Metabolic Signatures of Human Adipose Tissue Hypoxia in Obesity

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Adipose tissue (AT) hypoxia has been proposed as the cause of obesity-related AT dysfunction, moving the tissue toward a proinflammatory phenotype. In humans, AT oxygenation has been assessed by expression of hypoxia-sensitive genes or direct assessment of O_2 tension; the obvious read out of hypoxia, effects on intermediary metabolism, has not been investigated. We used tissue-specific venous catheterization of subcutaneous abdominal AT in humans to investigate oxygen-related metabolic processes, searching for metabolic signatures relating to hypoxia in obesity. O_2 delivery to AT was reduced in obesity ($P < 0.05$). However, O_2 consumption was low (<30% of resting forearm skeletal muscle [SM], $P < 0.001$); this was not related to obesity. AT primarily oxidized glucose, as demonstrated by a respiratory quotient close to 1.0 (higher than SM, $P < 0.05$). AT was a net producer of lactate, but there was an inverse relationship in venous outflow between lactate-to-pyruvate ratio (a marker of cytosolic redox state) and BMI, suggesting that AT is glycolytic but obese AT is not hypoxic. Although delivery of O_2 to the obese AT is reduced, O_2 consumption is low, and metabolic signatures of human AT do not support the notion of a hypoxic state in obesity. ***Diabetes*** **62:1417–1425, 2013**

Subcutaneous adipose tissue (SCAT) is a complex metabolic organ that responds dynamically to alterations in nutritional state. As the tissue expands, macrophages infiltrate and orchestrate inflammatory responses via molecules such as tumor necrosis factor-α, interleukin-6, and monocyte chemotactic protein-1, all of which have been implicated in pathological changes in AT physiology (1,2). Hypoxia within the tissue has been proposed as an underlying cause of AT dysfunction, moving the tissue toward a proinflammatory phenotype (3–5).

Tissue O_2 partial pressure (pO_2) reflects the balance between O_2 delivery and consumption. AT blood flow (ATBF) plays an integral role in oxygen supply. It is markedly lower in obese than in nonobese subjects in both the fasted (6–9) and postprandial (6,8) states. Thus, in obese individuals it could be anticipated that oxygen delivery to the tissue is compromised. Beyond this, it is also well established that expansion of fat tissue often leads to larger cells (6), increasing the diffusion distance for O_2.

The level of oxygen deficiency within human AT that defines hypoxia remains unclear. Direct measurements of AT pO_2 have been made in murine and human models. Ye et al. (4) reported a 70% lower interstitial pO_2 within epididymal fat pads of *ob/ob* compared with lean mice (4). The pO_2 of obese AT is reported to be significantly lower than nonobese AT in most (10–12) but not all studies. Recently, Goossens et al. (6) noted a markedly higher AT pO_2 in obese compared with lean individuals. The authors suggested that "hyperoxia" in the obese AT could be explained by lower AT O_2 consumption (6). This would fit with increasing evidence of a downregulation of expression of metabolic pathways in AT in obesity, including mitochondrial oxidative pathways (8,13). Thus, the influence of adiposity on arterial O_2 content, O_2 delivery to AT, or AT O_2 consumption remains unclear.

AT is not a large consumer of O_2. It accounts for ~5% of whole-body O_2 consumption in normal-weight individuals (14). Little is known about the processes that require O_2 in human AT or how they are altered in obesity. Measurements of the respiratory quotient (RQ) of AT in vitro suggest that glucose is the major metabolic fuel (15,16). Glucose taken up into AT can be partitioned between conversion to lactate; conversion to glycerol-3-phosphate, which is used for fatty acid (FA) reestification; oxidation; or storage as glycogen and possibly lipid. It could therefore be that in obese AT, with low oxygenation, there would be a repartitioning of glucose toward lactate rather than glycerol-3-phosphate production. A possible consequence could be less FA reesterification within the tissue and, hence, increased FA release.

At a cellular level, a pivotal signal of low oxygenation is hypoxia-inducible factor (HIF)-1α, which acts as a central controller of oxygen-related gene expression (5). In human obesity, it remains unclear whether the genetic hallmarks of hypoxia (i.e., HIF-1α or HIF-1α target gene expression) are reflected in AT (6,12).

It seems, therefore, that the question of AT hypoxia in obesity would be best addressed by looking at functional consequences in terms of a "metabolic signature" of hypoxia. An initial cellular response to low O_2 results in the cell switching to anaerobic glycolysis and increasing lactate production, which has to be exported from the cell (3). Lactate is released by human AT in vivo in the fasting (17–19) and postprandial (18–20) states. An even more specific marker of cellular redox state may be the blood lactate-to-pyruvate ratio (21).

Using arterio-venous difference methodology, with selective venous catheterization of abdominal SCAT, we recently described the metabolic characteristics of human AT after an overnight fast in a large cohort of healthy individuals (22). However, no information was available on O_2 delivery or consumption or its metabolic use, and studies were not made in the fed state either. We have therefore now examined gas exchange and substrate use

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in the fasting and postprandial state over 24 h in abdominal SCAT (22,23) and skeletal muscle (SM) (24) in human volunteers across a range of adiposity. The aim of the study was to investigate whether an expansion of fat mass alters oxygen-related metabolic processes in SCAT in humans in the fasting and postprandial state and to seek a metabolic signature of AT hypoxia in order to test the hypothesis of AT hypoxia in obesity as a potential driver of dysfunction.

RESEARCH DESIGN AND METHODS

Samples were taken during the course of several published (8,24–27) and unpublished studies. Volunteers were recruited by advertisement and from the Oxford BioBank (28). All were metabolically healthy, not taking medication affecting lipid or glucose metabolism, and gave signed, informed consent. However, based on fasting plasma glucose concentrations, three volunteers had impaired fasting glucose (>6.1 mmol/L), but none were diabetic (29).

