

Vascular Medicine

Increased Adipose Tissue Oxygen Tension in Obese Compared With Lean Men Is Accompanied by Insulin Resistance, Impaired Adipose Tissue Capillarization, and Inflammation

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Background—Adipose tissue (AT) dysfunction in obesity contributes to chronic, low-grade inflammation that predisposes to type 2 diabetes mellitus and cardiovascular disease. Recent in vitro studies suggest that AT hypoxia may induce inflammation. We hypothesized that adipose tissue blood flow (ATBF) regulates AT oxygen partial pressure (AT PO_2), thereby affecting AT inflammation and insulin sensitivity.

Methods and Results—We developed an optochemical measurement system for continuous monitoring of AT PO_2 using microdialysis. The effect of alterations in ATBF on AT PO_2 was investigated in lean and obese subjects with both pharmacological and physiological approaches to manipulate ATBF. Local administration of angiotensin II (vasoconstrictor) in abdominal subcutaneous AT decreased ATBF and AT PO_2 , whereas infusion of isoprenaline (vasodilator) evoked opposite effects. Ingestion of a glucose drink increased ATBF and AT PO_2 in lean subjects, but these responses were blunted in obese individuals. However, AT PO_2 was higher (hyperoxia) in obese subjects despite lower ATBF, which appears to be explained by lower AT oxygen consumption. This was accompanied by insulin resistance, lower AT capillarization, lower AT expression of genes encoding proteins involved in mitochondrial biogenesis and function, and higher AT gene expression of macrophage infiltration and inflammatory markers.

Conclusions—Our findings establish ATBF as an important regulator of AT PO_2 . Nevertheless, obese individuals exhibit AT hyperoxia despite lower ATBF, which seems to be explained by lower AT oxygen consumption. This is accompanied by insulin resistance, impaired AT capillarization, and higher AT gene expression of inflammatory cell markers.

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Key Words: blood flow ■ capillaries ■ hypoxia ■ inflammation ■ obesity

Converging data suggest that an impaired function of adipose tissue (AT) plays a crucial role in obesity-related complications.¹ In fact, AT inflammation, which is an important hallmark of AT dysfunction, may underlie type 2 diabetes mellitus, fatty liver disease, and cardiovascular disease.¹⁻⁵ Several pathways may trigger AT inflammation, including endoplasmic reticulum stress,⁶ activation of reactive oxygen species,⁷ and Toll-like receptor 4.⁸ However, the inciting event causing the metabolic and endocrine derangements in AT of obese individuals remains to be established.

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Recent cell culture experiments suggest that AT hypoxia may represent a unified cellular mechanism underlying AT

dysfunction in obesity. In mice, obesity is associated with lower interstitial oxygen partial pressure (PO_2) in AT.^{9,10} Exposure of 3T3-L1 adipocytes to hypoxia (1% O_2) adversely affected the expression of adipokines, with a shift toward a proinflammatory phenotype.^{10,11} In humans, PO_2 was lower in subcutaneous AT of the lateral upper arm in obese compared with nonobese patients during surgery.¹² Furthermore, abdominal subcutaneous AT PO_2 was slightly lower in overweight/obese compared with lean subjects, although values showed considerable overlap.¹³ Noteworthy, commercially available needle-type electrodes were used in these studies.^{9,10,12,13}

Oxygen supply may be an important determinant of AT PO_2 . Although AT development and vascularization seem to be closely linked,^{14,15} it has been proposed that the expansion

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of AT mass during the progressive development of obesity may lead to a relative oxygen deficit in certain parts of AT because angiogenesis is insufficient to maintain normoxia in the entire AT depot.¹⁶ It could be anticipated that impairments in both the structural and functional properties of the AT vasculature may result in lower oxygen supply due to decreased adipose tissue blood flow (ATBF). In fact, we and others have demonstrated previously that both fasting ATBF and the postprandial increase in ATBF^{17,18} are decreased in obese, insulin resistant, and type 2 diabetic subjects.^{19–22} Intriguingly, a decreased ATBF response to nutrient intake is closely associated with insulin resistance,^{21,22} suggesting that ATBF is of great importance in the regulation of metabolism.

In the present study, we investigated the effect of acute changes in ATBF on AT PO_2 in lean and obese subjects, using both pharmacological and physiological approaches to manipulate ATBF, and examined insulin sensitivity, *in vivo* AT oxygen consumption, AT capillarization, and AT gene expression of mitochondrial function, chemoattraction, macrophage infiltration, and inflammatory markers. To accomplish this goal, we developed an optochemical measurement system for the continuous monitoring of AT PO_2 using microdialysis. Our studies demonstrate that ATBF is an important regulator of AT PO_2 in humans. Nevertheless, obese subjects exhibit higher AT PO_2 despite lower ATBF, which seems to be explained by lower AT oxygen consumption. AT hyperoxia in obesity is accompanied by insulin resistance, lower AT capillarization, lower AT expression of mitochondrial function markers, and higher AT gene expression of inflammatory cell markers.

Methods

Subjects

Ten lean (body mass index <25 kg/m²) male subjects with normal glucose tolerance (plasma glucose <6.1 mmol/L) and 10 obese (body mass index >30 kg/m²) male subjects with impaired glucose tolerance (2-hour plasma glucose 7.8 to 11.1 mmol/L) participated in this study. Body composition was determined by underwater weighing.²³ Subjects had to have a stable weight for at least 3 months before participation. Exclusion criteria were smoking, diabetes mellitus, cardiovascular disease, cancer, lung disease, intentions to lose weight, alcohol/drug abuse, use of antioxidants, and use of medication known to affect glucose metabolism, body weight, or inflammation. The Medical-Ethical Committee of Maastricht University Medical Centre approved the study protocol, and all subjects gave written informed consent before participation.

Protocol

Subjects were asked to refrain from drinking alcohol and to perform no exercise for a period of 48 hours before the study. Subjects came to the laboratory by car or bus in the morning after an overnight fast on several occasions. All procedures were randomized and completed within 4 weeks after inclusion, with at least 1 week between measurements. For 2 experiments, a microdialysis catheter was inserted into abdominal subcutaneous AT for the measurement of AT PO_2 .

Continuous Monitoring of Abdominal Subcutaneous AT PO_2 In Vivo in Humans

We have developed, optimized, and validated an optochemical measurement system for the continuous monitoring of AT PO_2 in vivo in humans using microdialysis. The measurement system has been tested extensively *in vitro* and *in vivo* in pigs²⁴ and was miniaturized with improved production reproducibility of the disposable O_2 sensors. AT PO_2 can be measured in the range of 0 to

300 mm Hg with high resolution and accuracy (1 mm Hg). The system consists of a microdialysis catheter for the extraction of interstitial fluid, a miniaturized flow-through cell containing a planar O_2 -sensitive membrane, and an optoelectronic measuring unit, which is connected to a computer for data collection (continuous 27-second readings). An important advantage of this system is that AT PO_2 is measured over a larger AT area compared with commercially available needle-type oxygen sensors. Second, this system allows local administration of agents. To prevent oxygen diffusion with the use of commercially available microdialysis catheters, the outlet of the microdialysis catheter was replaced by Steel tubing (9 cm, outer/inner diameter 0.3/0.15 mm; Rometsch GmbH, Germany), which directed the microdialysate toward the flow-through cell containing the O_2 sensor. The oxygen sensors were prepared as described previously.²⁴

