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Dissociation of local and global skeletal muscle oxygen transport metrics in type 2 diabetes

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ABSTRACT

Aims: Exercise capacity is impaired in type 2 diabetes, and this impairment predicts excess morbidity and mortality. This defect appears to involve excess skeletal muscle deoxygenation, but the underlying mechanisms remain unclear. We hypothesized that reduced blood flow, reduced local recruitment of blood volume/hematocrit, or both contribute to excess skeletal muscle deoxygenation in type 2 diabetes.
Methods: In patients with ($n = 23$) and without ($n = 18$) type 2 diabetes, we recorded maximal reactive hyperemic leg blood flow, peak oxygen utilization during cycling ergometer exercise (VO_{2peak}), and near-infrared spectroscopy-derived measures of exercise-induced changes in skeletal muscle oxygenation and blood volume/hematocrit.
Results: We observed a significant increase ($p < 0.05$) in skeletal muscle deoxygenation in type 2 diabetes despite similar blood flow and recruitment of local blood volume/hematocrit. Within the control group skeletal muscle deoxygenation, local recruitment of microvascular blood volume/hematocrit, blood flow, and VO_{2peak} are all mutually correlated. None of these correlations were preserved in type 2 diabetes.
Conclusions: These results suggest that in type 2 diabetes 1) skeletal muscle oxygenation is impaired, 2) this impairment may occur independently of bulk blood flow or local recruitment of blood volume/hematocrit, and 3) local and global metrics of oxygen transport are dissociated.

1. Introduction

According to CDC estimates, nearly half of American adults now have type 2 diabetes or prediabetes.[1] The estimated lifetime risk of developing diabetes has risen to greater than 30%.[2] People with type 2 diabetes suffer disproportionate cardiovascular and all-cause mortality, in addition to potentially disabling complications such as diabetic retinopathy and diabetic foot ulcer. The pathological mechanisms leading to excess morbidity and mortality in the diabetic population are not yet fully understood, but vascular and microvascular dysfunction are a common theme. Consistent with this observation, aerobic exercise capacity (VO_{2max}, a powerful clinical predictor of mortality[3–5]), is impaired in type 2 diabetes.[6] Moreover, impaired aerobic exercise capacity is associated with diabetic complications[7] and insulin resistance,[8] suggesting that the causes of impaired exercise capacity are intimately related to the broader pathology of type 2 diabetes. This possibility mandates intensive investigation of the causes of impaired aerobic exercise capacity in type 2 diabetes.

Although the precise mechanisms by which VO_{2max} is reduced in type 2 diabetes are not yet fully understood, impaired oxygen delivery is a likely contributor. Rodent studies reveal skeletal muscle hypoxia at the onset of exercise in rodents with diabetes,[9] and these findings are corroborated by findings of increased skeletal muscle deoxygenation during exercise in humans with type 2 diabetes.[10] There are several plausible mechanisms that may contribute to impaired oxygen delivery to skeletal muscle in type 2 diabetes, including reduced capillary density,[11] reduced blood flow,[12] loss of capillary perfusion,[13] and heterogeneous distribution of blood flow.[14,15] Although each of these possible contributors has been previously noted, contradictory reports exist in the literature (especially with regard to bulk blood

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flow, e.g., Ref. 16), and it remains unclear which specific parameters, if any, may limit oxygen delivery.

Oxygen delivery to peripheral tissues consists of convective (i.e., arrival of oxygenated blood) and diffusive (i.e., transport of oxygen from blood to mitochondria) steps. The convective step in oxygen delivery is primarily determined by bulk blood flow to the exercising muscle, and also determined to some extent by distribution of blood flow within the skeletal muscle circulation. In this manuscript, we report a metric of maximal leg blood flow as recorded during reactive hyperemia (RH) by venous occlusion plethysmography. The diffusive step of oxygen delivery has many determinants. One important component is the net recruitment of tissue hemoglobin content during exercise whether through microvascular recruitment, increased capillary hematocrit, or any other mechanism. Reductions in microvascular blood volume/hematocrit or its recruitment are widely reported in animal models of type 2 diabetes,[11,13,17] and these changes are likely to contribute to any defect in oxygen diffusion.

In this study, we used near-infrared spectroscopy (NIRS) to monitor skeletal muscle deoxygenation/oxygenation and local recruitment of microvascular blood volume/hematocrit in response to exercise. Interpretation of the NIRS signal is complex because tissue composition (e.g., adipose tissue thickness) influences NIRS results[18] and also because a majority of signal may come from myoglobin rather than hemoglobin.[19] Furthermore, both hemoglobin and myoglobin can change in oxygenation status (i.e., oxygenated vs deoxygenated), but only hemoglobin content can acutely increase or decrease in the sampled tissue. Thus we interpreted changes in deoxygenation/oxygenation status (deoxy[hemoglobin + myoglobin], [HHb] and oxy[hemoglobin + myoglobin], [OHb]) as changes in *muscle* oxygenation, and interpreted changes in local signal intensity (total hemoglobin, [tHb]) as a change in a composite of local blood volume and microvascular hematocrit.

