Reduced adipose tissue oxygenation in human obesity evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response

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Reduced Adipose Tissue Oxygenation in Human Obesity
Evidence for Rarefaction, Macrophage Chemotaxis, and Inflammation Without an Angiogenic Response
Magdalena Pasarica, Olga R. Sereda, Leanne M. Redman, Diana C. Albarado, David T. Hymel, Laura E. Roan, Jennifer C. Rood, David H. Burk, and Steven R. Smith

OBJECTIVE—Based on rodent studies, we examined the hypothesis that increased adipose tissue (AT) mass in obesity without an adequate support of vascularization might lead to hypoxia, macrophage infiltration, and inflammation.

RESEARCH DESIGN AND METHODS—Oxygen partial pressure (AT pO2) and AT temperature in abdominal AT (9 lean and 12 overweight/obese men and women) was measured by direct insertion of a polarographic Clark electrode. Body composition was measured by dual-energy X-ray absorptiometry, and insulin sensitivity was measured by hyperinsulinemic-euglycemic clamp. Abdominal subcutaneous tissue was used for staining, quantitative RT-PCR, and chemokine secretion assay.

RESULTS—AT pO2 was lower in overweight/obese subjects than lean subjects (47 ± 10.6 vs. 55 ± 9.1 mmHg); however, this level of pO2 did not activate the classic hypoxia targets (pyruvate dehydrogenase kinase and vascular endothelial growth factor [VEGF]). AT pO2 was negatively correlated with percent body fat (R = −0.50, P < 0.05). Compared with lean subjects, overweight/obese subjects had 44% lower capillary density and 58% lower VEGF, suggesting AT rarefaction (capillary drop out). This might be due to lower peroxisome proliferator–activated receptor γ1 and higher collagen VI mRNA expression, which correlated with AT pO2 (P < 0.05). Of clinical importance, AT pO2 negatively correlated with CD68 mRNA and macrophage inflammatory protein 1α secretion (R = −0.58, R = −0.79, P < 0.05), suggesting that lower AT pO2 could drive AT inflammation in obesity.

CONCLUSIONS—Adipose tissue rarefaction might lie upstream of both low AT pO2 and inflammation in obesity. These results suggest novel approaches to treat the dysfunctional AT found in obesity. Diabetes 58:718–725, 2009

Both insulin resistance and β-cell failure are present in individuals with type 2 diabetes. Insulin resistance is closely linked to adiposity with a central or visceral pattern, providing a greater risk of insulin resistance and metabolic dysfunction. Adipose tissue (AT) serves as an endocrine organ secreting a variety of autocrine, paracrine, and endocrine factors that can produce or prevent insulin resistance (1). The failure of AT to adequately proliferate and/or differentiate to sequester lipids away from liver, skeletal muscle, and the pancreatic β-cell has been proposed as a precursor to type 2 diabetes, broadening the number of potential mechanisms linking obesity to insulin resistance (2).

The increase in body fat in obesity should be accompanied by an increase in vascularization, in order to provide adequate oxygen and nutrients (3). In contrast to expectations, obese mice have lower AT capillary density (rarefaction, also known as capillary drop out) and decreased vascular endothelial growth factor (VEGF), the most potent angiogenic factor (4,5). Consistent with this model, preclinical studies suggest that obese AT is hypoxic (6); however, the hypothesis that AT rarefaction might lead to hypoxia remains untested.

In humans, short-term whole-body hypoxia decreases insulin sensitivity (7) and short-term whole-body hyperoxygenation increases insulin sensitivity (8). In mice, obesity is associated with lower oxygen partial pressure in subcutaneous and visceral AT (6,9). Studies in postsurgical patients support the idea that AT oxygen partial pressure (AT pO2) is lower in obesity (10).