Pharmacological Manipulation of Adipose Tissue Blood Flow

To assess whether alterations in ATBF evoke changes in AT PO_2 , ATBF was manipulated by local administration of the vasoconstrictor angiotensin II (Ang II) (10^{-5} mol/L) and the vasodilator isoprenaline (10^{-5} mol/L) into abdominal subcutaneous AT ($n=6$). We have demonstrated previously that these dosages of Ang II and isoprenaline markedly affect ATBF.^{18,23,25}

On arrival, a microdialysis catheter (CMA 60, CMA Microdialysis AB, Stockholm, Sweden) was inserted in the abdominal subcutaneous AT under sterile conditions 6 to 8 cm right from the umbilicus. One hour before insertion of the probe, the skin was anesthetized by means of EMLA cream (AstraZeneca BV, Zoetermeer, Netherlands). After insertion, the probe was perfused with Ringer solution (Baxter BV, Utrecht, Netherlands), supplemented with 50 mmol/L ethanol, at a flow rate of 2.0 μ L/min (CMA400 microinfusion pump, CMA Microdialysis AB, Stockholm, Sweden).

Two to 3 hours after insertion of the probe, AT PO_2 had reached stable values (change in AT PO_2 <2 mm Hg within a 30-minute period). The probe was consecutively perfused with Ang II (at time 0 to 60 minutes) and isoprenaline (at time 180 to 240 minutes), each for 60 minutes. There was a washout period of 120 minutes (Ringer infusion) before isoprenaline administration was started. Throughout the experiment, AT PO_2 was measured continuously, and microdialysate was collected (30-minute fractions). Ethanol concentrations were determined in the ingoing and outgoing perfusion solvent to assess the ethanol outflow/inflow ratio as an indicator of local blood flow.^{23,25} Arterialized blood samples were obtained at 0, 60, 180, and 240 minutes.

Physiological Manipulation of Adipose Tissue Blood Flow

Baseline AT PO_2 was measured and the physiological importance of postprandial alterations in ATBF on AT PO_2 was examined in lean subjects with normal glucose tolerance ($n=9$) and obese subjects with impaired glucose tolerance ($n=9$). Therefore, ATBF and AT PO_2 were measured continuously under fasting conditions and for 2 hours after ingestion of an oral glucose load (75 g of glucose dissolved in 200 mL of water). ATBF was measured at the contralateral side of the abdomen, at the same level where the microdialysis probe was inserted, with the use of ¹³³Xe washout.^{19,26} The reason for this is that we and others have shown that ATBF is greater at the upper compared with the lower level of the abdomen, but is not different between the right and left sides at either level.^{17,18} Postprandial measurements were not available for 2 lean subjects because of technical problems.

Hyperinsulinemic-Euglycemic Clamp

A hyperinsulinemic-euglycemic clamp was performed to assess insulin sensitivity.²⁷ The mean glucose infusion rate during steady state (last 30 minutes) was used to assess insulin sensitivity.

Adipose Tissue Biopsy

Abdominal subcutaneous AT biopsies (≈ 1 g) were collected 6 to 8 cm lateral from the umbilicus under local anesthesia (2% lidocaine) by needle biopsy after an overnight fast in lean ($n=9$) and obese

($n=10$) subjects before the start of the hyperinsulinemic-euglycemic clamp. After immediate washing with saline, a small part of AT was fixed overnight in 4% paraformaldehyde and embedded in paraffin, whereas the other part was snap-frozen in liquid nitrogen and stored at -80°C until analysis.

Adipocyte Size

Histological sections ($8\text{ }\mu\text{m}$) were cut from paraffin-embedded tissue, mounted on microscope glass slides, and dried overnight in an incubator at 37°C . The sections were stained with hematoxylin and eosin. Digital images were captured with the use of a Leica DFC320 digital camera (Leica, Rijswijk, Netherlands) at $\times 20$ magnification (Leica DM3000 microscope, Leica, Rijswijk, Netherlands). Computerized morphometric analysis (Leica QWin V3, Cambridge, United Kingdom) of individual adipocytes was performed in a blinded fashion. Approximately 400 adipocytes per sample were measured.

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from adipose tissue ($\approx 500\text{ mg}$) with the use of Trizol chloroform extraction (Invitrogen, Cergy Pontoise, France). SYBR-Green–based real-time polymerase chain reactions were performed as 1-step reactions on the StepOne real-time polymerase chain reaction system (Applied Biosystems, Foster City, CA). Results were normalized to a housekeeping gene (18S ribosomal RNA).

Adipose Tissue Capillarization

Paraffin sections ($5\text{ }\mu\text{m}$) were stained with the primary antibodies CD31/CD34. The CD34 antibody (Thermo Scientific, Neomarkers, Clone QBE10/10) was diluted 1:500 in ready-to-use CD31 antibody (Dako, FLEX, Monoclonal Mouse Anti-Human CD31, Clone JC70A). Slides were incubated with peroxidase-conjugated secondary antibodies (FLEX/HRP, DAKO). Peroxidase activity was visualized with 3'-diaminobenzidine HCl, and sections were counterstained with hematoxylin and eosin. Digital images (10 images per sample) were captured at $\times 10$ magnification, and computerized morphometric analysis was performed to assess capillary density, the number of capillaries per adipocyte, and the area of supply (diffusion distance).

Biochemical Analyses

Blood samples were collected into ice-chilled EDTA tubes and centrifuged at $1000g$ at 4°C for 10 minutes. Plasma was immediately frozen in liquid nitrogen and stored at -80°C until analysis. Plasma glucose (ABX Pentra Glucose HK CP, Radiometer, Copenhagen, Denmark) and free fatty acids (Wako NEFA C kit, Wako Chemicals, Neuss, Germany) were measured with standard enzymatic methods. Plasma insulin was measured by a radioimmunoassay for human insulin (Linco Research Inc, St Charles, MO). Ethanol concentration in dialysate was measured as described previously.²³

Calculations

Baseline AT PO_2 was calculated by averaging the last 30 minutes before the start of local administration of vasoactive agents (pharmacological experiment) or ingestion of the glucose drink (physiological experiment). The effects of Ang II and isoprenaline on AT PO_2 were analyzed by averaging the last 10 minutes of each 60-minute infusion period (at time 50 to 60 and 230 to 240 minutes, respectively), when a steady state was reached, after subtraction of AT PO_2 at baseline (Ang II), or at the end of the washout period (at time 165 to 180 minutes) (isoprenaline). The effect of glucose intake on AT PO_2 was assessed by peak AT PO_2 values, calculated as the mean of 21 consecutive PO_2 recordings with the maximum in the middle, adjusted for baseline AT PO_2 . Quantitative values of ATBF were calculated as described previously.¹⁹ AT oxygen consumption in vivo was calculated by measuring arteriovenous differences of oxygen saturation and hemoglobin across abdominal subcutaneous AT in combination with ATBF, assuming an oxygen-binding capacity of hemoglobin of 1.34 mL O_2 per gram of hemoglobin.²⁸

Statistical Analysis

Data are presented as mean $\pm$ SEM. Comparisons between groups were made with the Student unpaired t test. Ang II and isoprenaline

