Adiponectin secretion and response to pioglitazone is depot dependent in cultured human adipose tissue

Susan A. Phillips,1,2 Theodore P. Ciaraldi,1,3 Deborah. K. Oh,1,3 Michelle K. Savu,1,4 and Robert R. Henry1,3

1Veterans Affairs San Diego Healthcare System, La Jolla; and Departments of 2Pediatrics, 3Medicine, and 4Surgery, University of California, San Diego, California

Submitted 11 April 2008; accepted in final form 23 July 2008

Phillips SA, Ciaraldi TP, Oh DK, Savu MK, Henry RR. Adiponectin secretion and response to pioglitazone is depot dependent in cultured human adipose tissue. Am J Physiol Endocrinol Metab 295: E842–E850, 2008. First published July 29, 2008; doi:10.1152/ajpendo.90359.2008.—The subcutaneous (S) and visceral (V) adipose tissue (AT) depots are increasingly recognized as distinct. To test the hypothesis that depot differences exist for adiponectin, fresh and cultured human VAT and SAT from obese type 2 diabetic (T2D) and obese non-diabetic (ND) subjects was examined to determine whether differences in adiponectin content and secretion occurred as a function of depot studied, diabetic status, and response to thiazolidinedione treatment. VAT and SAT were obtained by biopsy and AT explants cultured in defined media for 7 days. Protein expression was assessed by Western blot. Adiponectin content of conditioned medium was determined by radioimmunoassay. Diabetic status had no effect on adiponectin secretion over days 0–2 of culture. In ND SAT, secretion fell over days 2–4 but was sustained at greater levels vs. T2D SAT. In both ND and T2D VAT, adiponectin secretion was low, similar to T2D SAT. Over the 7-day culture period, cellular adiponectin increased in ND SAT and VAT; it remained unchanged in T2D SAT and VAT. Pioglitazone increased adiponectin secretion and content in all SAT. Pioglitazone failure to increase adiponectin secretion from either ND or T2D VAT and increased cellular content only in ND VAT. AT depot differences exist in the secretion of adiponectin and responsiveness to thiazolidinedione treatment. These data suggest that SAT, not VAT, appears to be the major contributor to increased circulating adiponectin levels in response to pioglitazone treatment. SAT and VAT explants. Given the low circulating levels of adiponectin associated with T2D and the increases associated with T2D treatment, we asked whether these differences might also reflect differences in depot responsiveness.

MATERIALS AND METHODS

Human subjects. A total of 50 obese subjects were recruited for this study. The Institutional Review Board at the University of California San Diego approved this research, and all subjects gave informed consent. Of the 50 subjects, 26 were diabetic. The determination of diabetic status was made on the basis of a fasting blood glucose >125 or a 2-h glucose >200 mg/dl following a 75-g oral glucose load [oral glucose tolerance test (OGTT)] (8). Characteristics of study subjects are shown in Table 1. Fasting serum lipids and glucose were determined using standard clinical assays. Waist circumference was measured as the abdominal circumference at the level of the iliac crest.

Adipose tissue biopsy. Human AT was obtained from a total 26 subjects (13 T2D and 13 non-diabetic (ND)) undergoing laparoscopic gastric bypass with Roux-en-Y gastroenterostomy surgery for the treatment of morbid obesity following an overnight fast. Diabetic obesity, insulin resistance, inflammation, and fatty acid metabolism. Energy homeostasis, and insulin sensitivity (55) via release of metabolically active products that regulate insulin sensitivity and fat metabolism (27). Many AT products exert opposing actions on insulin sensitivity, inflammation, and fatty acid metabolism. For example, AT products IL-6, TNF-α, and resistin may impair insulin sensitivity, increase circulating free fatty acids, and exert proinflammatory effects, whereas others such as adiponectin exert insulin-sensitizing effects, reduce free fatty acids, and dampen inflammation (28). The metabolic impact of a given AT depot is therefore related to the relative profile or balance of mediator release.

AT is distributed to a variety of locations, and in humans contributions of SAT and VAT can be distinguished (reviewed in Ref. 59). Individuals with increased visceral (V)AT are at higher risk of developing the metabolic syndrome, type 2 diabetes (T2D), and cardiovascular disease (32, 59) than those with similar amounts of AT as subcutaneous (S)AT (34). Underlying these findings are depot differences in gene and protein expression (36, 37). These findings lend support to the idea that different AT depots are functionally distinct metabolic units.