Volunteers attended our Clinical Research Unit having fasted from 10:00 P.M. the previous night. They were asked to refrain from strenuous exercise and alcohol for 24 h before the study. We measured arterio-venous differences across SCAT and forearm SM in the fasting state in 52 healthy subjects (42 males and 10 females) (Table 1). Of the 52 subjects, 40 had data for arterial and SCAT and 22 had complete data for arterial and forearm muscle. In a subgroup of 30 subjects (27 males and 3 females) (Table 1), we studied these processes in the postprandial state after subjects were fed three sequential meals over 24 h (8,24). Of these 30 subjects, 29 had complete arterial and SCAT data and 21 had data for arterial and forearm SM.

For studying tissue-specific metabolism, the superficial epigastric vein was cannulated as previously described (22,23) to sample the venous effluent of SCAT. Venous blood from forearm muscle was obtained from a cannula placed retrogradely in a deep antecubital vein (24,25). Arterial blood was sampled from a catheter in a femoral or radial artery. Blood sampling was performed simultaneously from all three sites. Abdominal subcutaneous ATBF was measured by the washout of ^{133}Xe (24).

Analytical methods. Blood samples were drawn into heparinized syringes (Sarstedt, Leicester, U.K.), and plasma was prepared rapidly at 4°C. Glucose concentrations were measured immediately and remaining samples frozen before analysis. Plasma triacylglycerol, nonesterified FA, and glucose were analyzed enzymatically using commercially available kits. A sample of whole blood was added to perchloric acid for analysis of lactate, glycerol, pyruvate, 3-hydroxybutyrate, and acetoacetate as previously described (30). Insulin was measured by radioimmunoassay (Millipore, Watford, U.K.).

Blood gas measurement. O_2 saturation in arterial and adipose venous blood was measured in all subjects, and 33 subjects had blood O_2 saturation measured additionally in muscle venous blood. In fasting-only studies, O_2 saturation and hemoglobin concentration were measured with a Corning 2500 cooximeter (Ciba-Corning Diagnostics, Halstead, U.K.). In the postprandial studies, an OPL GEM cooximeter was used (Instrumentation Laboratory, Warrington, U.K.) at times −30, 0, 150, 480, 840, and 1,440 min, where 0 is the time breakfast was given. In 19 subjects (17 males and 2 females) fasting and in 11 subjects (all male) postprandial, pO_2, pCO_2, and pH were measured in arterial and venous blood using either a GEM Premier 3000 blood gas analyzer (Instrumentation Laboratory) or a Radiometer ABL 700 Series (Radiometer, West Sussex, U.K.). All blood gas measurements were made in triplicate to reduce measurement variability. We attempted to use a Corning 965 Total CO_2 analyzer (Ciba-Corning Diagnostics) to measure whole-blood CO_2 content, but as reported also by Simonsen et al. (31) found it unreliable for measurement of the very small difference in CO_2 content between arterial and AT venous blood.

Calculations. We had fasting blood gas data and hemoglobin measurements in 37 of 41 subjects and full postprandial data (from 30 subjects) for 10 subjects, with the remainder of subjects missing either one time point (n = 4) or two or more time points (n = 10). We calculated the dissolved O_2, O_2 content, and tissue O_2 consumption for the individuals with complete data and found it to be stable over time; therefore, we interpolated missing data using time points before and after. If a baseline time point was missing and we had an end-of-study (24 h) time point, we used that and vice versa. We calculated O_2 content, O_2 delivery, tissue O_2 consumption, CO_2 delivery, CO_2 content of blood, tissue CO_2 production, tissue-specific RQ, and SCAT FA reesterificaiton as described in more detail in Supplementary Data.

Statistics. Data were analyzed using IBM SPSS Statistics, version 18 (IBM SPSS Products, Chertsey, U.K.). All data are presented as means ± SEM unless otherwise stated. All datasets were tested for normality with the Shapiro-Wilk test. Comparisons were made between AT and SM or within sites at different time points using a paired t test. Repeated-measures ANOVA, with time and site as factors, were used to investigate the change between sites over time with feeding. Where statistical significance was found, we did post hoc analysis with Bonferonni corrections. Correlations were assessed using the nonparametric Spearman rank correlation coefficient, r_s. To assess trends across BMI groups, we used polynomial contrasts across the groups that tested for a linear trend in the data.

RESULTS

Tissue O_2 delivery and consumption. O_2 saturation for arterial and venous blood draining SM and SCAT were significantly ($P < 0.001$) different from each other, with arterial being the most O_2-enriched compared with the venous blood from SM and SCAT at 97.2 ± 0.2, 56.1 ± 1.6, and 86.6 ± 0.5%, respectively (Fig. 1*A*). This was also true for the O_2 content (mmol/L), both fasting and postprandially, with the artery being significantly higher ($P < 0.001$) than the venous blood draining SCAT and SM (Fig. 1*B*). There was no change in O_2 content with feeding in the artery or venous blood draining SCAT, but there was an increase ($P = 0.029$) in O_2 content in SM venous blood (Fig. 1*B*). O_2 consumption was 3.7-fold greater in SM than in AT ($P < 0.001$). This marked difference highlights that SCAT is not a large consumer of O_2 compared with SM.

Tissue CO_2 release and RQ. The CO_2 content (mmol/L) of venous blood draining SM was significantly ($P < 0.05$)

TABLE 1
Subject characteristics

	Fasting data	Postprandial data
Males/females	42/10	27/3
Age (years)	42 (22–58)	42 (22–58)
BMI (kg/m^2)	25.5 (19.5–54.1)	25.5 (19.5–37.1)
Body fat (%)	26 (8–48)	26 (8–33)
Fasting biochemical parameters		
Arterial blood O_2 saturation (%)	98 (95.3–99.9)	97.7 (95.3–99.9)
Plasma glucose (mmol/L)	5.0 (4.3–6.8)	4.9 (4.3–5.4)
Plasma insulin (mU/L)	11.8 (0.8–76.4)	10.8 (2.5–25.2)
Plasma triacylglycerol (μmol/L)	973 (428–2,702)	904 (448–1,792)
Plasma NEFA (μmol/L)	551 (173–859)	505 (188–844)
Whole-blood 3-hydroxybutyrate (μmol/L)	105 (13–373)	113 (15–373)
ATBF (mL · 100 g^{-1} · min^{-1})	2.6 (0.3–11.3)	3.2 (1.2–11.30)
SM blood flow (mL · 100 mL^{-1} · min^{-1})	1.9 (1.0–3.4)	2.0 (1.1–3.4)

Data are median (range). NEFA, nonesterified FAs.