Table 1. Clinical Characteristics of the Study Population

	Lean	Obese
n	10	10
Age, y	55.8 $\pm$ 4.1	59.6 $\pm$ 3.1
Weight, kg	76.3 $\pm$ 2.7	106.9 $\pm$ 4.9†
Body mass index, kg/m ²	23.4 $\pm$ 0.3	34.2 $\pm$ 1.3†
Body fat, %	22.4 $\pm$ 1.8	33.6 $\pm$ 1.6†
Waist, cm	89.9 $\pm$ 1.4	118.3 $\pm$ 3.2†
Waist/hip ratio	0.95 $\pm$ 0.01	1.07 $\pm$ 0.01†
Systolic blood pressure, mm Hg	116.1 $\pm$ 6.6	144.6 $\pm$ 5.7†
Diastolic blood pressure, mm Hg	72.1 $\pm$ 3.8	82.5 $\pm$ 1.9*
Fasting plasma glucose, mmol/L	5.3 $\pm$ 0.1	5.9 $\pm$ 0.2*
2-h plasma glucose, mmol/L	4.8 $\pm$ 0.4	8.4 $\pm$ 0.4‡
Fasting insulin, mU/L	12.2 $\pm$ 0.9	22.0 $\pm$ 2.2†
GIR, $\mu\text{mol} \cdot \text{kg body wt}^{-1} \cdot \text{min}^{-1}$	38.5 $\pm$ 2.7	14.3 $\pm$ 2.5‡

Values are mean $\pm$ SEM. GIR indicates glucose infusion rate (hyperinsulinemic-euglycemic clamp).

* $P<0.05$, † $P<0.01$, ‡ $P<0.001$ vs lean.

effects were assessed with the Student paired t test. Relationships between clinical parameters were analyzed with the Pearson correlation coefficient, whereas associations with gene expression data were analyzed with the Spearman correlation coefficient. Calculations were performed with SPSS 15.0 for Windows (Chicago, IL). $P<0.05$ was considered statistically significant.

Results

Characteristics of the subjects are summarized in Table 1. Obese subjects with impaired glucose tolerance had a higher body weight, body mass index, body fat percentage, waist/hip ratio, blood pressure, fasting and 2-hour glucose concentration, and fasting insulin concentration, whereas insulin sensitivity was markedly lower compared with lean controls with normal glucose tolerance.

Pharmacological Manipulation of Adipose Tissue Blood Flow Induces Concomitant Changes in Adipose Tissue PO_2

We hypothesized that if ATBF controls AT PO_2 , pharmacological manipulation of ATBF would acutely evoke changes in AT PO_2 . As expected, local administration of the vasoconstrictor Ang II into abdominal subcutaneous AT of lean subjects increased the ethanol out/in ratio (baseline: 0.42 ± 0.06 versus Ang II: 0.51 ± 0.08 ; $P=0.050$), indicating that ATBF was decreased.²³ Furthermore, ATBF increased during local administration of the vasodilator isoprenaline, which was reflected by a decrease in the ethanol out/in ratio (washout: 0.40 ± 0.07 versus isoprenaline: 0.23 ± 0.05 ; $P=0.007$).

Manipulation of ATBF by Ang II markedly decreased local AT PO_2 ($-35.7 \pm 2.8\%$; $P<0.001$), reaching a steady state after ≈ 50 minutes of infusion (Figure 1). At the end of the washout period (at time 165 to 180 minutes), AT PO_2 had returned to baseline values (washout: 56.5 ± 1.5 versus baseline: $59.6 \pm 2.6\text{ mm Hg}$; $P=0.14$). Subsequently, isoprenaline administration induced a pronounced increase in AT PO_2 ($25.4 \pm 5.7\%$; $P<0.001$). Fasting arterialized oxygen tension ($75.3 \pm 1.5\text{ mm Hg}$), plasma glucose ($5.3 \pm 0.2\text{ mmol/L}$), and free fatty acid ($570 \pm 89\text{ }\mu\text{mol/L}$) concentrations were stable during the experiment, indicating that observed effects on

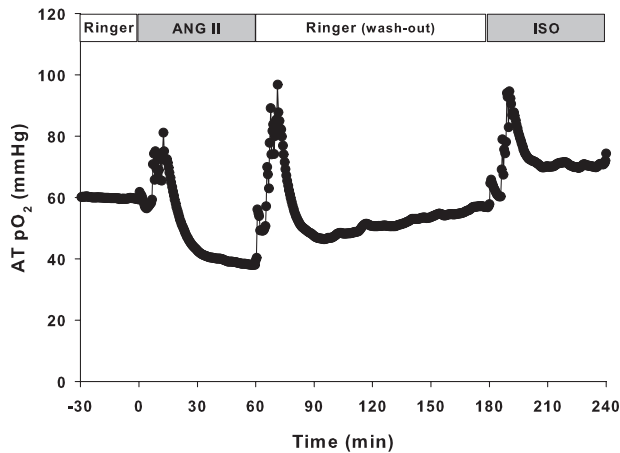


Figure 1. Fasting adipose tissue (AT) oxygen partial pressure (P_{O_2}) under stable baseline conditions and during local administration of the vasoconstrictor angiotensin II (ANG II) (10^{-5} mol/L) and vasodilator isoprenaline (ISO) (10^{-5} mol/L) into abdominal subcutaneous AT in lean subjects ($n=6$). Local angiotensin II administration markedly decreased ($-35.7\pm 2.8\%$; $P<0.001$), whereas isoprenaline administration increased ($25.4\pm 5.7\%$; $P<0.001$) AT P_{O_2} . The steep increase in AT P_{O_2} at the beginning of each infusion period is caused by environmental oxygen, which was introduced into the system during the change of infusion solution.

ATBF and AT P_{O_2} cannot be attributed to systemic effects. Thus, we demonstrated that alterations in ATBF acutely affect AT P_{O_2} in humans.

Adipose Tissue Hyperoxia and Blunted Postprandial Increase in Adipose Tissue Blood Flow and Adipose Tissue P_{O_2} in Obese Subjects

To determine whether physiological changes in ATBF also evoke alterations in AT P_{O_2} , we administered an oral glucose load (75 g of glucose dissolved in 200 mL of water) to lean and obese subjects. We and others have demonstrated previously that glucose ingestion increases ATBF in lean but not obese insulin resistant individuals.^{17,18,22} Fasting ATBF was significantly higher in lean compared with obese subjects (2.5 ± 0.5 versus 1.4 ± 0.2 mL $\cdot$ 100 g tissue $^{-1} \cdot$ min $^{-1}$, respectively; $P=0.029$). As anticipated, ATBF was markedly increased after ingestion of the glucose drink in lean subjects (baseline: 2.5 ± 0.5 versus postprandial: 4.6 ± 1.1 mL $\cdot$ 100 g tissue $^{-1} \cdot$ min $^{-1}$; $P=0.027$), but the postprandial increase in ATBF was completely blunted in obese subjects (baseline: 1.4 ± 0.2 versus postprandial: 1.7 ± 0.1 mL $\cdot$ 100 g tissue $^{-1} \cdot$ min $^{-1}$; $P=0.146$) (Figure 2A). This is further illustrated by the higher incremental area under the curve for ATBF in lean subjects ($P=0.034$) (Figure 2B).