Circulating levels of adiponectin are low in obese and insulin-resistant subjects (62) and are predictive for development of T2D (30). In contrast, adiponectin is high in lean and insulin-sensitive subjects (50). Interestingly, serum adiponectin levels are inversely correlated with waist circumference and intra-abdominal fat accumulation (48), suggesting that depot differences likely exist in adiponectin secretion. Depot differences in adiponectin gene expression have been reported by some (23, 46, 49) but not all investigators (18, 53, 63) and importantly are not always correlated with AT secretion (49) or circulating levels (30). Little is known regarding posttranscriptional regulation of adiponectin. However, thiazolidinediones (TZDs), potent insulin sensitizers, are recognized to augment cellular adiponectin and its circulating levels two- to threefold (13).

To determine whether reported in vivo correlations between AT depot distribution and circulating adiponectin reflect underlying tissue differences in adiponectin content and secretion, we examined adiponectin content and secretion in human SAT and VAT explants. Given the low circulating levels of adiponectin associated with T2D and the increases associated with T2D treatment, we asked whether these differences might also reflect differences in depot responsiveness.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

http://www.ajpendo.org

Address for reprint requests and other correspondence: S. A. Phillips, VA San Diego Healthcare System (9111G), 3350 La Jolla Village Dr., San Diego, CA 92161 (e-mail: saphillips@ucsd.edu).
subjects continued treatment with either metformin alone, metformin plus a sulfonylurea, or insulin; subjects on a TZD were excluded. Abdominal SAT was obtained from the lower abdominal region outside the fascia superficialis, and VAT was obtained from the greater omentum. In 13 (8 T2D AND 5 ND) subjects, paired samples of SAT and VAT were obtained. SAT was also obtained from 24 obese subjects (13 T2D and 11 ND) by needle biopsy as previously described (11).

Adipose explant culture. All procedures for AT explant culture were carried out using sterile technique. Following biopsy or surgery, AT was placed into sterile filtered HEPES-salts (HS) buffer containing (in mmol/l) 150 NaCl, 5 KCl, 1.2 MgSO4, 1.2 CaCl2, 2.5 Na2HPO4, 10 HEPES, and 2 pyruvate, pH 7.4, and supplemented with 4% BSA (Roche Diagnostic, Indianapolis, IN) washing buffer (WB) and immediately transported under aseptic conditions to the laboratory. AT was washed free of lipid and blood clots with WB and then processed further by cutting into 5- to 10-mg pieces, washed, and immediately transported under aseptic conditions to the laboratory. AT was washed free of lipid and blood clots with WB and then processed further by cutting into 5- to 10-mg pieces, washed, and placed in WB for 30-min recovery. AT was then weighed and divided for explant culture and studies on freshly isolated adipocytes. AT explants were cultured (1 g AT/30 ml) under an atmosphere of 5% CO2 in air at 37°C in defined medium (DM) (1:1 DME low/Ham’s explants were cultured (1 g AT/30 ml) under an atmosphere of 5% for explant culture and studies on freshly isolated adipocytes. AT was washed free of lipid and blood clots with WB and then immediately transported under aseptic conditions to the laboratory. All procedures for AT explant culture were performed as described previously.

For the leptin assay, the corresponding values were 0.5 ng/ml and 5 and 5%. Screening of cytokine content of the CM was preformed by hybridizing the medium with antibody-coated membranes according to manufacturer’s instructions (RayBiotech, San Diego, CA). Media IL-6, IL-8, IL-1β, TNF-α, monocyte chemoattractant protein-1 (MCP-1), and IL-10 were additionally quantified by ELISA (R&D Systems, Minneapolis, MN).

Statistical analysis. Results are presented as means ± SE for the indicated numbers of patients. Due to limitations in sample availability, not all analyses were performed in every subject. The number of subjects studied is given in the legend of each figure. Clinical characteristics of the subsets of subjects studied in the different experiments did not differ from the average of the total group. Statistical analysis was performed using the GraphPad Prism program v.4 (Intuitive Software, San Diego, CA). Statistical significance was evaluated with Student’s t-test for unpaired or paired data when appropriate and repeated-measures ANOVA. Significance was accepted at the P < 0.05 level.