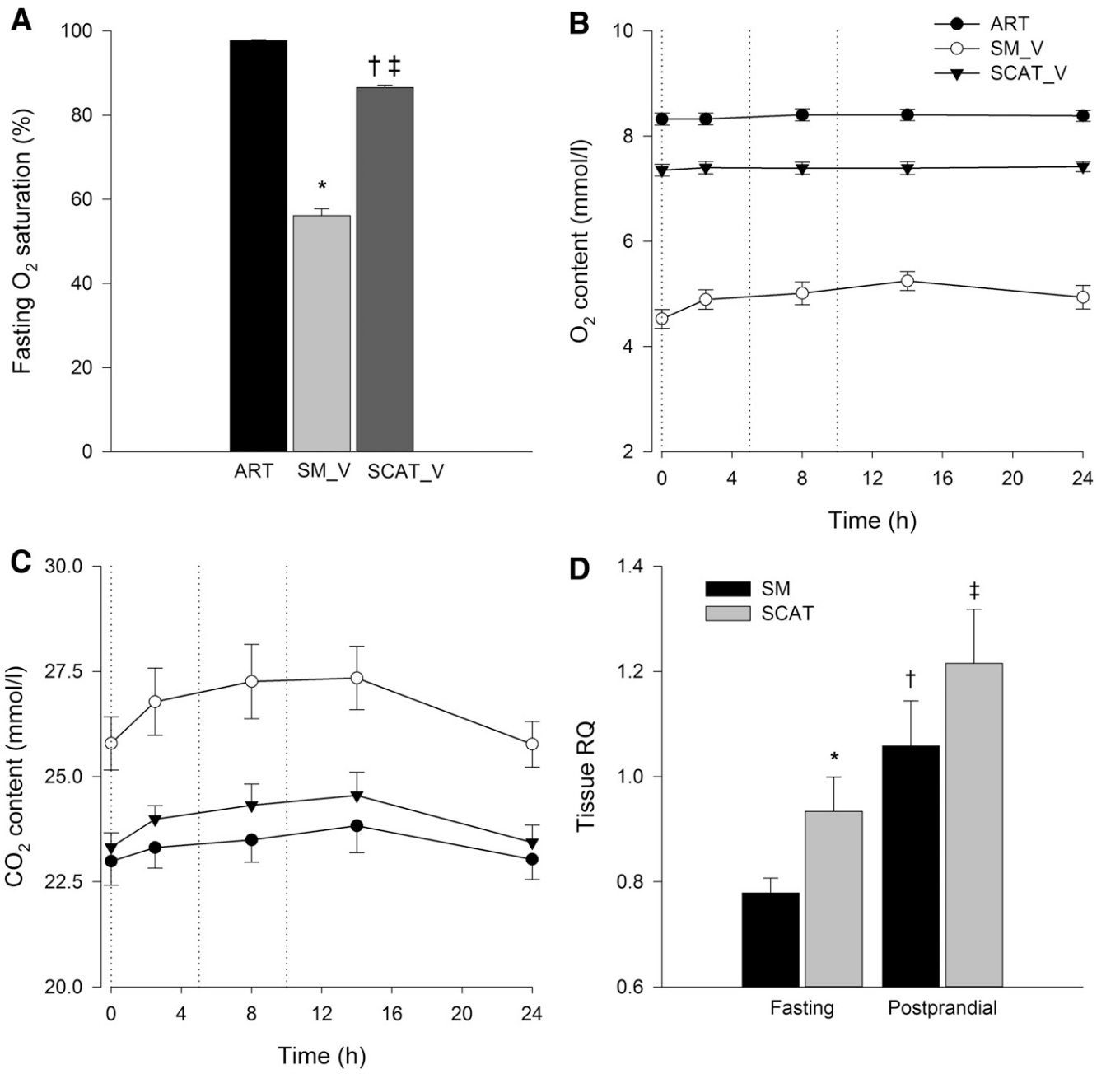

FIG. 1. *A–D*: Fasting and postprandial O_2 saturation and content and CO_2 content of arterial and venous blood. *A*: Fasting O_2 saturation (%) in arterial (ART), SM venous (SM_V), and SCAT venous (SCAT_V) blood. *$P < 0.001$ arterial vs. SM venous; †$P < 0.001$ arterial vs. SCAT venous; ‡$P < 0.001$ SM venous vs. SCAT venous. *B*: O_2 content (mmol/L) in arterial, SM venous, and SCAT venous blood over a 24-h period. Meals of similar energy and macronutrient composition were fed at 0, 5, and 10 h (dotted lines). $P < 0.001$ arterial vs. SM venous; $P < 0.001$ arterial vs. SCAT venous; $P < 0.001$ SM venous vs. SCAT venous, repeated measures over time. There was an increase in SM venous O_2 content (mmol/L) over time, $P = 0.029$. *C*: CO_2 content (mmol/L) in arterial, SM venous, and SCAT venous blood over 24 h. Meals of similar energy and macronutrient composition were fed at 0, 5, and 10 h (dotted lines). $P < 0.05$ arterial vs. SM venous; $P < 0.05$ SM venous vs. SCAT venous; $P < 0.05$ arterial vs. SCAT venous, repeated measures over time. There was an increase in the CO_2 content (mmol/L) over time in arterial, $P = 0.036$; SM venous, $P = 0.003$; and SCAT venous, $P = 0.005$. *D*: RQ for SM and SCAT in the fasting and postprandial state. *$P = 0.048$ fasting SM RQ vs. SCAT RQ; †$P < 0.001$ fasting RQ vs. postprandial RQ for SM; ‡$P = 0.015$ fasting RQ vs. postprandial RQ for SCAT; P = NS, postprandial SM vs. postprandial SCAT RQ.