Surprisingly, despite lower ATBF in obesity, fasting AT P_{O_2} was higher in obese compared with lean subjects (67.4 ± 3.7 versus 44.7 ± 5.8 mm Hg, respectively; $P=0.005$) (Figure 3A). In accordance with postprandial changes in ATBF, AT P_{O_2} significantly increased after the ingestion of glucose in lean subjects, whereas this response was blunted in obese individuals ($12.4\pm 3.5\%$ versus $3.7\pm 0.8\%$, respectively; $P=0.047$) (Figure 3B and 3C). It is noteworthy that the postprandial increase in ATBF preceded the rise in AT P_{O_2} , which is explained by the measurement delay due to transfer of the interstitial fluid toward the oxygen sensor.

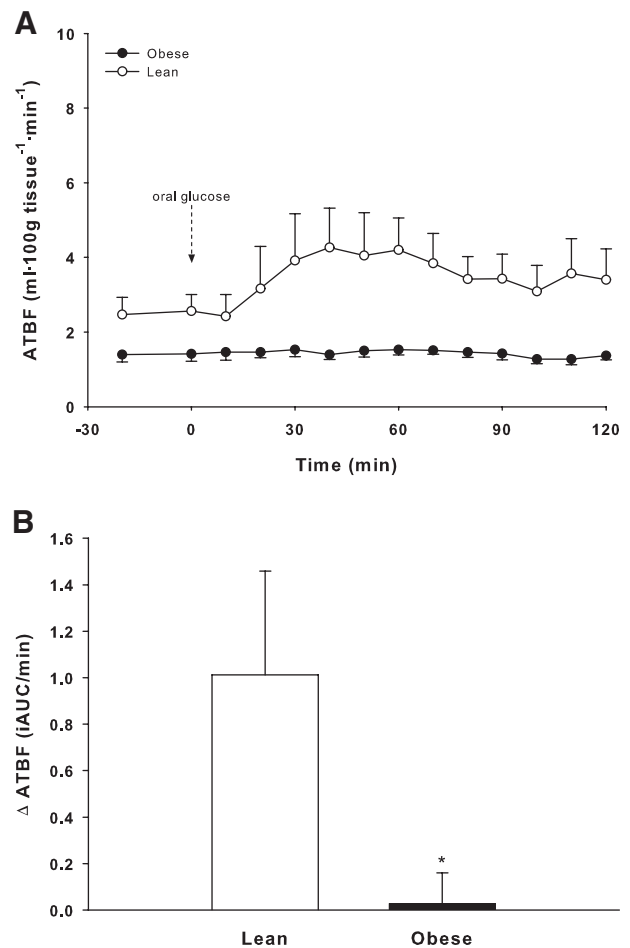


Figure 2. A, Fasting and postprandial adipose tissue blood flow (ATBF) in lean ($n=7$) and obese ($n=9$) subjects. Oral glucose (75 g) was ingested at 0 minutes to stimulate ATBF. B, The postprandial increase in ATBF (0 to 120 minutes) was blunted in obese compared with lean subjects. Values are mean $\pm$ SEM. * $P<0.05$ vs lean. iAUC indicates incremental area under the curve.

A strong positive correlation was found between both the postprandial increase in ATBF and AT P_{O_2} ($r=0.919$, $P<0.001$) (Figure 4A) and the postprandial incremental area under the curve for ATBF and incremental area under the curve for P_{O_2} ($r=0.725$, $P=0.001$). The postprandial increases in ATBF ($r=0.621$, $P=0.010$) and AT P_{O_2} ($r=0.709$, $P=0.002$) were positively associated with insulin sensitivity (Figure 4B and 4C). To summarize, a physiological increase in ATBF is associated with a concomitant increase in AT P_{O_2} in lean subjects, but these responses are blunted in obese individuals.

Lower Expression of Mitochondrial Function Markers in Obese Adipose Tissue

The finding of AT hyperoxia in obese subjects, despite lower ATBF, was unexpected. To assess whether lower AT oxygen consumption in obese subjects may underlie this observation, we first evaluated AT expression of mitochondrial function markers. Both peroxisome proliferator-activated receptor gamma coactivator-1 α (PGC-1 α), the main regulator of mitochondrial biogenesis and mitochondrial oxygen consumption,²⁹ and citrate synthase showed a lower AT mRNA expression in obese compared with lean subjects and were