RESULTS

Serum adiponectin levels in obese diabetic and obese ND subjects. To focus on the impact of T2D on adiponectin secretion, we recruited subjects who were matched for BMI, weight, waist circumference, and lipid levels. Only fasting glucose and Hb A1c levels differed between the subject groups (Table 1). There was a tendency (P = 0.16) for T2D subjects to have lower serum adiponectin levels (8.6 ± 1.4 vs. 10.9 ± 1.8 μg/ml). Serum adiponectin levels of a subset (n = 23) of subjects were correlated with glucose disposal rate (GDR) as determined from hyperinsulinemic euglycemic clamp (17) (r = 0.49, P = 0.01) a measure of whole body insulin action, although no correlation was found between BMI and serum adiponectin levels (data not shown).

Impact of depot and diabetic status on adiponectin secretion. Human SAT and VAT explants were cultured in defined medium for 1 wk. Over the first 2 days (days 0–2) of culture, no depot or diabetes-related differences were evident in the rate of adiponectin secretion by AT (Fig. 1, A and B). GDR was positively correlated with both serum adiponectin (r = 0.49, P = 0.01) and SAT days 0–2 adiponectin secretion (r = 0.56, P = 0.01), suggesting that days 0–2 culture is reflective of the in vivo environment including the impact of antidiabetic medication. By day 4 of culture, both SAT and VAT secreted less adiponectin. ND SAT (Fig. 1A), however, showed a greater

Table 1. Clinical characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Diabetic</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>Sex (m/f)</td>
<td>21/5</td>
<td>16/8</td>
</tr>
<tr>
<td>Age, yr</td>
<td>51±2</td>
<td>50±2</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>40.2±1.4</td>
<td>36.8±1.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>120.0±5.4</td>
<td>108.4±5.5</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>6.9±0.4*</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>Hb A1c, %</td>
<td>6.7±0.3*</td>
<td>5.5±0.1</td>
</tr>
<tr>
<td>HDL, mM</td>
<td>0.96±0.05</td>
<td>1.13±0.07</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>2.33±0.28</td>
<td>2.23±0.22</td>
</tr>
<tr>
<td>Waist Circumference, cm</td>
<td>122±4</td>
<td>111±7</td>
</tr>
</tbody>
</table>

Values are means ± SE. ND, nondiabetic; BMI, body mass index; HDL, high-density lipoprotein. To convert glucose mM to mg/dl, multiply by 18; HDL mM to mg/dl multiply by 38.67; and triglyceride mM to mg/dl, multiply by 88.57. Glucose, HDL, and triglyceride levels were measured after an overnight fast. *P < 0.05 vs. ND group.
of a potential artifact of the culture system. To address this concern, we assessed the secretion from AT of another key adipokine, leptin. In contrast to adiponectin, leptin secretion from ND SAT and VAT and from T2D SAT and VAT was sustained throughout the culture period (see Fig. 5), suggesting that the change in adiponectin secretion is a specific response. No diabetic impact was observed on the capacity of AT to maintain leptin secretion.

Impact of depot and diabetic status on cellular adiponectin content. Protein expression of adiponectin was compared in adipocytes isolated from fresh and 1-wk-cultured AT explants (Fig. 2). Adiponectin was detected by Western blot as a single band migrating at ~30 kDa. No correlation was found between serum adiponectin and adiponectin protein content in adipocytes isolated from fresh or explant cultures of VAT and/or SAT adipocytes (data not shown). No depot or diabetic differences were detected in adiponectin content of freshly isolated fat cells. However, and in contrast to the observed decreases in adiponectin secretion during culture, adiponectin content increased in cultured ND AT, regardless of depot. Adiponectin content remained unchanged in T2D AT over the culture period (Fig. 2). These results show that depot-dependent and diabetic-associated differences in adiponectin secretion seen with time in culture are not mirrored, or accounted for, by changes in cell content.