higher, both fasting and postprandially, than arterial and venous blood draining SCAT (Fig. 1*C*). The CO_2 content of arterial blood was significantly ($P < 0.05$) lower than that of venous blood draining SCAT (Fig. 1*C*). Blood CO_2 content increased ($P < 0.05$) over the postprandial period in all three sites ($P < 0.05$) (Fig. 1*C*). CO_2 production was significantly greater ($P < 0.001$) in SM than SCAT both fasting and postprandially, highlighting the oxidative metabolic activity of SM compare with SCAT.

We determined the RQ of SM and SCAT in the fasting and postprandial states. The fasting RQ of SM was 0.78 ± 0.03 ($n = 29$); for SCAT, it was 0.93 ± 0.06 ($n = 26$) ($P < 0.05$) (Fig. 1*D*). For SM, this value was significantly <1.0 ($P < 0.001$), but for SCAT this was not so. The RQ in both tissues increased significantly ($P < 0.01$) in the postprandial state (1.06 ± 0.09 and 1.22 ± 0.10 for SM [$n = 11$] and SCAT [$n = 9$], respectively) (Fig. 1*D*). For SM, this value was not significantly >1.0, but for SCAT it was ($P = 0.044$).

Tissue oxygenation in obesity. It has been proposed that hypoxia may occur with an expansion of AT mass. We found a significant inverse correlation ($r_s = -0.43$, $P = 0.002$, $n = 49$) between BMI and arterial blood O_2 saturation (Fig. 2*A*).

We investigated the influence of adiposity on fasting AT O_2 delivery and consumption in lean (BMI <25 kg/m^2), overweight (BMI >25 and <30 kg/m^2), and obese (BMI >30 kg/m^2) individuals. Their characteristics are reported in Supplementary Table 1. There was an inverse correlation between BMI and ATBF ($r_s = -0.40$, $P = 0.004$, $n = 50$). We found an inverse linear trend ($P = 0.05$) across the BMI groups for O_2 delivery to SCAT, indicating that with an increasing BMI (fat mass) there is a decrease in the amount of O_2 delivered (Fig. 2*B*). The trend for the amount of O_2 being consumed by SCAT across the BMI groups was less clear ($P = 0.099$) (Fig. 2*C*). ATBF increased after the meal ($P = 0.008$, repeated measures over time), whereas SM blood flow did not change as observed previously (24,32) (Supplementary Fig. 1).

Tissue glucose metabolism. We compared the uptake of glucose into SM and SCAT in fasting and across postprandial periods. In the fasting state, the arterio-venous difference for glucose uptake was greater in SM than in SCAT (0.37 ± 0.06 vs. 0.20 ± 0.02 mmol/L, respectively, $P = 0.006$) (Fig. 3*A*). In the postprandial state, the uptake of glucose into SM was greater and displayed a very different pattern compared with SCAT ($P < 0.001$, time × site interaction) (Fig. 3*A*). Plasma insulin concentrations increased in the postprandial state ($P < 0.001$, change over time) (Fig. 3*A*). The higher uptake of glucose into SM in the early (1–2 h) postprandial period was maintained in the late (3–5 h) postprandial period (Fig. 3*A*).

FAs that are taken up into SCAT require the provision of glycerol-3-phosphate for reesterification to triacylglycerol. Assuming that this glycerol-3-phosphate is derived from glucose, we calculated the proportion of measured glucose uptake used for reesterification. In the fasting state (time 0 and 24 h), FA reesterification as a percent of glucose uptake was between 3 and 7% (Fig. 3*B*). With feeding, FA reesterification as a percent of glucose uptake significantly increased ($P < 0.001$), and over the postprandial periods this was on average 9% (Fig. 3*B*) ($P < 0.001$, change over time).

We calculated the proportion of glucose taken up by the SM and SCAT that was released as lactate and pyruvate (Fig. 3*C*). At baseline and 24 h, ~35% of glucose taken up by SCAT was released as lactate and pyruvate (Fig. 3*C*). The proportion of glucose taken up and released as lactate and pyruvate at baseline in SM was significantly lower ($P < 0.001$) than for SCAT, at ~10%, and was negligible at 24 h (Fig. 3*C*). The release of lactate and pyruvate in the postprandial period was greater for AT (~23% of glucose taken up) than SM (~5%) (Fig. 3*C*). There was an effect of site ($P < 0.001$) and time ($P < 0.001$) but no time × site interaction for the proportion of glucose taken up and released as lactate and pyruvate from SCAT and SM (Fig. 3*C*). We did not find an association between BMI and fasting glucose uptake and release as lactate and pyruvate from SCAT.

We determined the maximal contribution that tissue glucose uptake could make to tissue O_2 consumption if all the glucose were oxidized. We did this after correction of glucose uptake for lactate and pyruvate release as previously described (33). In SCAT, we additionally corrected glucose uptake for that consumed in glycerol-3-phosphate production, calculated from the reesterification rate. In the fasting state, the potential maximal contribution of complete glucose oxidation to O_2 consumption was significantly higher for SCAT compared with SM (73 ± 5 vs. 58 ± 9% for SCAT [$n = 37$] and SM [$n = 21$], respectively; $P = 0.045$) (Fig. 3*D*). Transiting to the postprandial state resulted in a >1.5-fold increase ($P < 0.001$) in both tissues in the maximal contribution that glucose could make to O_2 consumption (Fig. 3*D*). For SM and SCAT, the postprandial value was significantly >100% ($P < 0.001$ SM and $P = 0.045$ SCAT), implying glucose storage.