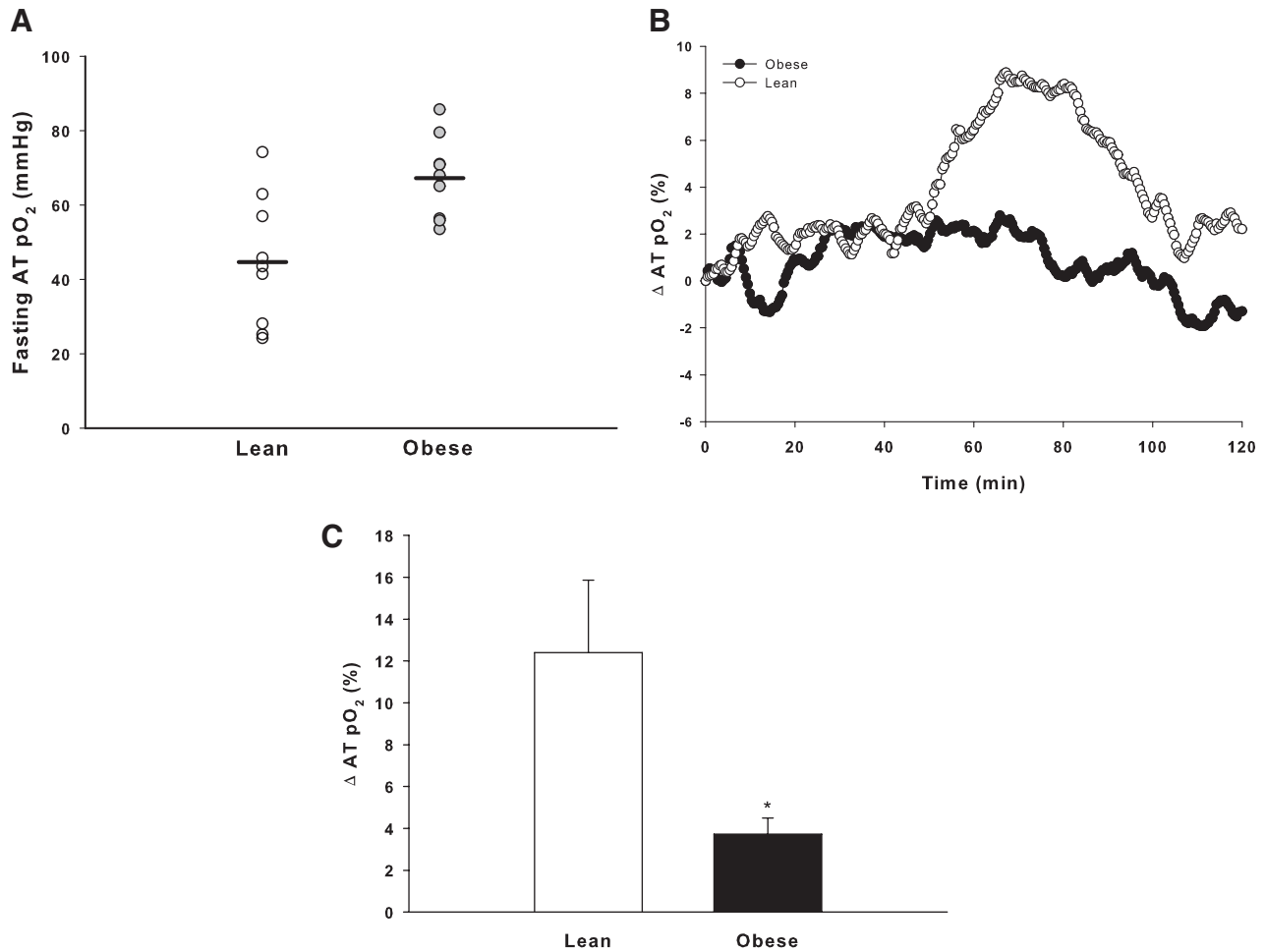


Figure 3. **A**, Fasting adipose tissue (AT) oxygen partial pressure (P_{O_2}) was significantly elevated in obese ($n=9$) compared with lean ($n=9$) subjects ($P=0.005$). **B**, The postprandial increase in AT P_{O_2} relative to baseline (%) after ingestion of an oral glucose load (75 g) in lean ($n=7$) and obese ($n=9$) subjects. **C**, The postprandial peak AT P_{O_2} relative to baseline was significantly lower in obese subjects. Values are mean $\pm$ SEM. * $P < 0.05$ vs lean.

inversely associated with body fat percentage and insulin sensitivity (Table 2). Furthermore, citrate synthase mRNA expression in AT was negatively associated with fasting AT P_{O_2} ($r = -0.529$, $P = 0.043$), suggesting that lower mitochondrial oxygen consumption may contribute to AT hyperoxia in obesity.

Adipose Tissue Oxygen Consumption In Vivo in Obese Individuals

To provide further evidence that AT oxygen consumption may be lower in obese subjects, we measured in vivo oxygen consumption across abdominal subcutaneous AT, using arteriovenous oxygen concentration differences in combination with ATBF, in lean and obese subjects with age, body mass index, body fat percentage, and glucose homeostasis²⁰ comparable to those of the participants of the present studies. Oxygen consumption was lower in abdominal subcutaneous AT of obese compared with lean subjects (0.67 ± 0.26 versus 1.78 ± 0.41 $\mu\text{mol} \cdot 100 \text{ g tissue}^{-1} \cdot \text{min}^{-1}$; $P = 0.044$).

AT Hyperoxia in Obesity Is Paralleled by Impaired Adipose Tissue Capillarization

Previous studies have shown that both high expression of PGC-1 α and low oxygen availability stimulate angiogenesis

in several cell lines, including differentiated 3T3-F442A adipocytes, and in skeletal muscle.^{30–32} Because we found lower AT expression of PGC-1 α and citrate synthase and higher AT P_{O_2} in obese AT, we hypothesized that angiogenesis and capillarization in AT may be lower in obese subjects. To test this hypothesis, we first examined AT expression of vascular endothelial growth factor (VEGF), the master regulator of vasculogenesis, angiogenesis, and remodeling of blood vessels.^{33,34} VEGF mRNA expression was significantly lower in AT of obese compared with lean subjects and was inversely associated with body fat percentage (Table 2). Both PGC-1 α ($r = 0.821$, $P < 0.001$) and citrate synthase ($r = 0.777$, $P < 0.001$) mRNA expression in AT were strongly associated with AT VEGF expression, which is in accordance with recent findings.^{30,32} In addition, PGC-1 α mRNA was positively correlated with fasting ($r = 0.600$, $P = 0.018$) and postprandial ATBF ($r = 0.650$, $P = 0.009$).

To assess whether AT capillarization was lower in obese subjects, we stained paraffin sections from abdominal subcutaneous AT with CD31/CD34 antibodies (Figure 5A and 5B). AT capillary density was inversely associated with adipocyte size ($r = -0.592$, $P = 0.008$) and body fat percentage ($r = -0.445$, $P = 0.056$) (Figure 5C and 5D, respectively),