In vitro hypoxic adipocytes secrete inflammatory molecules such as tumor necrosis factor (TNF) α, interleukin (IL)1, IL6, macrophage inflammatory protein (MIP), and plasminogen activator inhibitor-1 (6,11). Increased AT inflammation is a feature of obesity and type 2 diabetes (12). Hypoxic cells secrete chemokines, which attract macrophages, presumably to clear out necrotic cells and tissue (13). This suggests the hypothesis that the increase in AT macrophage content seen in human obesity (12) might be due to AT hypoxia.

These preclinical and cell culture experiments suggest that hypoxia might play a role in the inflammation and insulin resistance observed in human obesity. To test this hypothesis, we measured subcutaneous abdominal AT oxygenation (AT pO2) in lean and overweight/obese human subjects and related AT pO2 to the structure and function of AT.

RESEARCH DESIGN AND METHODS
Twenty-one subjects were recruited and screened based on their BMI: lean (20–25 kg/m2) or overweight/obese (27–35 kg/m2). Recruiting was conducted via newspaper, postcards, and the Pennington Biomedical Research Center (PBRC) webpage. Subjects were excluded if they had significant renal, cardiac, liver, lung, or neurological disease. Hypertension was acceptable if blood pressure was <140/90 mmHg on medications. Subjects were excluded for prior use of thiazolidinediones or injectable antihyperglycemic medication; drugs known to affect lipid metabolism, energy metabolism, or body weight; alcohol or other drug abuse; and smoking. The protocol was approved.
by the institutional review board at the PBRC, and all volunteers gave written informed consent.

Body composition was measured by dual-energy X-ray absorptiometry (DEXA) on a Hologic Dual Energy X-ray Absorptiometer in the fan beam mode (QDR 4500; Hologic, Waltham, MA). The coefficient of variation for the measurement of percentage of body fat was 1.7%. Two days prior and during an in-patient stay, participants were fed a standardized diet (50% carbohydrate, 15% protein, and 35% fat). The number of calories to be provided (and consumed) was calculated as 1.3 × basal energy expenditure using Harris-Benedict equation (665.10 + [9.66 × weight in kg] + [1.85 × height in cm] – [4.68 × age in year] for female and 60.47 + [13.75 × weight in kg] + [5.0 × height in cm] – [6.77 × age in years]) for male subjects.

Measurements were done at 2 cm from the umbilicus and the superior iliac crest (supplemental Fig. 1) and were made supine, on the left side of the abdomen, at one-third the distance of the body length. Bland-Altman analysis was used to compare the direct and indirect insertion methods. The direct method (supplemental Fig. 2) was used for the first 8 subjects, and the indirect method was used for the second 6 subjects, for technical reasons (see below).

Measurements of AT were done at 5-min interval. AT pO2 was measured using a polarographic micro Clark-type electrode, AT temperature was measured using a thermocouple concomitantly during these two distinct procedures. The measurements were done with a single oxygen probe (cat. no. C1), a single temperature probe (cat. no. C8), or a combined oxygen and temperature probe (cat. no. CCLP; Integra LifeSciences, Plainsboro, NJ). The probes were connected to an electronic unit (LI-COR CMP, brain oxygen-monitoring unit). This unit displays the AT temperature in °C and the AT pO2 in mmHg (after correcting for the contribution of temperature on AT pO2).

First, we measured AT pO2 (mmHg) with the probes inserted into a gas-permeable silastic tubing implanted in the subdermal space as described by Hopf (14) and optimized in our laboratory (indirect method, supplemental Fig. 2). Second, AT pO2 was measured using a direct insertion method developed by our laboratory (supplemental Fig. 2B). After cleaning the skin with povidone-iodine solution and removal of the dried iodine with sterile saline on gauze, a skin wheel was raised with 1% lidocaine. A combined oxygen and temperature probe was inserted through a 3.2-cm-long 14-Ga IV catheter (Medex) to a depth of 1 mm. The values obtained with the two methods were highly correlated (R = 0.64, P < 0.01; n = 13). However, the second method was less invasive and provided less discomfort to the volunteers. Therefore, after 13 subjects we only used the second method to measure AT pO2 and AT temperature. All values in this manuscript are from the “direct” measurement technique.

