Reduced Oxygenation in Human Obese Adipose Tissue Is Associated with Impaired Insulin Suppression of Lipolysis

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Context: Adipose tissue in obese individuals is characterized by reduced capillary density and reduced oxygenation.

Objective: Our objective was to test whether hypoxia is associated with reduced antilipolytic effect of insulin.

Participants, Design, and Setting: Twenty-one lean and obese individuals participated in this cross-sectional study at a university-based clinical research center.

Intervention: In all subjects, *in situ* adipose tissue (AT) oxygenation [AT oxygen partial pressure ($ATpO_2$)] was measured with a Clark electrode, insulin sensitivity as well as basal and insulin-suppressed lipolysis (continuous infusion of ($^2H_5$)glycerol) were measured during a euglycemic-hyperinsulinemic clamp, and abdominal sc AT biopsies were collected to assess fat cell size (Coulter counting of osmium-fixed cells), capillary density (by staining of histological sections), and gene expression (by quantitative RT-PCR).

Main Outcome Measure: *In situ* $ATpO_2$ was evaluated.

Results: The ability of insulin to suppress lipolysis (percent) was positively correlated with insulin sensitivity ($r = 0.43$; $P < 0.05$), $ATpO_2$ ($r = 0.44$; $P < 0.05$), vascular endothelial growth factor mRNA ($r = 0.73$; $P < 0.01$), and capillary density ($r = 0.75$; $P < 0.01$).

Conclusion: These results indicate that low capillary density and $ATpO_2$ in AT are potentially upstream causes of AT dysfunction. **(*J Clin Endocrinol Metab* 95: 4052–4055, 2010)**

Obesity is accompanied by insulin resistance in multiple organs including muscle, liver, and adipose tissue (AT) (1). There are multiple contributors to insulin resistance including AT hypoxia (2). Obese humans have reduced AT oxygen partial pressure ($ATpO_2$), which is positively correlated with local inflammation (3). The reduced $ATpO_2$ in obese adult humans may be due to decreased capillary density (rarefaction) in AT (3). Hypoxia interferes with insulin action in rodent models of obesity and cultured human adipocytes (4–6). Insulin, apart from its action in muscle and liver, inhibits lipolysis and efflux of nonesterified fatty acid in AT. Failure of insulin to suppress lipolysis has been proposed as one link between obesity and insulin resistance (7). In humans, however, reduced $ATpO_2$ does not correlate with whole-body insulin sensitivity as measured during a high-dose hyperinsulinemic-euglycemic clamp (3) designed to measure skeletal muscle insulin sensitivity (8).

Given the role of insulin to suppress lipolysis in AT and that hypoxia leads to insulin resistance *in vitro*, we hypothesized that reduced $ATpO_2$ *in situ* would be associ-

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Abbreviations: AT, Adipose tissue; $ATpO_2$, AT oxygen partial pressure; VEGF, vascular endothelial growth factor.