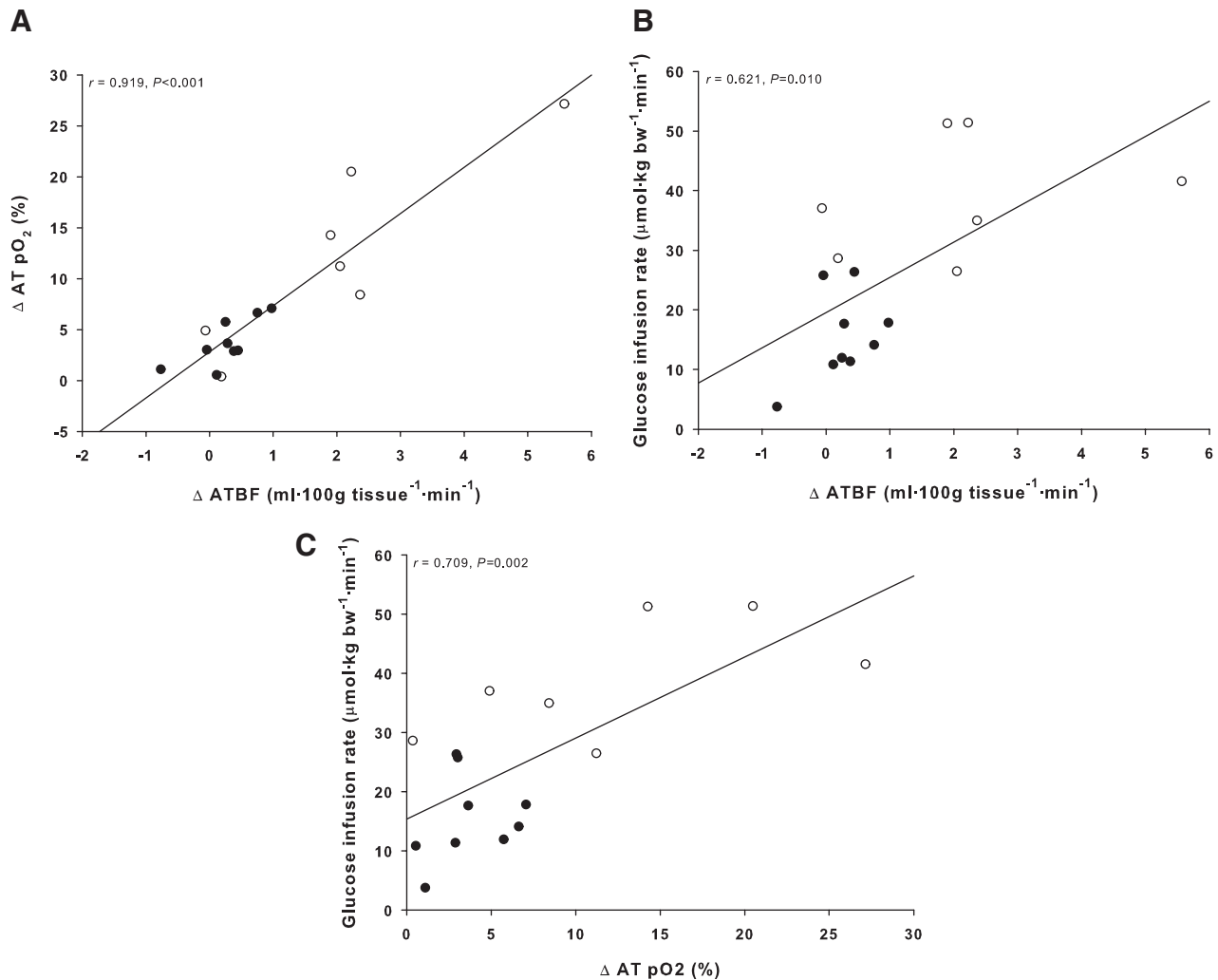


Figure 4. Correlation between postprandial adipose tissue blood flow (ATBF), adipose tissue (AT) oxygen partial pressure (PO_2), and insulin sensitivity in lean (open circles) and obese (closed circles) subjects ($n=16$). **A**, The postprandial increase in ATBF was associated with postprandial changes in AT PO_2 ($r=0.919$, $P<0.001$). Both the postprandial increase in ATBF ($r=0.621$, $P=0.010$) (**B**) and AT PO_2 ($r=0.709$, $P=0.002$) (**C**) were associated with insulin sensitivity.

whereas the mean area of supply (diffusion distance) was positively related to these parameters ($r=0.657$, $P=0.002$ and $r=0.495$, $P=0.031$, respectively). No significant differences in the number of capillaries per adipocyte were found between lean and obese subjects (1.77 ± 0.14 versus 1.89 ± 0.09 capillaries per adipocyte, respectively; $P=0.450$). VEGF mRNA expression and capillary density were negatively associated with several inflammatory cell markers in AT (data not shown). These data indicate that AT hyperoxia in obesity is accompanied by lower AT capillarization.

Adipose Tissue Hyperoxia in Obesity Is Associated With Insulin Resistance and Markers of Adipose Tissue Chemoattraction, Macrophage Infiltration, and Inflammation

Next we investigated whether AT hyperoxia is associated with insulin sensitivity and markers of AT dysfunction in obesity. Mean adipocyte size was significantly higher in obese compared with lean subjects (Figure 6A), with a shift toward a higher frequency of large adipocytes in the obese (Figure 6B). As expected, gene expressions of AT chemoat-

traction, macrophage infiltration, and inflammatory markers were higher in obese compared with lean subjects, although some did not reach statistical significance. Many of these genes were nevertheless positively associated with body fat percentage (Table 2) and adipocyte size (data not shown) while inversely associated with insulin sensitivity (Table 2).

AT PO_2 was inversely associated with insulin sensitivity ($r=-0.609$, $P=0.012$) and positively correlated with AT mRNA expression of chemokine ligand 5 (chemoattraction), CD11b (macrophage infiltration), and tumor necrosis factor- α (inflammation) (Table 2). In accord with a role for mitochondrial oxygen consumption in reducing AT PO_2 , we found significant inverse associations between PGC-1 α and citrate synthase mRNA expression in AT and gene expression of chemoattraction (monocyte chemoattractant protein-1, chemokine ligand 5), macrophage infiltration (CD68, CD163, CD11b), and inflammatory (tumor necrosis factor- α , plasminogen activator inhibitor-1) markers (data not shown). Thus, AT hyperoxia in obese humans is accompanied by insulin resistance and increased AT expression of inflammatory cell markers.

Table 2. Gene Expression in Abdominal Subcutaneous AT in Lean and Obese Subjects

	Lean	Obese	<i>P</i>	Correlation With Body Fat, %		Correlation With Insulin Sensitivity		Correlation With AT P _O ₂	
				<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Mitochondrial function									
PGC-1 α mRNA	0.89 (0.78–1.31)	0.66 (0.57–0.74)	0.009	−0.726	<0.001	0.807	<0.001	−0.286	0.302
CS mRNA	1.06 (0.64–1.77)	0.73 (0.59–0.95)	0.086	−0.440	0.059	0.893	<0.001	−0.529	0.043
Insulin sensitivity									
GLUT4 mRNA	1.17 (0.55–1.92)	0.45 (0.31–0.83)	0.050	−0.570	0.011			−0.357	0.191
Chemoattraction									
MCP-1 mRNA	0.81 (0.66–1.98)	1.88 (1.40–2.55)	0.050	0.584	0.009	−0.581	0.009	0.317	0.173
CCL5 mRNA	1.07 (0.50–1.45)	1.63 (0.57–2.71)	0.369	−0.084	0.732	−0.414	0.078	0.571	0.026
Macrophage infiltration									
CD163 mRNA	0.58 (0.45–3.15)	1.84 (1.31–2.66)	0.142	0.616	0.005	−0.718	0.001	0.436	0.104
CD11b mRNA	1.10 (0.50–2.83)	1.93 (1.07–2.94)	0.462	0.221	0.363	−0.753	<0.001	0.525	0.044
CD68 mRNA	0.83 (0.60–2.00)	1.59 (1.14–1.81)	0.165	0.477	0.039	−0.700	0.001	0.329	0.232
Inflammation									
PAI-1 mRNA	0.94 (0.40–2.52)	2.60 (2.01–4.14)	0.018	0.596	0.007	−0.688	0.001	0.268	0.334
TNF- α mRNA	1.00 (0.59–1.74)	1.25 (0.74–2.74)	0.369	0.684	0.100	−0.628	0.004	0.582	0.023
Interleukin-6 mRNA	1.20 (0.50–1.52)	1.52 (0.86–2.35)	0.191	0.575	0.010	−0.432	0.065	0.225	0.420
Interleukin-8 mRNA	1.12 (0.22–1.52)	1.79 (1.03–3.09)	0.221	0.191	0.448	−0.366	0.135	0.486	0.066
Angiogenesis									
VEGF mRNA	1.11 (0.69–1.41)	0.58 (0.48–0.75)	0.041	−0.479	0.038	0.886	<0.001	−0.221	0.428

Values are medians (interquartile range) from 9 lean and 10 obese subjects. PGC-1 α indicates peroxisome proliferator-activated receptor gamma coactivator-1 α ; CS, citrate synthase; GLUT4, glucose transporter 4; MCP-1, monocyte chemoattractant protein-1; CCL5, chemokine ligand 5; PAI-1, plasminogen activator inhibitor-1; TNF- α , tumor necrosis factor- α ; VEGF, vascular endothelial growth factor; AT Po₂, adipose tissue oxygen partial pressure; and *r*, Spearman's rho.

Discussion

AT dysfunction is implicated in the development of cardiovascular disease, fatty liver disease, and type 2 diabetes mellitus.^{1–5} Recent experiments suggest that AT hypoxia may underlie AT inflammation,^{10,11} a hallmark of AT dysfunction in obesity. In the present study, we demonstrate that ATBF is an important regulator of AT Po₂ in humans. Nevertheless, obese insulin resistant subjects exhibit higher AT Po₂ (hyperoxia) despite lower ATBF, which seems to be explained by lower AT oxygen consumption. AT hyperoxia in obese individuals was accompanied by insulin resistance, lower AT capillarization, lower AT expression of genes encoding mitochondrial biogenesis and function proteins, and higher AT gene expression of inflammatory cell markers.