Euglycemic-hyperinsulinemic clamp. The clamp was performed as previously described (15). Briefly, intravenous catheters were inserted in an antecubital vein for infusions and in a vein on the dorsum of the contra-lateral hand for liposuction (3- to 4-mm diameter “mercedes” liposuction needle; M.D. Benedict equation [665.10 + [9.66 × weight in kg] + [1.85 × height in cm] – [4.68 × age in year] for female and 60.47 + [13.75 × weight in kg] + [5.0 × height in cm] – [6.77 × age in years]) for male subjects).

RESULTS

Subject characteristics. Subject characteristics are listed in Table 1. The subjects were men (n = 11) and women (n = 10) with diverse ethnicity: Caucasian (n = 10), African American (n = 10), or Chinese (n = 1). The lean subjects were matched for age with the overweight/obese subjects without diabetes; however, both groups were younger than the overweight/obese subjects with diabetes (P < 0.05). Waist circumference was significantly larger in the overweight/obese versus lean group (P < 0.05). The range of BMI was 20.4–23.8 kg/m2 in the lean group and 28.9–34.7 kg/m2 in the overweight/obese group. By design, the overweight/obese group had greater BMI compared with the lean group (P < 0.05) and greater percent fat (P < 0.05). As expected, overweight/obese subjects had lower insulin sensitivity, as shown by the glucose disposal rates compared with lean subjects (P < 0.05).
O2 could be reduced via several potential mechanisms:
Evidence for rarefaction in overweight/obese AT. AT

<table>
<thead>
<tr>
<th>Lean subjects</th>
<th>Overweight/obese subjects</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>9 (5/4)</td>
<td>12 (6/6)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.6 ± 3.3</td>
<td>38.9 ± 15.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>64.8 ± 7.7</td>
<td>92.0 ± 12.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.1 ± 1.0</td>
<td>31.7 ± 1.9</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>73 ± 4.4</td>
<td>100 ± 10.4</td>
</tr>
<tr>
<td>Percent fat (DEXA)</td>
<td>20.9 ± 7.6</td>
<td>34.2 ± 8.2</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>89 ± 3</td>
<td>110 ± 27</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>4.9 ± 2.0</td>
<td>14.4 ± 9.6</td>
</tr>
</tbody>
</table>

Glucose disposal rate during euglycemic-hyperglycemic clamp (mg/min × kg fat-free mass) | 11.2 ± 3.4 | 6.0 ± 2.2 | <0.05 |

Abdominal subcutaneous AT

| AT pO2 (mmHg) | 55.4 ± 9.1 | 46.8 ± 10.6 | <0.05 |
| AT temperature (°C) | 34.0 ± 1.0 | 32.1 ± 1.4 | <0.05 |

Data are means ± SD. Percent body fat was measured by DEXA and represents mass as the percent of total body weight. The lean group included two African Americans, six Caucasians, and one Asian. The overweight/obese group included eight African Americans and four Caucasians. *\( P < 0.05 \).

**Overweight/obese subjects have lower AT pO2 and AT temperature without activation of hypoxia target genes.** We observed lower AT pO2 in overweight/obese than lean subjects (range 29.1–62.8 vs. 40.5–73.8 mmHg; \( P < 0.05 \)) (Fig. 1A; Table 1). In addition, AT pO2 was negatively correlated with percent fat (\( R = -0.50, P < 0.05 \)) (Fig. 1B) and fat mass (\( R = -0.48, P < 0.05 \)). AT temperature was lower in overweight/obese than lean subjects (\( P < 0.05 \)) (Table 1) and was negatively correlated with percent body fat (\( R = -0.62, P < 0.01 \)) (Fig. 1C).

Race, sex, and age were not significant contributors to AT pO2 or AT temperature (\( P = NS \)). There was no relationship between the use of antihypertensive medication and AT pO2 (\( P = NS \)).