Are there metabolic signatures of AT hypoxia? A possible consequence of hypoxia is that cellular metabolism may switch to anaerobic glycolysis and this would lead to an increase in lactate production. We measured the concentrations of lactate and pyruvate in arterial and venous blood samples. In fasting, there was a net release of lactate from SM and SCAT (Fig. 4*A*). Blood lactate concentrations increased with feeding in all three sites ($P <$ 0.001, time × site interaction) and tended to remain highest in the venous blood from SCAT (Fig. 4*B*). We found an

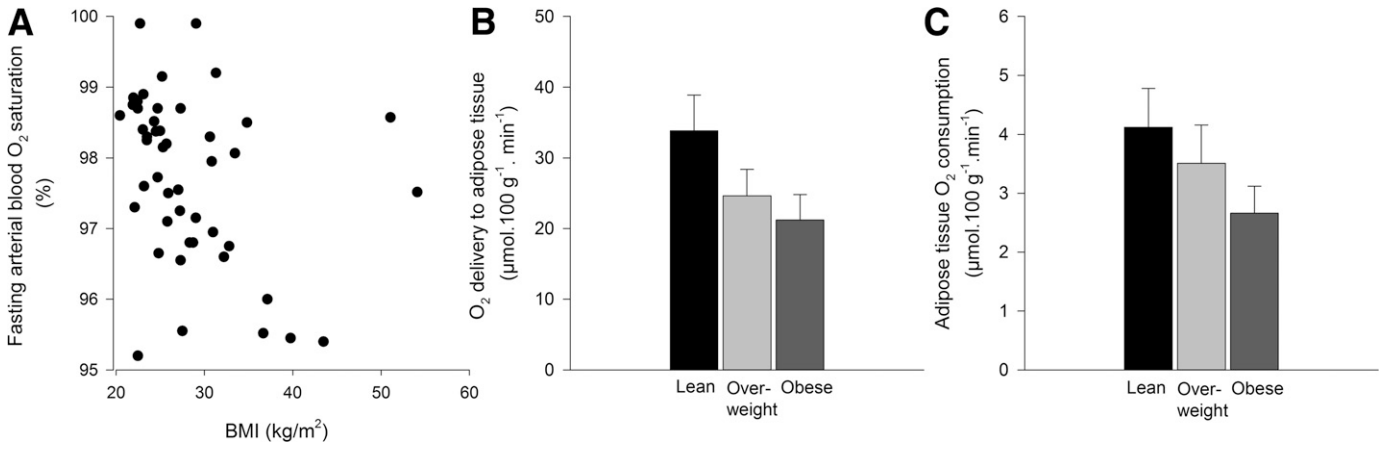

FIG. 2. *A–C*: Fasting arterial blood saturation, O_2 delivery, and O_2 consumption in relation to obesity. *A*: The correlation between fasting arterial blood O_2 saturation (%) and BMI was $r_s = -0.43$, $P = 0.002$ ($n = 49$). *B*: O_2 delivery to AT (µmol · 100 g^{-1} · min^{-1}) in lean (BMI <25 kg/m^2 [$n = 20$]), overweight (BMI >25 and <30 kg/m^2 [$n = 14$]), and obese (BMI >30 kg/m^2 [$n = 14$]) subjects' blood. Trend (linear using polynomial contrasts) across groups $P = 0.05$. *C*: AT O_2 consumption (µmol · 100 g^{-1} · min^{-1}) in lean (BMI <25 kg/m^2 [$n = 20$]), overweight (BMI >25 and <30 kg/m^2 [$n = 14$]), and obese (BMI >30 kg/m^2 [$n = 14$]) subjects' blood. Trend (linear using polynomial contrasts) across groups $P = 0.099$.