We developed, optimized, and validated an optochemical measurement system for the continuous monitoring of AT Po₂ in vivo in humans using microdialysis and showed that, using a pharmacological approach, local administration of the vasoconstrictor Ang II in abdominal subcutaneous AT markedly decreased ATBF and AT Po₂, whereas infusion of the vasodilator isoprenaline induced pronounced opposite effects. Next we demonstrated that physiological changes in ATBF evoked concomitant changes in AT Po₂. We and others have previously shown that both fasting ATBF and the increase in ATBF after the ingestion of glucose are decreased in obese, insulin resistant, and type 2 diabetic subjects.^{19–22} Fully in agreement with our hypothesis, both ATBF and AT Po₂ were increased after ingestion of the glucose drink in lean subjects, but these responses were markedly blunted in obese individuals. The strong positive association between the postprandial increase in

ATBF and AT Po₂ further indicates that ATBF determines postprandial alterations in abdominal subcutaneous AT Po₂.

Unexpectedly, despite lower ATBF, AT Po₂ was significantly higher in obese subjects, although it is noteworthy that AT Po₂ values showed considerable overlap between groups. This is in marked contrast to previous studies in rodents, demonstrating lower AT Po₂ in *ob/ob* and dietary-induced obese mice,^{9,10} and humans.^{12,13} This discrepancy may be explained at least in part by heterogeneity in the study population (with respect to age, ethnicity, gender, and the presence of type 2 diabetes mellitus) in the study of Pasarica et al¹³ or a different study setting (perioperative condition¹²). Furthermore, it is important to mention that in these studies, including the present study, AT Po₂ was measured at 1 place in the AT depot. Therefore, we cannot fully exclude the possibility that hypoxic areas may be present in certain parts of the abdominal subcutaneous AT depot in obese subjects. Our data challenge the concept of hypoxia-induced AT inflammation in obesity.³⁵ We questioned whether lower AT oxygen consumption may underlie AT hyperoxia in obesity because AT Po₂ is determined by the balance between AT oxygen consumption and supply. In fact, it has been demonstrated previously that mitochondrial morphology is abnormal, mitochondrial biogenesis and mass are reduced, and oxygen consumption is lower in both white and brown AT of obese Zucker rats,³⁶ *ob/ob* mice,³⁷ *db/db* mice, and high-fat diet-fed mice.³⁸ Experimental data on the bioenergetics and oxidative capacity of human adipocytes are scarce. It has been shown that oxygen consumption per adipocyte is higher but oxygen consumption per gram of AT is lower in obese

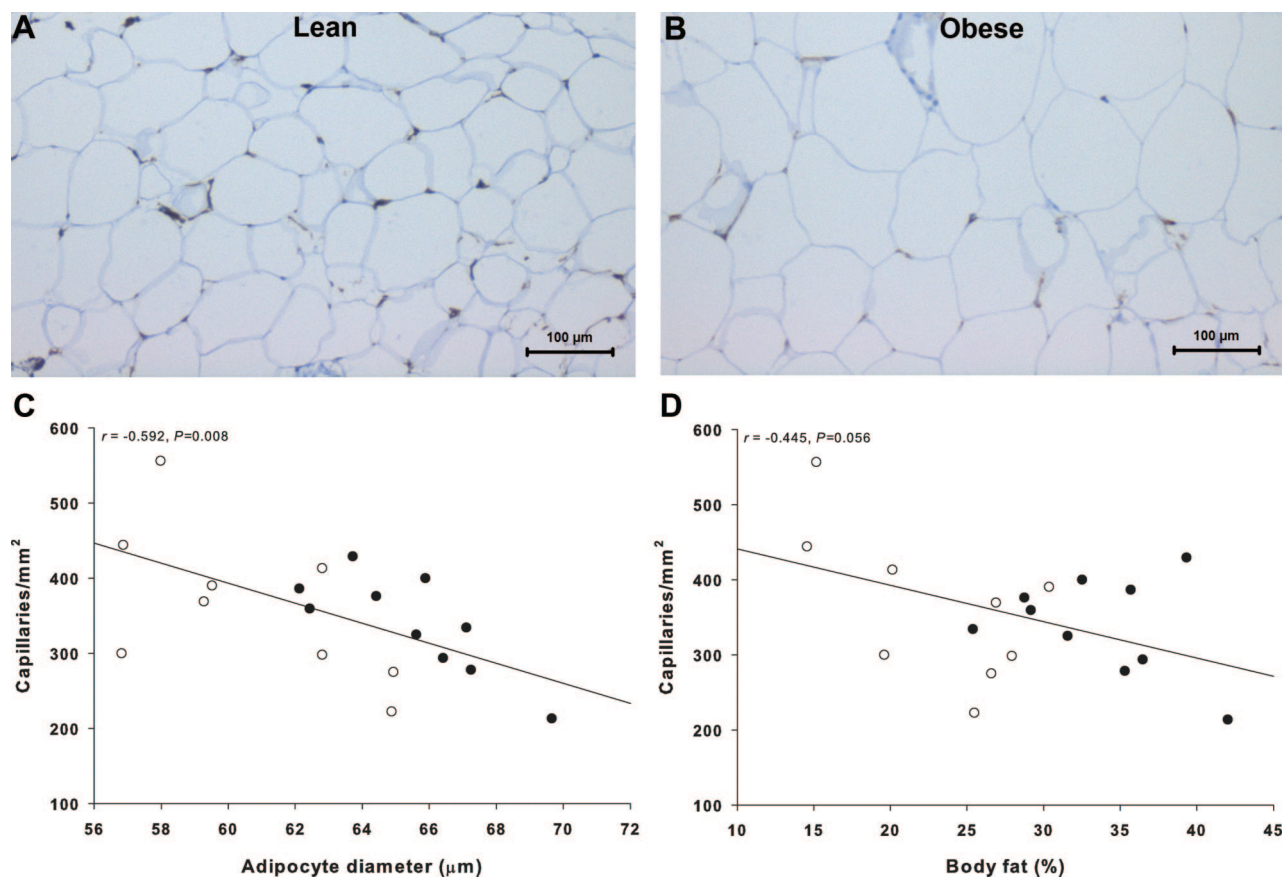


Figure 5. Capillarization in abdominal subcutaneous adipose tissue. Representative adipose tissue sections from lean (A) and obese (B) subjects were stained with hematoxylin and eosin to detect adipocyte plasmalemma (blue color) and anti-CD31/CD34 to detect capillaries (brown color). Adipose tissue capillary density was inversely associated with adipocyte size ($r = -0.592$, $P = 0.008$) (C) and body fat percentage ($r = -0.445$, $P = 0.056$) (D) in lean (open circles) and obese (closed circles) subjects ($n = 19$).

humans.³⁹ Our data confirm and extend these findings, showing that in vivo AT oxygen consumption was significantly lower in obese compared with lean subjects. In accordance, AT expression of mitochondrial function markers was lower in obese compared with lean subjects and was inversely associated with both body fat percentage and fasting AT Po_2 . Thus, lower mitochondrial oxygen consumption may underlie AT hyperoxia in human obesity. This may in turn contribute to reduced PGC-1 α expression in AT because it has been demonstrated that oxygen deprivation induces PGC-1 α expression in different tissues.³⁰

Recent studies have shown that PGC-1 α stimulates VEGF expression and angiogenesis.^{30,32} In agreement with this, we found that AT hyperoxia in obesity is paralleled by lower AT angiogenesis, capillarization, and ATBF. Although lower PGC-1 α expression in AT of obese individuals may explain these findings, the opposite may also hold true. In fact, it has been demonstrated previously that endothelial NO synthase expression in AT is positively associated with postprandial ATBF in humans,⁴⁰ and endothelial NO synthase-dependent NO production increased PGC-1 α expression, mitochondrial biogenesis, and function.^{41,42} Furthermore, the thermogenic response to cold was limited by the magnitude of the angiogenic response in brown AT.³⁴ Thus, it is tempting to postulate that a vicious circle operates between AT PGC-1 α expression, capillarization, and ATBF.