TZD regulation of adiponectin content and secretion. TZDs are selective PPARγ agonists that serve as potent insulin sensitizers. In vivo, administration of TZDs to diabetic subjects results in a two- to threefold increase in circulating adiponectin (40). To determine direct and depot-specific effects of TZD treatment on adiponectin secretion, ND and T2D SAT and VAT were cultured in the absence or presence of 10 μM Pio. In ND SAT, Pio treatment resulted in a two- to threefold
increase in adiponectin secretion by days 2–4 that was sustained through days 4–6 of culture, restoring adiponectin secretion by SAT to levels observed at days 0–2 (Fig. 3A). No Pio response was detected in ND VAT (Fig. 3B). Depot differences were also observed in T2D AT. Pio addition to T2D SAT resulted in a partial rescue of adiponectin secretion (Fig. 4A), increasing secretion 1.8-fold by days 2–4 and over twofold by days 4–6 of culture, although not to the levels seen in ND SAT (Fig. 3A), suggesting an impact of diabetic status on SAT responsiveness to Pio in its capacity for adiponectin secretion. Again, no response was detected in VAT; levels remained low throughout the culture period (Fig. 4B). These results suggest a difference in the relative responsiveness of the SAT vs. the VAT depot to TZD effects on adiponectin secretion.

Pio treatment significantly increased cellular adiponectin in adipocytes isolated from 7-day-cultured ND and T2D SAT explants (Table 2), mirroring the treatment associated increases in SAT adiponectin secretion (Figs. 3A and 4A). Interestingly, ND but not T2D VAT (Table 2) also responded to Pio treatment with an increase in cellular adiponectin, although the increase in ND VAT occurred without a parallel increase in adiponectin secretion (Fig. 3B). Thus, regardless of diabetic status, SAT retained the capacity to respond to Pio with an upregulation of cellular adiponectin, whereas in VAT diabetic status impacted Pio responsiveness (Table 2). These differences in AT depot behavior and TZD responsiveness may underlie observed differences in adiponectin levels and TZD responsiveness in both ND and T2D subjects. The failure of VAT to increase secreted and cellular adiponectin in response to Pio treatment was not due to a deficiency in PPARγ protein expression. Indeed, PPARγ expression in VAT was comparable to that in SAT for both T2D (0.63 ± 0.22 vs. 0.62 ± 0.10 AU/μg protein, VAT vs. SAT, respectively; n = 4) and ND (0.73 ± 0.17 vs. 0.78 ± 0.35 AU/μg protein; n = 11).

The above findings suggest a greater relative influence of AT depot differences compared with diabetic status in predicting changes in adiponectin. One potential mechanism for the

Table 2. Cellular adiponectin and response to pioglitazone

<table>
<thead>
<tr>
<th>SAT Culture</th>
<th>VAT Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 μM</td>
<td>0 μM</td>
</tr>
<tr>
<td>ND 164.3±50.5</td>
<td>318.5±51.9*</td>
</tr>
<tr>
<td>T2D 209.1±63.7</td>
<td>411.3±109.7*</td>
</tr>
<tr>
<td>10 μM</td>
<td>10 μM</td>
</tr>
<tr>
<td>ND 318.5±51.9*</td>
<td>491.3±69.2*</td>
</tr>
<tr>
<td>T2D 411.3±109.7*</td>
<td>128.5±14.7</td>
</tr>
</tbody>
</table>

Results are means ± SE in %; n = 11 for ND SAT and VAT, n = 8 for T2D SAT, and n = 6 for T2D VAT. Cellular adiponectin content in ND adipose tissue (AT) and type 2 diabetic (T2D) AT and treatment effect of pioglitazone (Pio). Explants were cultured for 7 days in defined medium ±10 μM Pio. Fat cell protein extracts were prepared from fresh and cultured ND and T2D subcutaneous (SAT) and visceral (VAT). Adiponectin expression is presented as a percentage of the value determined in fresh adipocytes from the same individual. *P < 0.05 vs. paired untreated control.
observed depot differences in adiponectin responsiveness could be a change in the secretion of inflammatory cytokines. To evaluate for this possibility, CM from cultured SAT and VAT explants was screened for inflammatory cytokine content using a commercial protein microarray; no differences between depots were detected. To quantitatively determine whether changes occurred in cytokines previously identified as products of AT, IL-8, IL-10, IL-6, MCP-1, TNF-α, and leptin content of the conditioned medium, over time in culture, were determined by ELISA or Bio-Plex. All cytokines except leptin decreased in parallel with adiponectin (Table 3). Leptin secretion, in contrast to adiponectin, increased at days 2–4 in SAT (Fig. 5A) but not in VAT (Fig. 5B). Interestingly, TZDs act to reduce leptin expression in adipocytes (16). Consistent with this reported activity of PPARγ agonists on leptin gene regulation, Pio treatment of SAT and VAT explants significantly reduced the secretion of leptin into the medium (Fig. 5, A and B). Moreover, this response was earlier and of a greater magnitude in VAT, suggesting a greater sensitivity of the VAT depot to this TZD effect, in contrast to its failure to respond to TZD augmentation of adiponectin secretion (Figs. 3B and 4B).