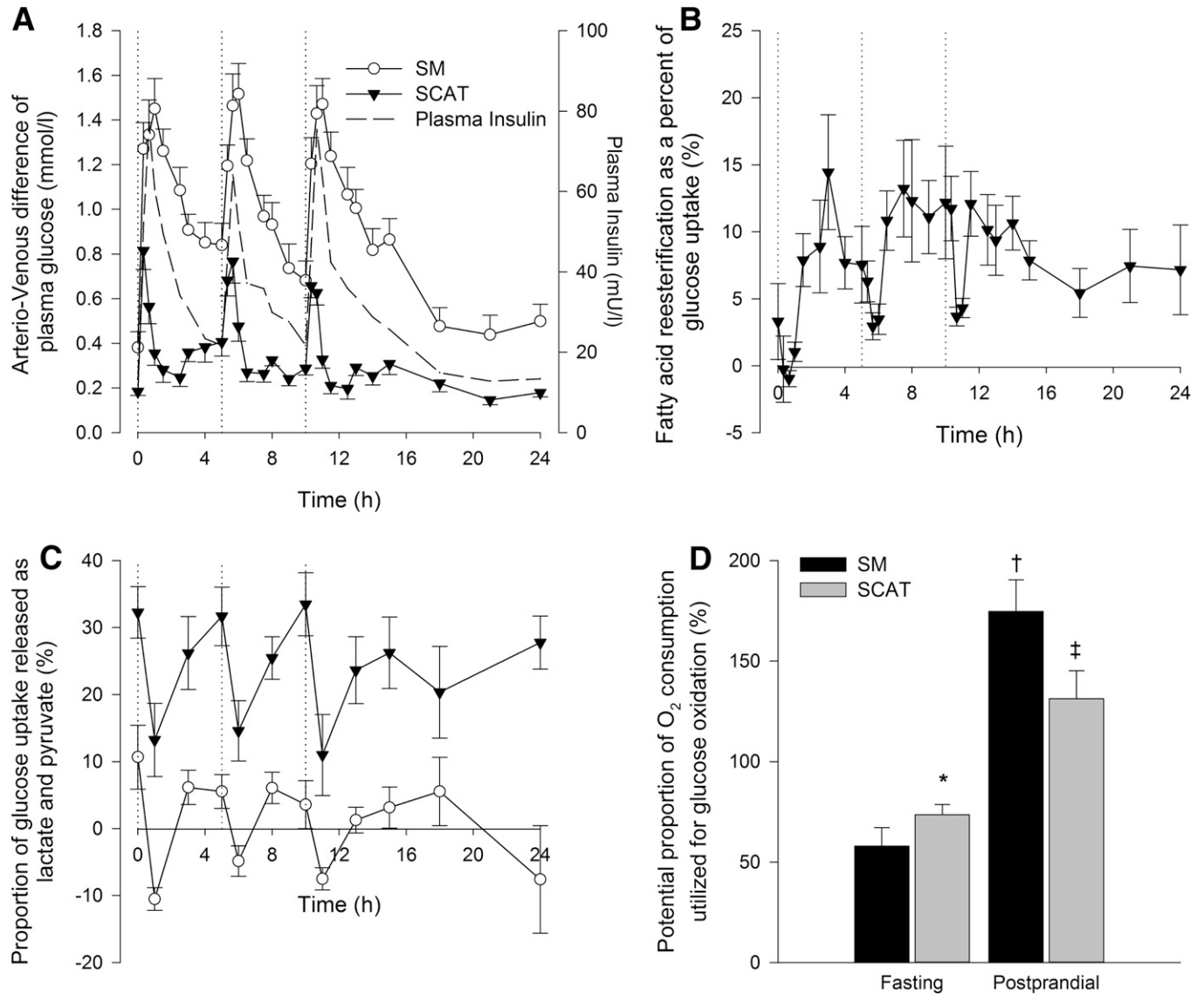

FIG. 3. *A–D*: Fasting and postprandial glucose metabolism in SCAT and SM. *A*: Uptake of plasma glucose (mmol/L) across SM and SCAT during fasting and postprandial periods. Plasma insulin concentrations (mU/L) during fasting and postprandial periods. Meals of similar energy and macronutrient composition were fed at 0, 5, and 10 h (dotted lines). For both SM and SCAT, there was an increase in glucose uptake with feeding ($P < 0.001$, repeated measures over time). The uptake of glucose was significantly greater for SM than SCAT ($P < 0.001$, repeated measures, time × site interaction). Plasma insulin concentrations increased with feeding ($P < 0.001$, repeated measures over time). *B*: FA reesterification as a proportion of glucose uptake in SCAT ($P < 0.001$, repeated measures over time). *C*: Proportion of glucose that is taken up into SM and SCAT released as lactate and pyruvate over time ($P < 0.001$, repeated measures over time for both sites, and $P < 0.001$, repeated measures between sites; no time × site interaction). *D*: The potential proportion of O_2 consumption used for glucose oxidation in SM and SCAT in the fasting and postprandial state. *$P = 0.045$ fasting SM vs. SCAT; ††$P < 0.001$ fasting vs. postprandial for both tissues.

association between arterial lactate concentration and BMI ($r_s = 0.28$, $P = 0.046$) but not with percentage body fat.

In fasting, there was net uptake of pyruvate from the blood into SM, while there was a net release of pyruvate from AT (Fig. 4*C*). Blood pyruvate concentrations increased after the consumption of a meal, and the increment was greatest in arterial blood and venous blood from SCAT ($P < 0.001$, for all three sites a site × time interaction) (Fig. 4*D*). Fasting arterial blood pyruvate concentrations were positively associated with BMI ($r_s = 0.60$, $P < 0.001$) and percentage body fat ($r_s = 0.51$, $P = 0.001$).

The lactate-to-pyruvate ratio is a potential metabolic "signature" of hypoxia, as it reflects the cytosolic redox state (34). The fasting lactate-to-pyruvate ratio in arterial blood was inversely correlated with BMI ($r_s = -0.49$, $P = 0.001$) and percentage body fat ($r_s = -0.41$, $P = 0.025$). This inverse correlation was driven by the positive relationship between BMI and arterial pyruvate concentration. We calculated the change across SCAT in lactate-to-pyruvate ratio as a measure of tissue-specific hypoxia as follows: (veno–arterio difference lactate)/(veno–arterio difference pyruvate). We found no association between this tissue-specific change in lactate-to-pyruvate ratio and BMI ($r_s = 0.11$) (Fig. 4*E*). However, the proportion of glucose released as lactate and pyruvate in SCAT was strongly negatively correlated with BMI (Fig. 4*F*). A lactate-to-pyruvate ratio of 10:1 or below is considered normal (21). The lactate-to-pyruvate ratio in arterial and SCAT venous blood was not significantly different from 10, suggesting adequate systemic and cellular oxygenation.