The differences in AT pO2 cannot be explained by differences in AT temperature. A 6°C difference in temperature leads to a 1-mmHg difference in pO2 at atmospheric pressure (supplemental Fig. 3B), a value that is trivial compared with the range of values for AT pO2 (~35 mmHg). Next, we measured the expression of known HIF-1 targets: pyruvate dehydrogenase kinase (PDK) 1 and VEGF. We found that PDK1 and VEGF were not upregulated in overweight/obese subjects (Table 2), suggesting that the decrease in AT pO2 is not sufficient to activate the hypoxia pathway that would increase AT pO2 to normal values.

Within the overweight/obese group, there were six overweight/obese subjects without type 2 diabetes and six overweight/obese subjects with type 2 diabetes. The groups had similar BMI and insulin sensitivity, and we found similar AT pO2 and AT temperature. Data in humans suggests that whole-body hypoxia might cause insulin resistance (7,8); however, the relationship with AT pO2 has not been explored. We found that AT pO2 did not correlate with glucose disposal rate, a gold-standard measure of skeletal muscle insulin resistance (\( R = 0.21, P = NS \)).

**Evidence for rarefaction in overweight/obese AT.** AT pO2 could be reduced via several potential mechanisms: increased demand for oxygen, decreased blood flow due to vasoconstriction, or decreased blood flow due to rarefaction. To explore the latter possibility, we measured capillary density in AT samples collected from the contralateral abdomen. Capillary density in AT was reduced by 44% in overweight/obese subjects compared with lean subjects (172 ± 60 vs. 308 ± 135, \( P < 0.05 \)) (Fig. 2C).

We found that VEGF mRNA was 58% lower in overweight/obese versus lean subjects (\( P < 0.05 \)) (Fig. 2D). Capillary density and VEGF expression were strongly correlated (\( R = 0.81, P < 0.01 \)) (Fig. 2E). AT pO2 was also positively correlated with VEGF mRNA (\( R = 0.54, P < 0.05 \)).

Total body fat (%) was negatively correlated with capillary density (\( R = -0.69, P < 0.01 \)) and VEGF mRNA (\( R = -0.78, P < 0.01 \)) (Fig. 2F). To determine the effect of sex, we used capillary density as the dependent variable and percent fat and sex as the independent variables. The effect of sex on capillary density was not significant (data not shown).

Classically, peroxisome proliferator–activated receptor (PPAR)γ is known as a nuclear hormone receptor that increases adipogenesis and turns on lipogenesis (25). However, recent evidence strongly suggests that PPARγ also drives angiogenesis (26–28). We found that PPARγ was strongly correlated with VEGF (\( R = 0.94, P < 0.01 \)) (Fig. 2G) and capillary density (\( R = 0.72, P < 0.01 \)). Ultimately, PPARγ1 was correlated with AT pO2 (\( R = 0.60, P < 0.05 \)).

Angiopoetin 1 (ANG1) is involved in vascular remodeling and is negatively correlated with the rate of body weight change in animals (4,29). We found that ANG1 negatively correlated with AT pO2 (\( R = -0.57, P < 0.05 \)) and positively with percent fat (\( R = 0.73, P < 0.05 \)). These suggest that overweight/obese subjects with lower AT pO2 have less vascular remodeling compared with lean subjects. Overweight/obese subjects also had a 66% greater expression of collagen VI (collagen VI α3 subunit [COL6]), an extracellular matrix collagen (\( P < 0.05 \), Table 2). Expression of COL6 was positively correlated with percent body fat (\( R = 0.55, P < 0.05 \)) and inversely correlated with AT pO2 (\( R = -0.81, P < 0.01 \)) (Fig. 2H). In addition to increased AT mass, overweight/obese subjects had greater mean adipocyte size compared with lean subjects (0.86 ± 0.2 vs. 0.43 ± 0.13 μl, \( P < 0.05 \)). Mean adipocyte size was negatively correlated with capillary density (\( R = -0.66, P < 0.01 \)) and VEGF (\( R = -0.69, P < 0.01 \)).