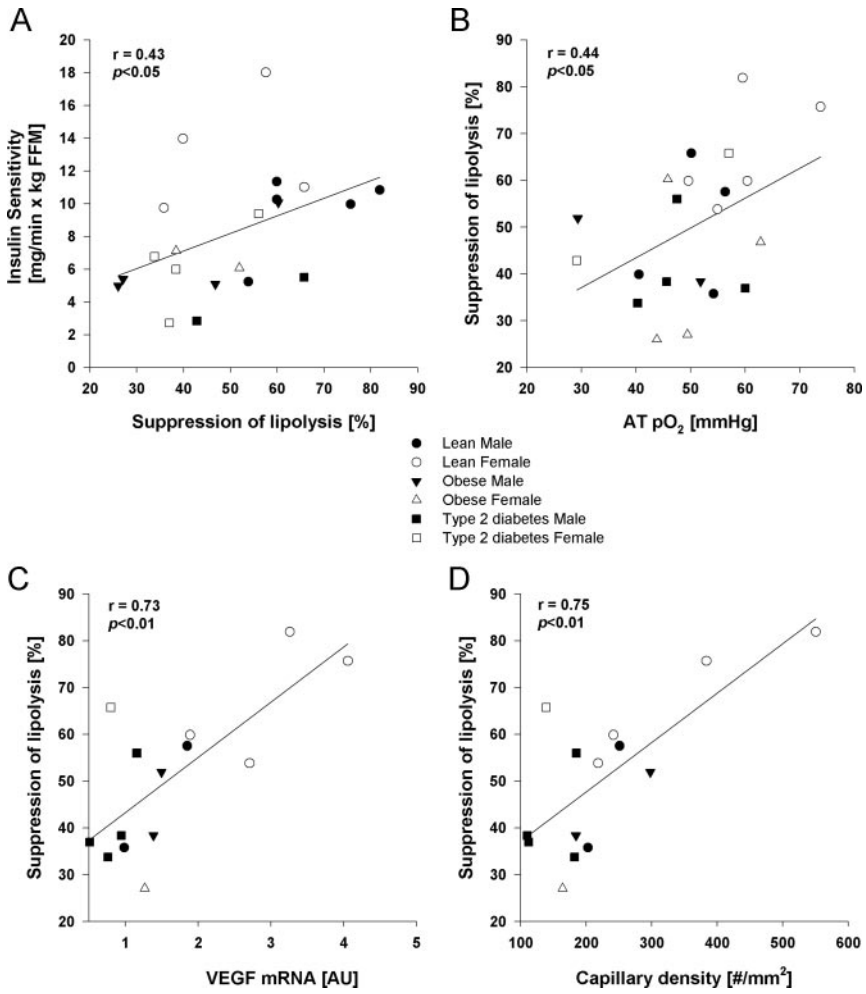

FIG. 1. Pearson correlation between suppression of lipolysis and insulin sensitivity for glucose disposal (A); AT oxygenation, $ATpO_2$ (B); VEGF (C); and capillary density by image analysis (D). Males are represented by *squares* and females by *circles*: *white* for lean, *gray* for obese without type 2 diabetes, and *black* for obese with type 2 diabetes. AU, Arbitrary units.

ated with reduced whole-body suppression of AT lipolysis by insulin.

Subjects and Methods

Twenty-one lean, obese, and type 2 diabetes mellitus subjects were recruited as previously described (3). Subjects were excluded for previous use of thiazolidinediones or drugs known to affect lipid metabolism or body weight. The protocol was approved by our Institutional Review Board, and all volunteers gave written informed consent.

Body composition was measured by dual-energy x-ray absorptiometry (QDR 4500; Hologic, Inc., Waltham, MA). As detailed previously, approximately 2 g sc AT was obtained by liposuction, 5 cm from the umbilicus (3). Mean fat cell size of sc abdominal AT was measured by Coulter counting of osmium-fixed cells (9), and $ATpO_2$ was measured with a combined oxygen and temperature probe (micro Clark type electrode with a thermocouple, catalog item CC1.P1; Integra Lifesciences Corp., Plainsboro, NJ) inserted at 1 cm depth (3).

A euglycemic-hyperinsulinemic clamp and glycerol turnover were performed after an overnight fast and a 3-d standardized diet. Baseline blood samples were obtained for substrate and hormone concentrations and background isotope enrichments. A primed continuous infusion of $[^2H_5]$glycerol (0.1 mg/kg · min; Cambridge Isotope Laboratories, Andover, MA) was started and continued for 360 min. After 240 min, a euglycemic (~90 mg/dl), hyperinsulinemic (80 mU/m^2 · min) clamp was conducted for 120 min. Exogenous glucose disposal was calculated during the final 30 min of the clamp. The rate of appearance of glycerol (enrichment of $[^2H_5]$glycerol) was calculated from isotopic enrichment in four baseline and insulin-stimulated blood samples as previously described (10). Lipolysis was assessed as percentage change in rate of appearance of glycerol from the basal to insulin-stimulated state. The clinical characteristics, insulin sensitivity, $ATpO_2$, and vascular endothelial growth factor (VEGF) have been previously reported (3).

Paraffin-embedded AT was incubated in tetraethylrhodamine isothiocyanate-conjugated lectin from *Ulex europaeus* (10 μg/ml) (catalog item L4889; Sigma-Aldrich, St. Louis, MO) and lectin-fluorescein isothiocyanate conjugate from *Griffonia simplicifolia* (25 μg/ml) (catalog item L2895; Sigma-Aldrich) for 30 min. Images were taken with a Zeiss Axioplan 2 upright microscope and microvessels counted using MBF ImageJ Bundle software (microvessel density = number of microvessels per square millimeter of section area, averaged across six to 10 images).

