasting led to a decrease in adiponectin mRNA in chow-fed mice, and similarly fasting led to a decrease in adiponectin mRNA in diet-induced obese mice as well (Fig. 3, A and B). The reduction in adiponectin expression with fasting is most likely because of the decrease in total adipose tissue as energy stores are depleted with nutritional deprivation.

The most surprising result in the current study was the high degree of correlation between resistin and adiponectin mRNA levels across all conditions. This was true even after removal of other statistical variables such as body weight, adiposity, and other hormones. Thus, as can be observed in the regression shown in Fig. 4, resistin and adiponectin are correlated even when analyzed only in chow ad libitum-fed mice. Because in the second study resistin and adiponectin mRNAs were regulated concomitantly across all conditions, the high degree of correlation is readily apparent. In the first study, this correlation may perhaps seem paradoxical, since the regulation of these mRNAs differs in a key respect: that adiponectin mRNA is downregulated in ob/ob mice, whereas resistin mRNA is not. However, as with diet-induced obesity, inspection of the regression plot (Fig. 2) reveals that the regression occurs robustly within each group. This high degree of correlation leads us to conclude that adiponectin and resistin are regulated in a virtually identical fashion, at least in our two model systems. These results suggest that the regulation of expression of these two genes involves similar molecular mechanisms.

REFERENCES