**AT pO2 and AT inflammation.** Based on in vitro studies in cultured adipocytes (6,11), we hypothesized that lower AT pO2 might lead to macrophage recruitment and the secretion of inflammatory cytokines. We have previously validated the use of macrophage-associated antigen [MAC2/CD163 and CD68 as markers of AT macrophage infiltration (\( R^2 = 0.77, P < 0.001 \)) (B. Kozak, J. Gimble, S.R. Smith, unpublished data). Both MAC2/CD163 and CD68 were inversely correlated with AT pO2 (Fig. 3A and B; \( R = -0.60, P < 0.05 \) and \( R = -0.51, P = NS \)) consistent with accumulation of macrophages in AT with reduced oxygenation. Furthermore, MIP1α was higher in the overweight/obese subjects (Table 2) and AT pO2 was inversely correlated with MIP1α expression (\( R = -0.41, P = NS \)) (Fig. 3C) and MIP1α secretion in the media (\( R = -0.79, P < 0.05 \)) (Fig. 3D).

There was a trend toward higher monocyte chemotactic protein-1 and IL1α in obesity and negative correlations with AT pO2, but these did not reach statistical significance. However, IL1α secretion was negatively correlated with VEGF mRNA (\( R = -0.70, P < 0.001 \)) and capillary density (\( R = -0.61, P < 0.05 \)).
DISCUSSION

Trayhurn and Wood (30) first hypothesized that hypoxia of AT might play a role in insulin resistance. This hypothesis was based on experiments demonstrating that surrogate markers of hypoxia were increased in the AT of obese animals (30). We found that reduced AT pO$_2$ in overweight/obese subjects and AT pO$_2$ strongly correlates with percent body fat. We also found a significant reduction in AT temperature in overweight/obese subjects and a strong inverse correlation with percent total fat. This is in accordance with previous findings (31) showing a lower skin temperature in obesity. The presence of capillary rarefaction suggests that decreased AT perfusion might also play a role. Indeed, there is evidence to suggest that obese subjects have lower blood flow in abdominal adipose subcutaneous tissue (32). Further work using direct measures of both AT pO$_2$ and direct measures of AT blood flow such as xenon washout are needed to formally address this hypothesis. There is additional evidence that obese mice have decreased oxygenation in epididymal and retroperitoneal AT (6,9) and that weight loss increases oxygenation (6). Choban et al. (33) observed an increased incidence of postsurgical wound infections in obese patients. This same group, measuring AT pO$_2$ in the upper arm, subsequently showed that decreased oxygenation of AT contributes to the increased risk of infection (34). AT mass and adipocyte hypertrophy are closely related to the metabolic complications of obesity (35). In vivo data suggested a role for hypoxia in insulin resistance even though AT pO$_2$ has never been measured in human subjects (7,8). We were unable to demonstrate a correlation between insulin sensitivity and AT pO$_2$. A lack of correlation of AT pO$_2$ and insulin sensitivity may be due to lack of power because this AT pO$_2$ and inflammation are just two of many factors that induce insulin resistance or perhaps because there is simply no meaningful biological relationship. The correlation between macrophage content and insulin resistance is modest (36). One limitation of our study is that only one region of the body was measured and only at one depth.

Most hypoxic tissues develop strong transcriptional, metabolic, and secretory responses to reduced oxygenation in order to increase capillary density and to correct the hypoxia. Hypoxia turns on genes that act to increase oxygen availability by decreasing oxygen consumption (switching “on” anaerobic glycolysis) and stimulating angiogenesis. Hypoxia target genes expressed in AT include PDK1 and VEGF (37). Consistent with this data, VEGF and

FIG. 1. AT pO$_2$ and AT temperature are inversely correlated with percent body fat. AT pO$_2$, measured by direct insertion of a micro Clark-type electrode into abdominal subcutaneous AT, was lower in overweight/obese group (O/O) compared with lean subjects (A) and inversely correlated with percent body fat (B). AT temperature measured by a thermocouple inserted into the abdominal AT was inversely correlated with percent body fat (C). Males are represented by squares and females by circles, filled with different colors as follows: white for lean, gray for O/O without type 2 diabetes, and black for O/O with type 2 diabetes.
PPAR and capillary density in omental and mesenteric AT is needed to test this hypothesis.