Human total RNA was isolated by column purification (QIAGEN, Valencia, CA). VEGF sequences were AGCCTTGCCTTGCTGCTCT (forward), ACCTCCACCATGCCAAGTGGTCCC (probe), and TCCTTCTGCCATGGGTGC (reverse). Quantitative RT-PCR was performed on a ABI PRISM 7900 with *Cyclophilin-β* as housekeeping gene.

Comparisons between lean, obese nondiabetic, and obese type 2 diabetic were tested with ANOVA. Relationships between lipolysis and measures of AT oxygenation were modeled with linear regression. Analyses were controlled for sex and race. If the sex and race interaction terms were not significant, these terms were removed. Analyses were conducted using JMP (version 5.0.1; SAS, Cary, NC).

Results

The clinical characteristics are presented in supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Mean fat cell size was negatively correlated with VEGF

mRNA ($r = -0.70$; $P < 0.008$) and capillary density ($r = -0.67$; $P < 0.01$) but not with $ATpO_2$. Glycerol turnover during fasting (glycerol appearance, micromoles per kilogram fat mass per minute) was positively correlated with $ATpO_2$ ($r = 0.48$; $P < 0.05$) but not with VEGF or capillary density ($r = 0.30$; P value not significant). Insulin suppressed glycerol turnover (glycerol appearance, micromoles per kilogram fat mass per minute) was inversely correlated with VEGF ($r = -0.55$; $P < 0.05$) and only marginally with capillary density ($r = -0.50$; $P = 0.06$) but not with $ATpO_2$ ($r = 0.01$; P value not significant). Insulin suppression of lipolysis was lower in obese *vs.* lean (44 ± 3 *vs.* $59 \pm 15\%$, $P < 0.05$) and correlated with whole-body glucose disposal rate ($r = 43$; $P < 0.05$; Fig. 1A) and fat cell size ($r = 0.53$; $P < 0.06$). Insulin suppression of lipolysis was positively correlated with $ATpO_2$ ($r = 0.44$; $P < 0.05$; Fig. 1B), VEGF mRNA ($r = 0.73$; $P < 0.01$; Fig. 1C), and capillary density ($r = 0.75$; $P < 0.01$; Fig. 1D). Sex and race were not significant contributors to suppression of lipolysis.

Discussion

AT dysfunction consisting of infiltration of macrophages and inflammation (11), impaired regulation of lipolysis (12), and disordered secretion of adipokines (13) is recognized as a precursor to diabetes (14) and cardiovascular disease (15). Recent data in cell culture systems (4, 5), animal models (4, 6), and humans (3) suggest that AT hypoxia lies upstream of AT dysfunction. In this study, we explored the role of $ATpO_2$ in the dysregulation of adipose lipolysis that occurs in diabetes as evidence of decreased sensitivity to insulin-suppressed lipolysis in human sc AT. Low $ATpO_2$ was associated with decreased whole-body lipolysis as measured by glycerol turnover. The ability of insulin to suppress lipolysis was defective in subjects with a low $ATpO_2$, and impaired lipolysis was associated with low AT capillary density and low VEGF mRNA. Taken together, these results implicate AT rarefaction and $ATpO_2$ as potentially causative for AT dysfunction.

These results have implications for the treatment of AT inflammation and impaired lipolysis commonly described in obesity and insulin resistance. Strategies to increase AT blood flow by drugs blocking angiotensin II, a potent vasoconstrictor shown to be up-regulated in obesity (16), may improve AT oxygenation and possibly lipolysis. The observed reduction in AT VEGF and capillary rarefaction suggests that the reduced $ATpO_2$ does not elicit an angiogenic response. Strategies that increase AT angiogenesis might be employed to increase $ATpO_2$ and reverse the dysfunctional AT.

In summary, low $ATpO_2$ is associated with a reduced insulin suppression of glycerol turnover, a hallmark of dysfunctional AT. These results provide new insight into the origins of AT dysfunction in obesity and suggest that defective angiogenesis might lie upstream of AT insulin resistance and therefore type 2 diabetes.

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