Next we examined whether AT hyperoxia in obesity is accompanied by derangements in AT function and found that AT hyperoxia was associated with insulin resistance and increased gene expression of AT chemoattraction, macrophage infiltration, and inflammatory markers. In accord with a role for mitochondrial oxygen consumption in reducing AT Po_2 , we found inverse associations between genes encoding mitochondrial function proteins and inflammatory cell markers in AT. Although increased macrophage infiltration and inflammation in obese AT is well established,^{1,4,5} our data are in contrast with previous findings showing that exposure of 3T3-L1 adipocytes to hypoxia induced a proinflammatory phenotype.^{10,11} Importantly, our data show that the oxygen availability used in previous cell culture experiments (1% O_2) does not reflect human physiology because AT Po_2 ranged from 3.2% to 11.3% in the present study. This may explain these conflicting findings. It remains to be established whether AT hyperoxia may evoke derangements in AT function or, alternatively, whether AT dysfunction contributes to AT hyperoxia. In support of the latter, tumor necrosis factor- α decreased AT mitochondrial biogenesis in animal models of obesity.⁴³ Furthermore, it has been shown that reactive oxygen species, which are increased in obese AT,⁴⁴ diminish oxygen consumption in rat primary adipocytes, whereas the reactive oxygen species scavenger *N*-acetylcysteine increased oxygen consumption and decreased fat mass in mice.⁴⁵

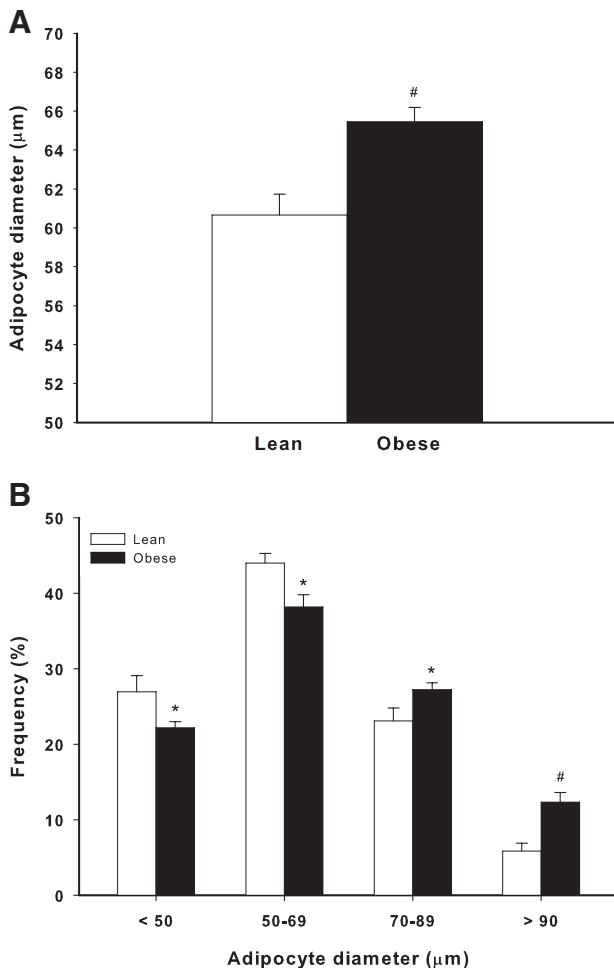


Figure 6. Mean adipocyte diameter and adipocyte size distribution in lean ($n=9$) and obese ($n=10$) subjects. **A**, Adipocyte size was higher in obese compared with lean subjects, with **(B)** a shift toward a higher proportion of large/very large adipocytes. Values are mean $\pm$ SEM. $^*P<0.05$, $^{\#}P<0.01$ vs lean.

In conclusion, we demonstrate for the first time that ATBF is an important regulator of AT PO_2 in humans. Nevertheless, obese insulin resistant individuals exhibit AT hyperoxia despite lower ATBF, which seems to be explained by lower AT oxygen consumption. This is accompanied by insulin resistance, impaired AT capillarization, and higher AT gene expression of inflammatory cell markers. Our data are preliminary in nature and require confirmation. Future studies should elucidate whether all areas of obese AT are hyperoxic and whether hyperoxia is causally related to AT dysfunction or is secondary to the obese insulin resistant state. Our findings may suggest that targeting AT oxygen tension in obese subjects with impaired glucose tolerance could restore the metabolic and endocrine derangements in AT and subsequently improve insulin sensitivity, thereby reducing the risk of developing obesity-related type 2 diabetes mellitus and cardiovascular disease. Treatment strategies to increase AT oxidative capacity (ie, modulation of ATBF and/or PGC-1 α activity in AT) should be explored as modulators of insulin resistance.

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Disclosures

None.

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CLINICAL PERSPECTIVE

The increase in adipose tissue mass during the development of obesity is accompanied by impaired adipose tissue function, which may underlie type 2 diabetes mellitus and cardiovascular disease. The inciting event causing the metabolic and endocrine derangements in adipose tissue of obese individuals remains to be established. Recent cell culture experiments suggest that a reduced oxygen tension in adipose tissue (adipose tissue hypoxia) may be involved. It has been proposed that the expansion of adipose tissue mass during weight gain may lead to adipose tissue hypoxia because angiogenesis is insufficient to maintain normoxia. Although adipose tissue hypoxia has been demonstrated in rodent models of obesity, evidence for this in humans is scarce. We hypothesized that decreased adipose tissue blood flow in obese humans may lower adipose tissue oxygen tension, thereby affecting adipose tissue inflammation and insulin sensitivity. In the present study, we describe a novel system for the continuous monitoring of adipose tissue oxygen tension in humans using microdialysis. Using both pharmacological and physiological approaches, we demonstrate that adipose tissue blood flow regulates adipose tissue oxygen tension in humans. Nevertheless, obese individuals exhibit adipose tissue hyperoxia (increased oxygen tension) despite lower adipose tissue blood flow, which seems to be explained by lower oxygen consumption in adipose tissue. This was accompanied by insulin resistance, impaired adipose tissue capillarization, and higher adipose tissue gene expression of inflammatory cell markers. Although these findings are preliminary in nature and require confirmation, this work sheds new light on the role of adipose tissue oxygen tension in metabolic disease.