Previous studies have shown that COL6 is abundantly expressed by adipocytes (42), and obese mice have increased COL6 expression in the extracellular matrix (43). We found that overweight/obese subjects with low AT pO2 have greater expression of COL6, and COL6 expression increased with increased body fat and fat-cell size. Scherer et al. (44) suggests that proteolytic fragments of COL6 promote tumor growth through prosurvival and proliferation signaling pathways such as Akt and β-catenin. This suggests that new blood vessel formation is restricted by increased extracellular matrix or that a reduction in angiogenesis leads to increases in the formation of the extracellular matrix as exemplified by COL6. Further work is needed to separate these two possibilities.

AT inflammation has received much attention as an important factor in insulin resistance and type 2 diabetes (6,12,45). Previous in vitro and in vivo preclinical studies showed that hypoxia induces inflammation that might contribute to insulin resistance (6,11). We found that in humans, AT pO2 correlates with macrophage markers (CD68 and MAC2/CD163). In addition, AT secretion of MIP1α, a potent macrophage chemokine (46,47), increased as AT pO2 decreased. This is consistent with recent data showing upregulation of MIP1α in obesity (48). This is supportive of the hypothesis that lower oxygenation drives inflammation by upregulating adipocyte chemokine secretion but, as discussed previously, not by activating the classic hypoxia pathway and VEGF. However, it is possible that inflammation could drive hypoxia. Given that MIP1α has been implicated in angiogenesis, it is unclear why MIP1α is up when capillary density is down. Further work is needed to understand the factors regulating angiogenesis in human AT.

In summary, we provide direct evidence of lower AT pO2 in overweight/obese subjects and that the most likely causes are decreased capillary density and reduced expression of the angiogenic factors like VEGF and PPARγ1 and increased expression of COL6. This suggests that low AT pO2 in overweight/obese subjects does not result in a

**TABLE 2**

<table>
<thead>
<tr>
<th>Genotype (AU)</th>
<th>Lean</th>
<th>Overweight/obese</th>
<th>P</th>
<th>Correlation with</th>
<th>percent fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td>r</td>
</tr>
<tr>
<td>Leptin mRNA</td>
<td>0.19 ± 0.11</td>
<td>0.57 ± 0.12</td>
<td>&lt;0.05</td>
<td>0.74</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PDK1 mRNA</td>
<td>0.63 ± 0.24</td>
<td>0.45 ± 0.21</td>
<td>&lt;0.05</td>
<td>-0.10</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VEGF mRNA</td>
<td>2.46 ± 1.11</td>
<td>1.04 ± 0.34</td>
<td>&lt;0.05</td>
<td>-0.78</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ANG1 mRNA</td>
<td>0.41 ± 0.13</td>
<td>0.69 ± 0.11</td>
<td>&lt;0.05</td>
<td>0.73</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PPARG1 mRNA</td>
<td>1.33 ± 0.36</td>
<td>0.74 ± 0.21</td>
<td>&lt;0.05</td>
<td>-0.73</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>COL6 mRNA</td>
<td>0.32 ± 0.17</td>
<td>0.53 ± 0.16</td>
<td>&lt;0.05</td>
<td>0.55</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CD68 antigen mRNA</td>
<td>0.23 ± 0.05</td>
<td>0.62 ± 0.27</td>
<td>&lt;0.05</td>
<td>0.64</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MAC2/CD163 mRNA</td>
<td>0.56 ± 0.22</td>
<td>1.49 ± 0.63</td>
<td>&lt;0.05</td>
<td>0.72</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MIP1α mRNA</td>
<td>0.02 ± 0.01</td>
<td>0.21 ± 0.18</td>
<td>&lt;0.05</td>
<td>0.60</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCP1 mRNA</td>
<td>0.12 ± 0.05</td>
<td>0.25 ± 0.11</td>
<td>&lt;0.05</td>
<td>0.43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cytokine release (pg/mg tissue × h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>MIP1α</td>
<td>0.44 ± 0.38</td>
<td>0.96 ± 0.69</td>
<td>&lt;0.05</td>
<td>0.60</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MCP1</td>
<td>2.26 ± 0.83</td>
<td>2.36 ± 1.18</td>
<td>&lt;0.05</td>
<td>0.67</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL1α</td>
<td>0.09 ± 0.08</td>
<td>0.20 ± 0.07</td>
<td>&lt;0.05</td>
<td>0.12</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are means ± SD. MCP1, macrophage chemotractant protein 1.