DISCUSSION

Adipose tissue is a dynamic tissue whose function impacts a number of key physiological processes, including inflammation, reproduction, energy homeostasis, and lipid and glucose metabolism. Complicating this evolving and important role of AT in whole body physiology is the observation that AT is not homogeneous in function.

AT is distributed to a number of distinct regional depots. The literature suggests that this pattern of distribution, particularly in regard to the relative proportion of VAT and SAT, may be an important indicator of metabolic abnormalities (35). The size of the VAT depot has been positively correlated with insulin resistance and cardiovascular risk (19) and is a better determinant of insulin sensitivity than SAT (12). Direct evidence for regional differences in AT behavior comes from studies of preadipocytes, adipocytes, and cultured tissue explants that extend to adipocyte gene (44) and protein expression (44, 59), as well as to functional measures (5, 20). Depot differences also exist at the level of secretion (reviewed in Ref. 59). A high degree of correlation exists between VAT mass and circulating levels of proinflammatory cytokines (31, 41, 42). At the tissue level, depot differences have been docu-

Table 3. Changes in cytokine secretion over days 2–4 of culture: depot effect

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>SAT</th>
<th>VAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>32.3±8.8*</td>
<td>39.6±11.4*</td>
</tr>
<tr>
<td>IL-6</td>
<td>88.9±17.2</td>
<td>5.6±14.0*</td>
</tr>
<tr>
<td>IL-8</td>
<td>19.6±3.9*</td>
<td>22.3±4.0*</td>
</tr>
<tr>
<td>MCP-1</td>
<td>48.4±12.7*</td>
<td>124.9±13.3*</td>
</tr>
<tr>
<td>TNF-α</td>
<td>11.0±0.4*</td>
<td>6.1±1.1*</td>
</tr>
<tr>
<td>IL-10</td>
<td>9.5±1.0*</td>
<td>52.7±8.2*</td>
</tr>
<tr>
<td>Leptin</td>
<td>220±53*</td>
<td>125±23</td>
</tr>
</tbody>
</table>

Values are reported as percentage of the value on day 0–2 levels ± SE. Media content of cytokines were determined as described in MATERIALS AND METHODS. Results from ND and T2D were combined; n = 14–21 for SAT and n = 22 for VAT cytokine analysis; n = 14–16 for SAT and VAT leptin analysis. *P < 0.05 vs. days 0–2.

Fig. 5. Leptin secretion by cultured SAT and VAT explants: effect of PPARγ agonist Pio treatment. Leptin secretion by SAT (A) and VAT (B) from combined ND and T2D AT explants. Explants were cultured for 7 days in DM containing 30 nM Dex and 1 nM insulin ± 10 μM Pio. Medium was sampled every 2 days and analyzed by RIA for leptin content. Results are reported as average ± SE. SAT: n = 16 − Pio and n = 8 + Pio; VAT: n = 14 − Pio and n = 10 + Pio. VAT. Open bars, control; hatched bars, + Pio. *P < 0.05 vs. secretion on day 2; †P < 0.05 vs. paired control.

Adiponectin is a novel adipocyte-specific protein possessing important insulin-sensitizing, antiatherogenic (64), and anti-inflammatory properties (47). Circulating levels of adiponectin are positively correlated with insulin sensitivity (27, 54), and decreases in adiponectin parallel the development of insulin resistance (30). Unlike other adipokines, such as TNF-α, resistin, leptin, and IL-6, adiponectin circulates in inverse proportion to fat mass (3). However, the regional distribution of AT between the SAT and the intra-abdominal or VAT depot may be a more important factor. In visceral adipocytes, reductions in circulating levels of adiponectin have been associated with both reduced (23, 39) and unchanged (18, 49, 63) levels of gene expression. Moreover, changes in adiponectin secretion may occur in the absence of parallel changes in gene expression (30, 49), suggesting that circulating adiponectin may be regulated at the posttranslational and/or secretory level.