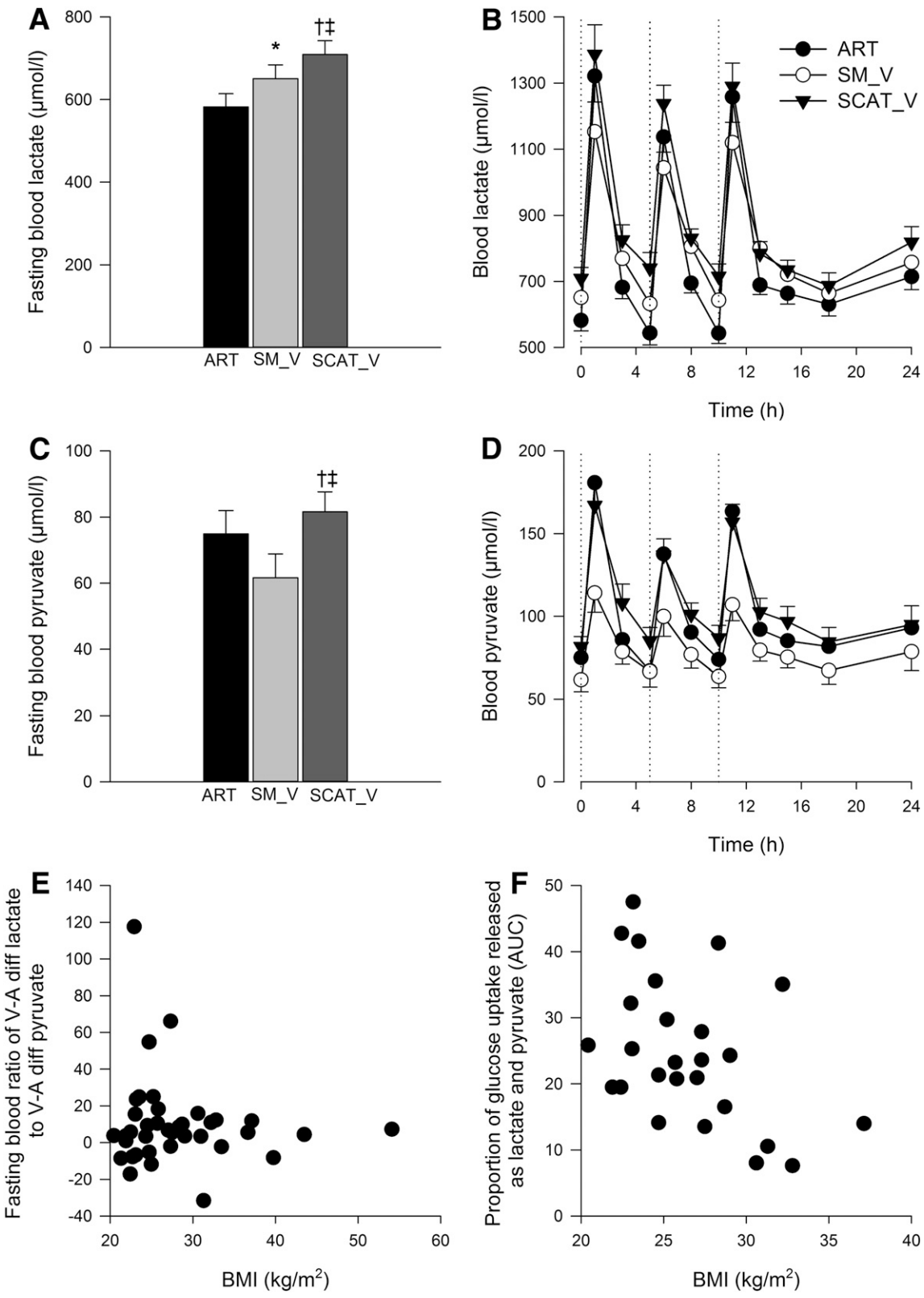

FIG. 4. *A–F*: Fasting (*A* and *C*) and postprandial (*B* and *D*) concentrations of lactate and pyruvate in arterial (ART), SM venous (SM_V), and SCAT venous (SCAT_V) blood and associations with BMI (kg/m^2) (*E* and *F*). *A*: Fasting blood lactate concentrations (μmol/L) in arterial, SM venous, and SCAT venous. **P* = 0.004 arterial vs. SM venous; †*P* < 0.001, arterial vs. SCAT venous; ‡*P* = 0.004, SM venous vs. SCAT venous. *B*: The postprandial concentrations (μmol/L) of lactate in arterial, SM venous, and SCAT venous blood. Meals of similar energy and macronutrient composition were fed at 0, 5, and 10 h (dotted lines); *P* < 0.001, repeated measures over time for all sites, and *P* < 0.001, time × site interaction for all sites. *C*: Fasting blood pyruvate concentrations (μmol/L) in arterial, SM venous, and SCAT venous; *P* = NS, arterial vs. SM venous; †*P* = 0.01, arterial vs. SCAT venous; ‡*P* < 0.001, SM venous vs. SCAT venous. *D*: The postprandial concentrations (μmol/L) of pyruvate in arterial, SM venous, and SCAT venous blood. Meals of similar energy and macronutrient composition were fed at 0, 5, and 10 h (dotted lines). *P* < 0.001, repeated measures over time for all sites, and *P* < 0.001, time × site interaction for all sites. *E*: The correlation for the (veno–arterio difference lactate) / (veno–arterio

DISCUSSION

We performed an in vivo human study to investigate whether an expansion of AT mass alters oxygen-related metabolic processes in SCAT in the fasting and postprandial states to test the hypothesis of AT hypoxia in obesity as a potential driver of dysfunction. We did not detect a tissue-specific metabolic signature indicative of obesity-related AT hypoxia. Our data highlight the remarkably low oxygen requirement of AT to undertake its many metabolic processes compared with SM.

Angiogenesis may play a critical role in providing expanding AT with adequate oxygen (35). AT from obese individuals has lower expression of angiogenic genes and capillarization compared with lean individuals (6,12). Additionally, fasting and postprandial ATBF is decreased in obese compared with lean individuals (6–9). We found indications that with increasing obesity, arterial O_2 saturation and O_2 delivery to AT are decreased. Low oxygenation is well recognized as a mechanical effect of severe obesity (36).

Experimental data on human AT O_2 consumption are limited. We found a nonsignificant trend for O_2 consumption by AT to decrease with increasing obesity. However, the proportion of inactive triacylglycerol is greater in AT from obese subjects (37,38), so the delivery of blood and oxygen as well as oxygen consumption per unit of active cytosolic weight may not be as different as our data indicate. Goossens et al. (6) reported AT O_2 consumption to be significantly lower in obese compared with lean subjects. The AT O_2 consumption of obese individuals in the current study was 2.66 μmol $\cdot$ 100 g tissue^{-1} $\cdot$ min^{-1} higher than that reported by Goossens et al. (6) (0.67 μmol $\cdot$ 100 g tissue^{-1} $\cdot$ min^{-1}). A plausible explanation for the discrepancy in findings is that Goossens et al. (6) measured pO_2 in arterialized rather than arterial blood. Arterialized blood pO_2 does not provide a valid estimation of arterial pO_2 (39). Our measurements emphasize the small O_2 requirement of AT compared with SM.