PDK1 are upregulated in adipocytes cultured in 1% oxygen (a hypoxic environment) (6,9,11). In contrast to our expectations, we found that lower AT pO2 in overweight/obese subjects did not induce an increase in hypoxia targets (PDK1, VEGF). Also, capillary density and VEGF were decreased in overweight/obese AT along with a lower AT pO2. This suggests that the transcriptional counterregulatory system was not activated. One should note, however, that the lowest value of AT pO2 in the overweight/obese group was 29 mmHg; this corresponds to 3.8% oxygen compared with the 1% oxygen used in the cell culture experiments. This suggests that overweight/obese subjects have low AT pO2 but not low enough to mount a counterregulatory response driven during a response to hypoxia. Consistent to our results, Lijnen et al. (5) found that obese mice have both lower VEGF and lower vascular density in AT. In addition, it is known that spleen, thymus, and retina have low pO2 in normal rats (38), suggesting that angiogenesis is activated at different levels of oxygenation for different types of tissue or that oxygenation could influence angiogenesis. One limitation of our study is that we did not measure VEGF protein, and this should be addressed in future experiments.

Recent data suggests that PPARG1 might be required for angiogenesis in AT (39). PPARGγ drives VEGF (and angiogenesis) (26–28). We found a strong positive correlation between PPARG1 and VEGF and between VEGF and AT pO2. One way to interpret this data is that PPARG1 drives angiogenesis in human AT and therefore is a key controller of AT pO2. More work is needed to test this hypothesis.

AT expansion (adipogenesis) during development is preceded by a wave of neovascularization (40). Vascular plasticity may play a role in the ability of AT to increase or decrease in size (29,41). Our data suggests that reduced capillary density might restrict the growth of AT. Our finding of reduced capillary density in subcutaneous AT in obesity suggests the hypothesis that the failure of the vasculature to expand with increasing subcutaneous obesity might limit adipogenesis in subcutaneous depot. If visceral AT were not similarly restricted, this might allow for the growth of visceral AT. Further work measuring AT pO2 and capillary density in omental and mesenteric AT is needed to test this hypothesis.
FIG. 2. Vascularization of AT. Representative AT sections from lean (A) and O/O (B) subjects stained with UEA lectin (orange) to label capillaries and with GS lectin (green) to label the adipocyte plasmalemma. Capillary density (C) was measured and averaged across 6–10 histological sections for each subject and VEGF mRNA expression measured by quantitative RT-PCR (D); both were lower in O/O versus lean subjects. VEGF mRNA was positively correlated with capillary density (E) and PPARγ1 mRNA (G) and inversely with percent body fat (F). H: Collagen VI (COL6) mRNA was negatively correlated with AT pO2.

Males are represented by squares and females by circles, filled with different colors as follows: white for lean, gray for O/O without type 2 diabetes, and black for O/O with type 2 diabetes. (Please see http://dx.doi.org/10.2337/db08-1098 for a high-quality digital representation of this figure.)
complete counterregulatory response to reduced AT \( \text{pO}_2 \) and that neovascularization is not activated. Decreased AT \( \text{pO}_2 \) was paralleled by an increase in the expression and secretion of the chemokine and markers of macrophage infiltration. These data suggest that proangiogenic therapies might reduce AT inflammation, improve insulin action, and reduce cardiovascular disease risk in obesity and type 2 diabetes.

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