To better understand the impact of regional fat distribution on adiponectin production, we compared adiponectin secretion from freshly obtained and cultured SAT and VAT explants of individual obese subjects. Our findings of no depot or diabetic differences in adiponectin secretion over days 0–2 (Fig. 1, A and B) are consistent with previous reports on cultured adipo-
To investigate properties intrinsic to AT, independent from the in vivo environment, we proceeded to study AT depot behavior over time in culture. In ND AT, adiponectin secretion from SAT was sustained in culture, whereas that from VAT fell (Fig. 1A). In contrast, in T2D AT, neither SAT nor VAT was capable of sustained adiponectin secretion over the culture period, a finding mirroring in vivo observations of lower circulating levels of adiponectin in T2D vs. ND subjects matched for BMI (29). Our results in ND AT differ from those of Perrini et al. (49), who report greater adiponectin release by visceral adipocytes at 48 h. Important methodological differences, however, exist between our studies. First, and most importantly, our studies reflect results from explant compared with isolated adipocyte culture. Although each method has advantages, we chose explant culture for its proven utility in the analysis of long-term regulation of adipocyte function in vitro (25). Explant culture uniquely preserves both the extracellular matrix, providing structural support for adipocytes, and the paracrine influence of the many constituent cell types of AT that influence adipocyte function. Second, our studies reflect AT behavior over a 7-day compared with a 48-h period, permitting AT function to be studied in isolation from the influence of in vivo metabolism and hormonal influences.

Circulating levels of adiponectin and leptin have a reciprocal relationship (43). In the present studies, depot and diabetes-related differences in adiponectin secretion were not observed to occur with leptin secretion. In contrast to adiponectin, leptin secretion remained stable in culture regardless of depot source or diabetic status. Consistent with published reports (51), both ND and T2D SAT had a greater tendency for leptin secretion compared with VAT (Fig. 5), further supporting the validity of our experimental system.

One possible explanation for the reduction in adiponectin secretion is a parallel depletion of cellular adiponectin content. However, cellular adiponectin content did not decrease over time in culture. Therefore, observed reductions in media adiponectin suggest a change in the regulation of adiponectin secretion distal to protein synthesis.

TZDs are PPARγ agonists widely used for their insulin-sensitizing activity (38). In vivo (40, 50) and in vitro (40), TZDs enhance the expression of adiponectin mRNA and protein. Consistent with its in vivo effects on circulating adiponectin levels, we report in human AT direct effects of TZDs to significantly increase adiponectin secretion from cultured SAT explants obtained from both ND and T2D subjects. In contrast, VAT was resistant to TZD-mediated stimulation of adiponectin secretion, suggesting that depot differences may reflect not only the responsiveness of SAT but also the lack of the same in VAT. Intriguingly, in vivo TZD treatment is associated with an increase in SAT and a stabilization or possible reduction in VAT mass (33). Although the underlying molecular mechanism for this depot remodeling is not known, it may relate to an elevation in the expression of PPARγ and increased responsiveness to TZD agonism by SAT preadipocytes (52). These demonstrated direct effects of TZDs on AT are consistent with the prevailing view of AT as the primary target of TZD action. Our findings, together with those of Bodles et al. (4), differ from those of Motoshima et al. (45), who report that TZD augments adiponectin secretion from visceral but not subcutaneous adipocytes. The difference in results likely relates to a difference in experimental methods. Our use of explant culture, wherein isolated pieces of fat are placed in culture, has the advantage of ensuring that stromal or regulatory factors remain associated with adipocytes. In isolated cell culture this relationship of the adipocyte to the nonadipocyte components is lost, potentially impacting paracrine regulation of adiponectin secretion. With regard to SAT responsiveness, our findings are in agreement with the previous report (45), where no TZD-mediated increase in SAT adiponectin release was seen by 48 h (Figs. 3A and 4A).

Depot specificity was also observed in TZD responsiveness for cellular content. Of interest, ND VAT increased cellular adiponectin following PIO treatment but in contrast to SAT, there was no parallel increase in adiponectin secretion. In T2D VAT there is a failure to both increase and secrete cellular adiponectin in response to TZDs. Several insights are suggested from these studies. First, only SAT, regardless of diabetic status, exhibits the capacity to respond to TZDs by increases in both cellular and secreted adiponectin. Second, given the dissociation between increases in cellular adiponectin and secretion observed in VAT, depot differences likely also exist in the regulation of adiponectin secretion.