Our overarching hypothesis was that during exercise, either reduced blood flow, reduced local recruitment of microvascular blood volume/hematocrit, or both contribute to impaired oxygen delivery to skeletal muscle in type 2 diabetes. Based on our assessments of oxygen delivery and interpretations of NIRS signals, we formulated several sub-hypotheses to test the relationship of the convective and diffusive steps in oxygen delivery to reduced exercise capacity in type 2 diabetes:

- *Sub-hypothesis:* RH leg blood flow correlates with VO_{2peak} in both type 2 diabetes and in BMI-matched controls.
  - *Interpretation:* blood flow limits oxygen uptake with or without type 2 diabetes.
  - *Rationale:* oxygen uptake cannot exceed oxygen delivery.
- *Sub-hypothesis:* increase in total hemoglobin correlates with VO_{2peak} in type 2 diabetes but not in BMI-matched controls.
  - *Interpretation:* local recruitment of microvascular blood volume/hematocrit limits oxygen diffusion only in type 2 diabetes.
  - *Rationale:* reduced microvascular recruitment could explain blood-flow independent limitations in tissue oxygenation.
- *Sub-hypothesis:* RH leg blood flow correlates with increase in total hemoglobin in both type 2 diabetes and in BMI-matched controls.
  - *Interpretation:* blood flow and local recruitment of microvascular blood volume/hematocrit are coordinated.
  - *Rationale:* coordination of central and peripheral cardiovascular responses would maximize the efficiency of oxygen delivery.
- *Sub-hypothesis:* skeletal muscle deoxygenation correlates inversely with VO_{2peak} in type 2 diabetes but not in BMI-matched controls.
  - *Interpretation:* skeletal muscle deoxygenation limits oxygen uptake in type 2 diabetes.
  - *Rationale:* a correlation between muscle oxygenation and oxygen uptake would suggest a muscle-level limitation in oxygen uptake.

2. Methods

2.1. Source of Data

The source of data analyzed in this manuscript is the INSITE study (Reusch (JEBR), Regensteiner (JGR) and Bauer (TAB), unpublished), which was designed to investigate differences in, and the effects of antioxidant treatment or exercise training on, exercise capacity and insulin sensitivity in overweight, middle aged men and premenopausal women. In this study, middle aged, overweight, and sedentary (defined as <1 hour of exercise per week) subjects either with ($n = 23$) or without ($n = 18$) type 2 diabetes underwent an incremental maximal exercise test on a cycling ergometer to assess VO_{2peak} by metabolic cart (Medgraphics CPX/D, Medical Graphics Corp., St. Paul, MN, USA) (JEB, JGR and TAB manuscript in progress). On a subsequent date, participants also performed two separate five-minute bouts of constant work rate cycling at 85% of lactate threshold, as determined by the V-slope method. Bouts were separated by a 10-min rest period. Changes in muscle concentrations of [tHb], [OHb], and [HHb] were monitored by NIRS for the duration of the constant work rate exercise protocol. Values for [tHb] and [HHb] used in this study were recorded in the vastus lateralis at rest and during constant work rate cycling at 85% of lactate threshold. In addition, maximal blood flow during reactive hyperemia (RH) was recorded using venous occlusion plethysmography. Study subject characteristics are included in Table 1.

2.2. NIRS Data Acquisition

Tissue total hemoglobin + myoglobin ([tHb]), deoxy[hemoglobin + myoglobin] ([HHb]), and oxy[hemoglobin + myoglobin] ([OHb]) were assessed by a frequency domain multi-distance NIRS monitor (Optiplex TS, ISS, Champaign, IL, USA) during each constant work rate exercise test. The NIRS monitor emits two wavelengths (690 and 830 nm) and measures absorbance at distances of 2.0, 2.5, 3.0 and 3.5 cm. The NIRS data were sampled continuously and recorded at 50 Hz. Upon export, data were down-sampled to 1 Hz using a running average of the higher-resolution 50 Hz data. During cycling exercise tests, the NIRS probe was positioned on the distal third of the vastus lateralis of the dominant limb, secured using a Velcro strap, and covered with a cloth bandage to exclude ambient light. The NIRS monitor was calibrated prior to each visit using a calibration phantom of known scattering and optical properties.

2.3. NIRS Data Analysis

Resting values of tissue [tHb], [HHb], and [OHb] were obtained by averaging the 30 s prior to the onset of exercise. Exercise values of these parameters were obtained by averaging values between 270 s and 300 s following the onset of exercise. The absolute change in [tHb], [HHb], and [OHb] from rest to steady-state exercise was recorded as well. The change in [tHb] reflects local recruitment of microvascular blood volume/hematocrit (only the hemoglobin

Table 1
Study subject characteristics.

	Control	T2DM
Age (years)	44.8 ± 6.1	46.9 ± 5.2
Height (cm)	173.1 ± 9.9	174.8 ± 9.5
Weight (kg)	91.2 ± 10.4	92.6 ± 17.5
BMI	30.4 ± 2.7	30.1 ± 3.9
HbA1c (%)	5.3 ± 0.4	6.9 ± 0.8*
% Male	56	78*
Duration of diagnosis (years)	NA	3.9 ± 3.4

All values expressed as mean ± standard deviation.
* indicates $p < 0.05$.