Adipocytes, like other cells, require energy for metabolic activities. In vitro work has suggested that this energy is primarily obtained by glucose catabolism (15,16). We have been able to corroborate in vivo the hypothesis that in the fasting state, human white AT oxidizes predominantly glucose. We found resting SM, in the fasting state, to have a significantly lower RQ than AT, highlighting the difference in substrate use between the tissues. SM RQ has previously been reported, and our data are in agreement with most (31,40,41) but not all (42) previous studies. The maximal contribution glucose uptake could make to O_2 consumption, if it were all oxidized, in the fasting state was notably higher in AT than SM. Previous work in which the proportion of O_2 consumption that reflects glucose oxidation was calculated for SM (17,40) and AT (17) found lower values than we report here. With feeding, the RQ in both tissues increased markedly, with the RQ for AT being significantly >1.0, indicative of net lipogenesis (43). The capacity of human AT for de novo lipogenesis (DNL) is now well recognized (44–46), but this is the first indication that the pathway becomes active after meals during normal energy balance. We attempted to quantitate fat synthesis using the gas-exchange data (33). An approximate calculation for the peak postprandial period suggests net synthesis of ~100 nmol FA $\cdot$ 100 g tissue^{-1} $\cdot$ min^{-1}. At the same time, net FA import (from transcapillary FA flux [24]) was ~600–1000 nmol FA $\cdot$ 100 g tissue^{-1} $\cdot$ min^{-1}. The relationship between DNL and import of circulating FAs is not dissimilar to the figure of 20% of fat stored from the de novo pathway in AT derived from labeling with ^2H_2O (46). Interestingly, the RQ of human adipocytes in vitro is not affected by the addition of insulin, although glucose uptake significantly increased (15). In the postprandial period, there was a robust increase, to $>100\%$, in the maximal contribution glucose uptake could make to O_2 consumption in both tissues. These data demonstrate that glucose uptake exceeded O_2 consumption, indicating net glucose storage, which in SM may be as glycogen and in AT as glycogen or lipid (via DNL) (16,17). The conditions favoring glycogen deposition in AT are generally the same as those enhancing fat deposition, although glycogen deposition is thought to precede fat deposition (16,47). Consumption of a high-carbohydrate diet (800 g for 2.5 days) significantly increased the content of glycogen in human AT (48).

In vitro hypoxia work suggests that a compensatory response of adipocytes switching to anaerobic glycolysis is an increased demand for glucose (3). As demonstrated here, the postprandial uptake of glucose by SM is considerably greater than that of SCAT, and we found no evidence of an increase in glucose uptake across SCAT with increasing obesity; others also have not (9). The possible fates for glucose within SCAT are conversion to glycerol-3-phosphate, which is used for FA reestification; conversion to lactate released into the blood; oxidation; or storage as glycogen and possibly lipid (17,22). In the fasting state, the conversion of glucose to glycerol-3-phosphate was 3–7%, increasing up to peaks between 9–14% at ~3 h after meal consumption. During the postprandial period(s) the FAs being reesterified will be predominantly, although not exclusively, derived from intravascular lipolysis (lipoprotein lipase action). We found no evidence for a lack of provision of glycerol-3-phosphate, suggesting that glucose partitioning toward this pathway is not impaired with increasing obesity. We have previously reported that the overall quantity of lipoprotein lipase–mediated FAs delivered to AT is much smaller for abdominally obese than for lean men, and they appear to be readily reesterified (8).

In the fasting state, approximately one-third of the glucose taken up by AT is converted to lactate, with a smaller amount to pyruvate (22), as we found. In the postprandial state, 5 h after meal consumption ~30% of the glucose taken up was converted to lactate and pyruvate. If there were an increase in glycolysis, then production of pyruvate, the immediate precursor of lactate, would be increased. If cellular hypoxia were present, then the NADH-to-NAD$^+$ ratio (cytosolic redox state) would be increased, which would drive the equilibrium from pyruvate toward lactate, accelerating lactate production (21). If obesity-related AT hypoxia were present, it would result in a positive association between the proportion of glucose taken up released as lactate and pyruvate and BMI. However, we found a negative association. Although we found an association between BMI and arterial lactate concentrations, the difference between lean and obese groups was not statistically different, as shown previously by some (18) but not all (19).

difference pyruvate) and BMI (kg/m^2) was $r_s = 0.11$; P = NS. F: The correlation between the area under the curve for the proportion of glucose taken up into AT over 24 h released as lactate and pyruvate and BMI (kg/m^2) was $r_s = -0.44$; $P = 0.016$.

Whole-body lactate production is between 1–1.5 mol/24 h in healthy individuals (49). The overall contribution of SCAT to systemic lactate concentrations is minor (0.8 mmol/24 h by extrapolating our data to whole-body fat mass) such that in the early postprandial period, when systemic lactate concentrations are increased, the reduced output from AT will not impact greatly. We found a stronger association between BMI and pyruvate. Doar et al. (50) reported that fasting blood pyruvate concentrations increased with adiposity in women but could not distinguish whether the higher pyruvate noted with increased obesity was the result of increased production or impaired removal. Most importantly, we found no evidence that the proportion of glucose uptake released as lactate and pyruvate was increased in obesity; in fact, the opposite was true. Furthermore, the tissue-specific change in lactate-to-pyruvate ratio was unrelated to BMI. Both these observations strongly argue against functional consequences of AT hypoxia in obesity.

We report here for the first time the use of oxygen in human AT. Our data clearly demonstrate that AT is a low consumer of O_2 compared with SM in lean and obese individuals. We found no evidence of a metabolic signature to support the notion of obesity-related AT hypoxia being a potential driver of dysfunction.

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