Similar to another report (21), TZD treatment was associated with inhibition of leptin secretion by explants. These findings of increased adiponectin and decreased leptin following TZD treatment mirror not only the in vivo reciprocal regulation of these factors but further demonstrate depot-specific responsiveness and highlight that, in regard to TZD treatment, leptin secretion from VAT remains responsive.

Although descriptive, this study of AT in culture has revealed several properties of adiponectin secretion that are depot dependent. Adiponectin secretion over the first 48 h correlates with in vivo GDR and may be thought to be reflective of the in vivo environment; conversely, the behavior of AT over the remainder of the culture period reveals characteristics intrinsic to the tissue. Given the greater mass of the SAT depot and its now demonstrated greater capacity for adiponectin secretion, SAT likely exerts a greater influence on circulating adiponectin than VAT. In the presence of diabetes, this greater capacity for adiponectin secretion is lost and may contribute to the negative impact of diabetes on circulating adiponectin levels seen in vivo (3, 30, 62). The ability of PIO to directly stimulate adiponectin secretion in SAT suggests that this depot may also be responsible for adaptive responses of circulating adiponectin as observed in vivo (50). VAT manifests two defects with regard to regulation of adiponectin: an inability to sustain secretion and a selective nonresponsiveness to TZDs. This observation would amplify the benefit derived from AT remodeling noted after TZD treatment (15) i.e., selective loss of the nonresponsive depot (VAT) with gain in the more responsive (SAT) depot. The fact that PIO treatment can induce changes in cellular adiponectin in VAT while not altering adiponectin secretion indicates that adiponectin secretion is a regulated process.

There are several possible mechanistic explanations for our observations. Depot differences may exist both in the machinery responsible for the regulated release of adiponectin (61) and in the nonadipocyte components of AT (14, 57). For example, Wang et al. (61) reported that AT depots can differ in their expression of ERp44 and Ero1-Lα, endoplasmic reticulum
chaperone proteins that modulate the secretion of adiponectin, and that these chaperones are PPARγ targets (61). Regarding nonadipocyte constituents of AT, greater macrophage infiltration and inflammatory cytokine production reported in VAT compared with SAT could negatively impact adiponectin secretion and contribute to the observed depot differences (6), although the differences in cytokine secretion that we observed would not explain the decreases in adiponectin that we report.

A consideration regarding these studies is that observed changes in adiponectin and leptin might reflect effects of hypoxia on explants over the 7-day culture period (26, 60). Reports of the effects of hypoxia on AT are notable for increases in the expression and secretion of leptin, TNF-α, IL-6, macrophage migration inhibitory factor, PAI-1, anaeorobic glycolysis, and, importantly, a decrease in expression of adiponectin and PPARγ2 and secretion of adiponectin. In our studies, we found an increase in adiponectin content throughout the culture period, and no elevations of IL-6, TNF-α, MCP-1, or vascular endothelial growth factor. A lack of change of media pH or lactate levels during culture suggests that aneorobic glycolysis was stable, ruling out a potential influence of hypoxia.

The results suggest that that regional adipose tissue distribution, because of its impact on adiponectin responsiveness, may be a predictor of the clinical response to insulin-sensitizing therapies. One limitation to these studies is that they reflect changes in total adiponectin and do not recognize the potential influence of hypoxia. The concurrent accumulation of intra-abdominal and subcutaneous fat explains the association between insulin resistance and plasma leptin concentrations: distinct metabolic effects of two fat compartments. Diabetes 51: 1005–1015, 2002.

ACKNOWLEDGMENTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Disease Grants K08-DK-061987 (S. A. Phillips) and RO1-DK-258291 (R. R. Henry); a Distinguished Clinical Scientist Award from the American Diabetes Association (R. R. Henry); a VA Merit Review grant from the Medical Research Service, Department of Veterans Affairs and VA San Diego Healthcare System; and Grant M01-RR-00827 in support of the General Clinical Research Center from the General Clinical Research Branch, Division of Research Resources, NIH.

DISCLOSURES

Dr. Robert R. Henry is on the Speaker’s Bureau and is a retained consultant for Takeda, the maker of pioglitazone.

REFERENCES


46. Weyer C, Funahashi T, Tanaka S, Hotta K, Matsuzawa Y, Pratley RE, Tataranni PA. Hypoadiponectinemia in obesity and type 2 diabetes:
