HHS Public Access

Author manuscript

J Diabetes Complications. Author manuscript; available in PMC 2018 August 01.

Published in final edited form as:

J Diabetes Complications. 2017 August ; 31(8): 1311–1317. doi:10.1016/j.jdiacomp.2017.05.004.

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

P. Mason McClatchey[a,b,c], **Timothy A. Bauer**[d], **Judith G. Regensteiner**[d,e], **Irene E. Schauer**[a,c], **Amy G. Huebschmann**[d,e], and **Jane E.B. Reusch**[a,c,e,*]

[a]Division of Endocrinology, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

[b]Department of Bioengineering, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

[c]Department of Medicine, Denver Veterans Affairs Medical Center, Denver, CO, United States

[d]Division of General Internal Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

[e]Center for Women's Health Research, University of Colorado Anschutz Medical Campus, Aurora, CO, United States

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.

[*]Corresponding author at: Denver VAMC, ENDO-111H, Rm 9c120b, 1055 Clermont St, Denver, CO 80220, United States. Tel.: +1 303 399 8020x2775/3137; fax: +1 303 393 5271. jane.reusch@ucdenver.edu (J.EB. Reusch).

Disclosures: There is no conflict of interest related to this work for P.M.M., T.A.B., J.G.R., I.E.S., A.G.H., or J.E.B.R.

Author Contributions

P.M.M. performed the analysis and wrote the manuscript. T.A.B., helped design the analysis, helped design the original study, and extensively edited the manuscript. J.G.R. designed the original study, provided data, and edited the manuscript. I.E.S., designed the original study, provided data, and edited the manuscript. A.G.H. assisted in interpretation of the data and edited the manuscript, and J.E.B.R. designed the original study, mentored P.M.M, assisted in interpretation of the data, and edited the manuscript.

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.

Keywords

Type 2 diabetes; Exercise capacity; Near-infrared spectroscopy; Oxygen delivery; Oxygen transport; Perfusion heterogeneity

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 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 portion of the [tHb] signal can change acutely). The changes in [HHb] (deoxy[hemoglobin + myoglobin] accumulation) and [OHb] (oxy[-hemoglobin + myoglobin] depletion) represent changes in local skeletal muscle deoxygenation and oxygen availability, respectively. Data which included negative values for concentrations of any hemoglobin species were discarded. A visualization of the NIRS parameters employed in this analysis is included in Fig. 1.

2.4. Data Analysis

Values for [OHb] depletion (as assessed by change from rest to steady-state exercise), [HHb] accumulation (as assessed by change from rest to steady-state exercise), increase in total hemoglobin, and RH blood flow were compared between type 2 diabetes and control groups. Increases in total hemoglobin and VO_2peak were compared within each group, as were [HHb] accumulation and VO_2peak, RH blood flow and VO_2peak, and RH blood flow and increase in total hemoglobin. Unpaired t-tests were used for between-group comparisons, and a standard f-test was used to assess possible differences in group variance. Where significant differences in variance were found ($p < 0.05$), Welch's correction[20] was applied to the original t-test to compare group means. Pearson's R was used to assess the significance of correlations. Because each hypothesis was assessed independently (i.e., single comparison for each hypothesis), multiple comparison corrections were not used in this analysis.

3. Results

3.1. Group Differences in Skeletal Muscle Deoxygenation/Oxygenation and Limb Blood Flow

Group comparisons of muscle deoxygenation/oxygenation, RH limb blood flow, and local recruitment of microvascular blood volume/hematocrit are shown in Fig. 2. [HHb] accumulation from rest to steady-state exercise is significantly increased in type 2 diabetes relative to control ($p = 0.003$, Fig. 2A), consistent with our hypothesis of increased skeletal muscle deoxygenation in type 2 diabetes. [OHb] depletion from rest to steady-state exercise is significantly increased in type 2 diabetes relative to control ($p = 0.01$, Fig. 2B), consistent with our hypothesis of impaired skeletal muscle oxygen availability during exercise in type 2 diabetes. No significant difference (p = NS) was observed between type 2 diabetes and control in increase in total hemoglobin, failing to support our hypothesis of impaired blood volume recruitment in type 2 diabetes. There was also no significant difference observed (p = NS) between type 2 diabetes and control in RH blood flow, failing to support our hypothesis of impaired limb blood flow in type 2 diabetes. Variance was significantly increased ($p < 0.05$) in type 2 diabetes for all local NIRS metrics (increase in [tHb], [HHb] accumulation, and [OHb] depletion), but not for RH blood flow (p = NS).

3.2. Within-Group Comparisons of Muscle Deoxygenation and Increase in Total Hemoglobin to VO_2peak

Comparisons within each group of deoxygenation and increase in total hemoglobin metrics to VO_2peak are shown in Fig. 3 below. Increase in total hemoglobin significantly correlates

($p = 0.027$) with VO_2peak in control subjects (Fig. 3A) but not in type 2 diabetes subjects (Fig. 3B, p= NS), failing to support our hypothesis that local recruitment of microvascular blood volume/hematocrit limits oxygen uptake in type 2 diabetes but not in controls. Local deoxygenation significantly correlates ($p = 0.0036$) with VO_2peak in control subjects (Fig. 3C) and does not correlate (p = NS) with VO_2peak in type 2 diabetes (Fig. 3D), failing to support our hypothesis that skeletal muscle deoxygenation limits VO_2peak in type 2 diabetes but not in controls.

3.3. Within-Group Comparisons of RH Blood Flow with VO_2peak and Increase in Total Hemoglobin

Comparisons of the correlations of the various predictor variables used in this analysis with VO_{2peak} or increase in total hemoglobin within each group are shown in Fig. 4. RH blood flow correlates significantly ($p = 0.002$) with VO_2peak in controls (Fig. 4A) but not in type 2 diabetes (p = NS, Fig. 4B), consistent with our hypothesis that blood flow limits oxygen uptake in controls, but failing to support our hypothesis that blood flow limits oxygen uptake in type 2 diabetes. RH blood flow also correlates significantly ($p = 0.018$) with increase in total hemoglobin in controls (Fig. 4C) but not in type 2 diabetes (p = NS, Fig. 4D), consistent with our hypothesis that limb blood flow and local recruitment of microvascular blood volume/hematocrit are coordinated in controls, but failing to support our hypothesis that the same is true in type 2 diabetes.

4. Discussion

In this analysis, we found evidence that skeletal muscle oxygen availability is reduced and skeletal muscle deoxygenation is increased in exercise with type 2 diabetes as compared to a similarly overweight and sedentary control group, consistent with previous reports.[9,10,21,22] We also found that the increase in total hemoglobin during exercise is not reduced in type 2 diabetes. This finding is consistent with reports of preserved microvascular recruitment (measured using contrast enhanced ultrasound) during exercise with type 2 diabetes.[23] We also found that RH blood flow was not impaired in type 2 diabetes. Previous studies have found conflicting results when investigating blood flow limitations in type 2 diabetes,[12,16] and the degree to which health status differs between disease and control groups may account for these inconsistencies. Finally, we observe that variance of all local oxygenation metrics ([HHb] accumulation, [OHb] depletion, and increase in [tHb]) was increased in type 2 diabetes, despite no increase in variance of global or whole-limb oxygen transport metrics (VO_{2peak} and RH blood flow). Collectively, these results suggest that skeletal muscle oxygenation during exercise is impaired in type 2 diabetes, and that this impairment does not appear to be related to either decreased limb blood flow or local recruitment of microvascular blood volume/hematocrit.

Our correlation analysis returned unexpected results. As hypothesized, maximal RH blood flow and VO_{2peak} were correlated in control subjects, but this correlation was lost in type 2 diabetes. Meanwhile, increase in total hemoglobin was correlated with VO_{2peak} in control subjects but not in type 2 diabetes, contrary to our hypothesis that local recruitment of microvascular blood volume/hematocrit would limit exercise capacity only in type 2

diabetes. The recurring theme of correlation in health and dissociation in type 2 diabetes continued with dissociation of skeletal muscle deoxygenation from VO_{2peak} and dissociation of maximal RH blood flow from increase in total hemoglobin. Collectively, these findings suggest that in type 2 diabetes, local metrics of tissue oxygen transport do not reflect whole-limb or whole-body oxygen transport, blood flow is dissociated from local recruitment of microvascular blood volume/hematocrit, and factors besides bulk blood flow and local recruitment of microvascular blood volume/hematocrit are likely to limit oxygen uptake during exercise. Any plausible and complete explanation of these data must satisfy three observations: 1) skeletal muscle oxygenation is impaired in type 2 diabetes, 2) skeletal muscle oxygenation is more heterogeneous in type 2 diabetes, and 3) these changes appear to be unrelated to bulk blood flow or local recruitment of microvascular blood volume/hematocrit.

While our NIRS data do not allow us to draw definitive conclusions about the causes of impaired skeletal muscle oxygenation in type 2 diabetes, the literature does provide a plausible explanation that could satisfy all three of these requirements. Distribution of blood flow is more spatially heterogeneous in the obese Zucker rat model of type 2 diabetes, and this perfusion heterogeneity is associated with impaired oxygen uptake.[14,15,24,25] Simulation studies reveal that heterogeneous perfusion results in impaired muscle oxygenation on average, because over-perfused vessels cannot fully compensate for under-perfused vessels.[15,26–28] By definition, heterogeneous perfusion not resulting from heterogeneous tissue demand would result in flow/VO_2 mismatch. Moreover, these effects would influence skeletal muscle deoxygenation/oxygenation even in cases of normal bulk blood flow and local recruitment of microvascular blood volume/hematocrit. Although the findings referenced above have not yet been translated to human type 2 diabetes, it has been shown in humans that increased perfusion heterogeneity correlates with reduced oxygen extraction, and perfusion heterogeneity decreases in response to endurance training,[29,30] supporting the plausibility of this explanation. Moreover, the effects of age on the vasculature, which parallel the influence of type 2 diabetes,[11,31] are themselves spatially heterogeneous.[32] Visualizations of skeletal muscle perfusion in type 2 diabetes reported by Zheng et al.[33] appear more heterogeneous qualitatively.

It is worth noting, however, that the studies cited in the previous paragraph pertain to *spatial* heterogeneity within a single muscle, whereas the heterogeneity we observed in our NIRS was measured as *population* heterogeneity, and only one site per muscle was assessed. Although spatial heterogeneity could produce population heterogeneity in local but not global metrics as a statistical artifact if only one site were observed (which is exactly what our study design entailed), we cannot rule out the possibility that population heterogeneity was observed due to variance among study subjects rather than observed as an artifact of variance among locations. Future studies will be required to test the hypothesis that heterogeneous distribution of blood flow on both microvascular and macrovascular scales during exercise may contribute to reduced oxygen delivery to skeletal muscle in type 2 diabetes. Additionally, the NIRS signal heterogeneity we observed may also have been caused by heterogeneous muscle metabolism rather than heterogeneous blood flow. Future studies with alternative measurement methods (e.g., arterial spin labeling) will be required to

determine the relative contributions of spatial heterogeneities in metabolism and blood flow to potential blood flow/metabolism mismatch in type 2 diabetes.

There are some limitations of our study design. First, leg blood flow, VO_{2peak}, and skeletal muscle oxygenation measures were each recorded under separate conditions (following venous occlusion, during maximal cycling exercise, and during submaximal cycling exercise, respectively). It is possible that these discrepancies influenced our results. However, given that these disparate metrics were correlated in health but not in type 2 diabetes, the recurring theme of dissociation in type 2 diabetes remains relevant. The use of NIRS to assess skeletal muscle deoxygenation/oxygenation may also introduce complexity to the interpretation of our data, given that there is controversy in the field about the relative contributions of hemoglobin (i.e., vascular) and myoglobin (i.e., muscular) contributions to the NIRS signal.[19] Our analysis avoided this issue by reporting local deoxygenation/oxygenation without any attempt to distinguish vascular/intramuscular contributions and by reporting only the change in [tHb] from rest to exercise as a vascular-specific measure. This parameter must be vascular in origin, given that myoglobin does not acutely enter or leave the muscle upon contraction. Additionally, NIRS measurements are subject to influences from adipose tissue.[18] If excess subcutaneous adipose tissue in type 2 diabetes patients influenced our results, we would expect to see less deoxygenation and less hemoglobin recruitment, both as a result of there being less skeletal muscle in the optode light path. In fact, we found that the degree of deoxygenation observed was greater and the degree of microvascular recruitment was preserved in type 2 diabetes. Thus, it is unlikely that differences in subcutaneous adipose tissue thickness confounded our results.

Finally, the sex imbalance in our type 2 diabetes group would be expected to influence our results. Women generally have more adipose tissue, thus likely reducing the magnitude of NIRS signals, and also may experience greater age-related reduction in capacity for maximal blood flow.[34] If sex differences were driving the observed correlations in health and dissociations in type 2 diabetes, however, one would expect *reduced* variance in the type 2 diabetes group, and in fact we observed *increased* variance in type 2 diabetes. Thus the dissociations in type 2 diabetes occurred despite a greater dynamic range over which correlations could be observed. Additionally, we did not find a statistically significant sex difference in any NIRS parameter within the control group (all $p > 0.05$, data not shown), thus making it improbable that sex differences accounted for the associations observed in the control group. Although the data presented in this paper do not allow us to definitively rule out the possibility that sex differences may substantially alter the interpretation of the NIRS signal, no sex differences are required to account for the type 2 diabetes related differences that we observed. Additional studies with larger sample sizes will be required to assess the possibility that the findings reported here generalize to within-sex comparisons.

In summary, we found that skeletal muscle oxygenation during exercise is impaired in type 2 diabetes, and that this impairment can occur independently of changes in limb blood flow or local recruitment of microvascular blood volume/hematocrit. We also found that correlations between local and global oxygen transport metrics were abolished in type 2 diabetes, and that local muscle oxygenation is more heterogeneous. Although our data do not allow definitive conclusions as to the cause of these changes, it is plausible that heterogeneous

blood flow distribution may account for dissociation of local and global oxygen transport in type 2 diabetes. Future studies will be required to more fully understand the heterogeneity and impaired coordination of skeletal muscle oxygenation in type 2 diabetes. In light of the previously discussed association of impaired exercise capacity with premature mortality and excess morbidity,[3–5] understanding the mechanisms leading to impaired oxygen transport in type 2 diabetes holds great translational potential.

Acknowledgments

Sources of Funding: Sources of funding for this study were provided by CCTSI-UL1RR025780 (JEBR, MS, KSH and PMM), the Center for Women's Health Research (JEBR, JGR), VA Merit Review (JEBR) and the Department of Bioengineering (PMM), American Diabetes Association 1-12-CT-64 (JGR).

The authors wish to acknowledge Drs. Amy Keller, Rebecca Scalzo, Pete Watson, Robert Roach, and Andrew Subudhi, all at the University of Colorado Anschutz Medical Campus, for their support and input during creation of this manuscript.

References

1. National Diabetes Statistics Report: estimates of diabetes and its burden in the United States, 2014. Atlanta, GA: U.S. Department of Health and Human Services, Centers For Disease Control And Prevention; 2014.
2. Narayan KM, Boyle JP, Thompson TJ, Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. JAMA. 2003; 290:1884–90. [PubMed: 14532317]
3. Church TS, Cheng YJ, Earnest CP, Barlow CE, Gibbons LW, et al. Exercise capacity and body composition as predictors of mortality among men with diabetes. Diabetes Care. 2004; 27:83–8. [PubMed: 14693971]
4. Myers J, Prakash M, Froelicher V, Do D, Partington S, Atwood JE. Exercise capacity and mortality among men referred for exercise testing. N Engl J Med. 2002; 346:793–801. [PubMed: 11893790]
5. Nylen ES, Kokkinos P, Myers J, Faselis C. Prognostic effect of exercise capacity on mortality in older adults with diabetes mellitus. J Am Geriatr Soc. 2010; 58:1850–4. [PubMed: 20929462]
6. Reusch JE, Bridenstine M, Regensteiner JG. Type 2 diabetes mellitus and exercise impairment. Rev Endocr Metab Disord. 2013; 14:77–86. [PubMed: 23299658]
7. Estacio RO, Regensteiner JG, Wolfel EE, Jeffers B, Dickenson M, Schrier RW. The association between diabetic complications and exercise capacity in NIDDM patients. Diabetes Care. 1998; 21:291–5. [PubMed: 9539998]
8. Nadeau KJ, Zeitler PS, Bauer TA, Brown MS, Dorosz JL, et al. Insulin resistance in adolescents with type 2 diabetes is associated with impaired exercise capacity. J Clin Endocrinol Metab. 2009; 94:3687–95. [PubMed: 19584191]
9. Behnke BJ, Kindig CA, McDonough P, Poole DC, Sexton WL. Dynamics of microvascular oxygen pressure during rest-contraction transition in skeletal muscle of diabetic rats. Am J Physiol Heart Circ Physiol. 2002; 283:H926–32. [PubMed: 12181120]
10. Bauer TA, Reusch JE, Levi M, Regensteiner JG. Skeletal muscle deoxygenation after the onset of moderate exercise suggests slowed microvascular blood flow kinetics in type 2 diabetes. Diabetes Care. 2007; 30:2880–5. [PubMed: 17675540]
11. Groen BB, Hamer HM, Snijders T, van Kranenburg J, Frijns D, et al. Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes. J Appl Physiol (1985). 2014; 116:998–1005. [PubMed: 24577061]
12. Kingwell, BA., Formosa, M., Muhlmann, M., Bradley, SJ., McConell, GK. Type 2 diabetic individuals have impaired leg blood flow responses to exercise. 2003.
13. Padilla, DJ., McDonough, P., Behnke, BJ., Kano, Y., Hageman, KS., et al. Effects of type II diabetes on capillary hemodynamics in skeletal muscle. 2006.

14. Frisbee JC, Wu F, Goodwill AG, Butcher JT, Beard DA. Spatial heterogeneity in skeletal muscle microvascular blood flow distribution is increased in the metabolic syndrome. Am J Physiol Regul Integr Comp Physiol. 2011; 301:R975–86. [PubMed: 21775645]
15. McClatchey PM, Wu F, Olfert IM, Goldman E, Reusch JEB, Frisbee JC. Impaired tissue oxygenation in metabolic syndrome requires increased microvascular perfusion heterogeneity. J Cardiovasc Transl Res. 2017; 10:69–81. [PubMed: 28168652]
16. Baldi JC, Aoina JL, Oxenham HC, Bagg W, Doughty RN. 2003. Reduced exercise arteriovenous O_2 difference in type 2 diabetes. J Appl Physiol. 1985; 94:1033–8.
17. Kindig CA, Sexton WL, Fedde MR, Poole DC. Skeletal muscle microcirculatory structure and hemodynamics in diabetes. Respir Physiol. 1998; 111:163–75. [PubMed: 9574868]
18. van Beekvelt MC, Borghuis MS, van Engelen BG, Wevers RA, Colier WN. Adipose tissue thickness affects in vivo quantitative near-IR spectroscopy in human skeletal muscle. Clin Sci (Lond). 2001; 101:21–8. [PubMed: 11410110]
19. Davis ML, Barstow TJ. Estimated contribution of hemoglobin and myoglobin to near infrared spectroscopy. Respir Physiol Neurobiol. 2013; 186:180–7. [PubMed: 23357615]
20. Welch BL. The generalization of student's' problem when several different population variances are involved. Biometrika. 1947; 34:28–35. [PubMed: 20287819]
21. Tagougui S, Leclair E, Fontaine P, Matran R, Marais G, et al. Muscle oxygen supply impairment during exercise in poorly controlled type 1 diabetes. Med Sci Sports Exerc. 2015; 47:231–9. [PubMed: 24983346]
22. Frisbee JC. Impaired dilation of skeletal muscle microvessels to reduced oxygen tension in diabetic obese Zucker rats. Am J Physiol Heart Circ Physiol. 2001; 281:H1568–74. [PubMed: 11557545]
23. Womack L, Peters D, Barrett EJ, Kaul S, Price W, Lindner JR. Abnormal skeletal muscle capillary recruitment during exercise in patients with type 2 diabetes mellitus and microvascular complications. J Am Coll Cardiol. 2009; 53:2175–83. [PubMed: 19497445]
24. Frisbee JC, Goodwill AG, Butcher JT, Olfert IM. Divergence between arterial perfusion and fatigue resistance in skeletal muscle in the metabolic syndrome. Exp Physiol. 2011; 96:369–83. [PubMed: 21123363]
25. Frisbee JC, Goodwill AG, Frisbee SJ, Butcher JT, Wu F, Chantler PD. Microvascular perfusion heterogeneity contributes to peripheral vascular disease in metabolic syndrome. J Physiol. 2014
26. Jespersen SN, Østergaard L. The roles of cerebral blood flow, capillary transit time heterogeneity, and oxygen tension in brain oxygenation and metabolism. J Cereb Blood Flow Metab. 2012; 32:264–77. [PubMed: 22044867]
27. Ostergaard L, Kristiansen SB, Angleys H, Frøkiær J, Michael Hasenkam J, et al. The role of capillary transit time heterogeneity in myocardial oxygenation and ischemic heart disease. Basic Res Cardiol. 2014; 109:409. [PubMed: 24743925]
28. McClatchey, PM., Frisbee, JC., Reusch, JEB. A conceptual framework for predicting and addressing the consequences of disease-related microvascular dysfunction. Microcirculation. 2017. http://dx.doi.org/10.1111/micc.12359. [in press]
29. Kalliokoski KK, Oikonen V, Takala TO, Sipilä H, Knuuti J, Nuutila P. Enhanced oxygen extraction and reduced flow heterogeneity in exercising muscle in endurance-trained men. Am J Physiol Endocrinol Metab. 2001; 280:E1015–21. [PubMed: 11350784]
30. Kalliokoski KK, Knuuti J, Nuutila P. Blood transit time heterogeneity is associated to oxygen extraction in exercising human skeletal muscle. Microvasc Res. 2004; 67:125–32. [PubMed: 15020203]
31. Stansberry KB, Hill MA, Shapiro SA, McNitt PM, Bhatt BA, Vinik AI. Impairment of peripheral blood flow responses in diabetes resembles an enhanced aging effect. Diabetes Care. 1997; 20:1711–6. [PubMed: 9353614]
32. Barton M, Cosentino F, Brandes RP, Moreau P, Shaw S, Lüscher TF. Anatomic heterogeneity of vascular aging: role of nitric oxide and endothelin. Hypertension. 1997; 30:817–24. [PubMed: 9336378]
33. Zheng J, Hasting MK, Zhang X, Coggan A, An H, et al. A pilot study of regional perfusion and oxygenation in calf muscles of individuals with diabetes with a noninvasive measure. J Vasc Surg. 2014; 59:419–26. [PubMed: 24080129]

34. Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Proctor DN. Sex-specific influence of aging on exercising leg blood flow. J Appl Physiol (1985). 2008; 104:655–64. [PubMed: 18162481]

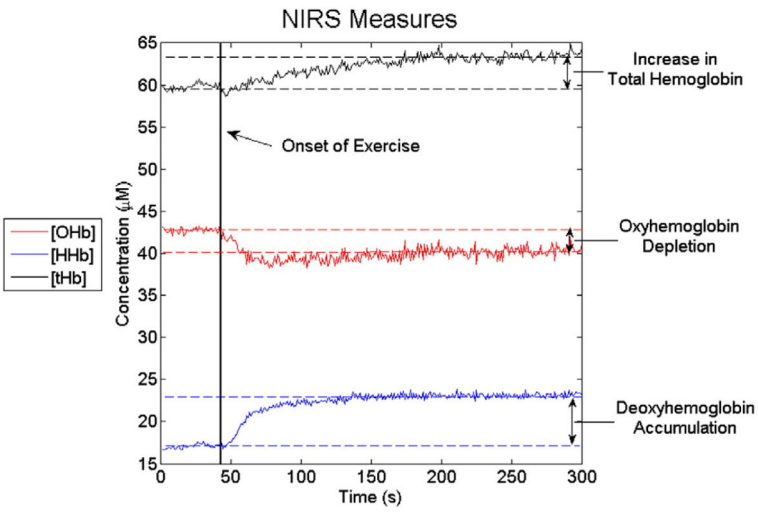

Fig. 1.

Illustration of NIRS measures used for analysis. Following the onset of exercise, oxyhemoglobin (OHb) acutely decreases and then gradually returns partway to baseline. The depletion of oxy(hemoglobin + myoglobin) at steady-state was recorded as a metric of oxygen availability. Deoxy(hemoglobin + myoglobin) (HHb), meanwhile, increases gradually to steady state. The increase in [HHb] was recorded as a metric of muscle deoxygenation. Finally, total hemoglobin + myoglobin (tHb) comprises the sum of the [OHb] and [HHb] signals. The increase in [tHb] from rest to steady state exercise reflects the local increase in total hemoglobin concentration (i.e., local recruitment of microvascular blood volume/hematocrit).

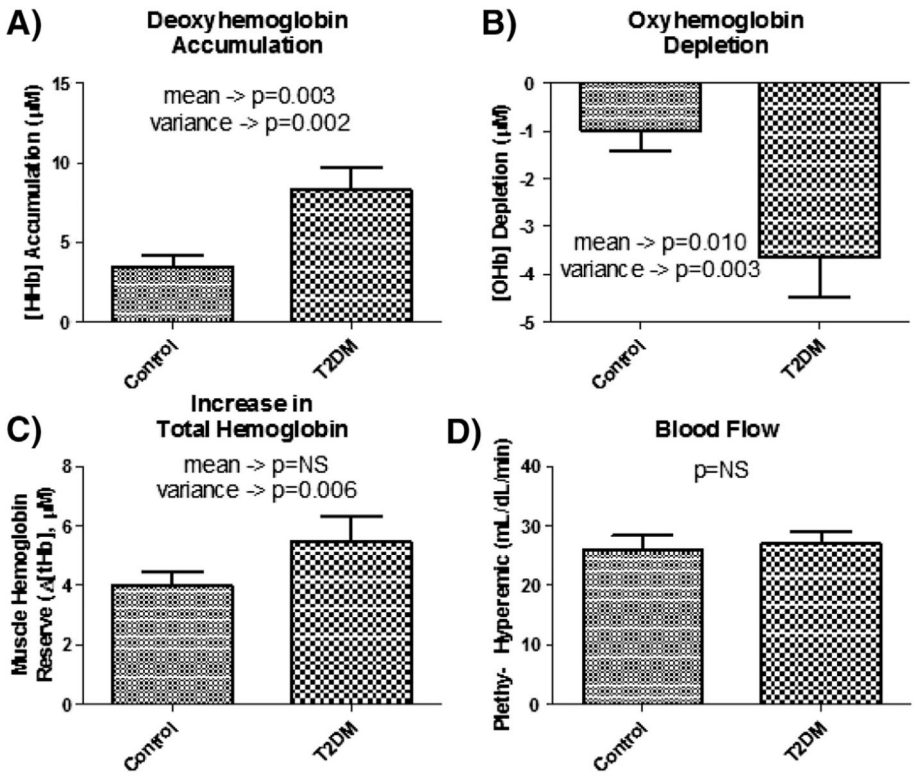

Fig. 2.

Impaired muscle oxygenation despite normal RH blood flow and increase in total hemoglobin in type 2 diabetes. (A) [HHb] accumulation is significantly ($p = 0.003$) increased in type 2 diabetes. In addition, the variance of [HHb] accumulation is significantly ($p = 0.002$) increased in type 2 diabetes. (B) [OHb] depletion is significantly ($p = 0.01$) increased in type 2 diabetes. In addition, the variance of [OHb] depletion is significantly ($p = 0.003$) increased in type 2 diabetes. (C) Increase in total hemoglobin is not significantly different from controls in type 2 diabetes (p = NS). However, the variance in the increase in total hemoglobin is significantly ($p = 0.006$) increased in type 2 diabetes. (D) RH blood flow does not significantly differ (p = NS) between controls and type 2 diabetes.

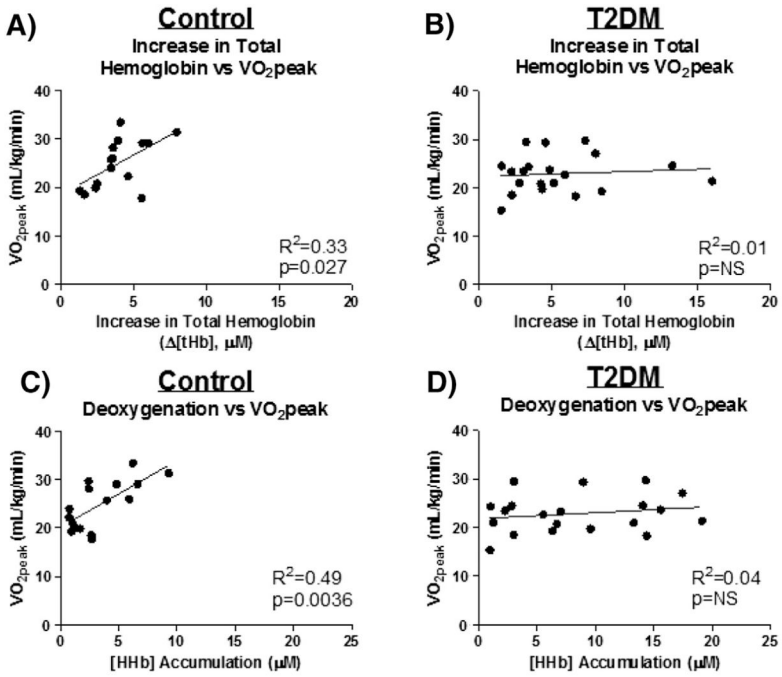

Fig. 3.

Increase in total hemoglobin and muscle deoxygenation do not correlate with VO_2peak in type 2 diabetes. (A) Increase in total hemoglobin and VO_2peak are significantly ($p = 0.027$) correlated in control subjects. (B) Increase in total hemoglobin and VO_2peak are not correlated (p = NS) in type 2 diabetes. (C) Local deoxygenation and VO_2peak are significantly ($p = 0.0036$) correlated in control subjects. (D) Local deoxygenation and VO_2peak are not correlated (p = NS) in type 2 diabetes.

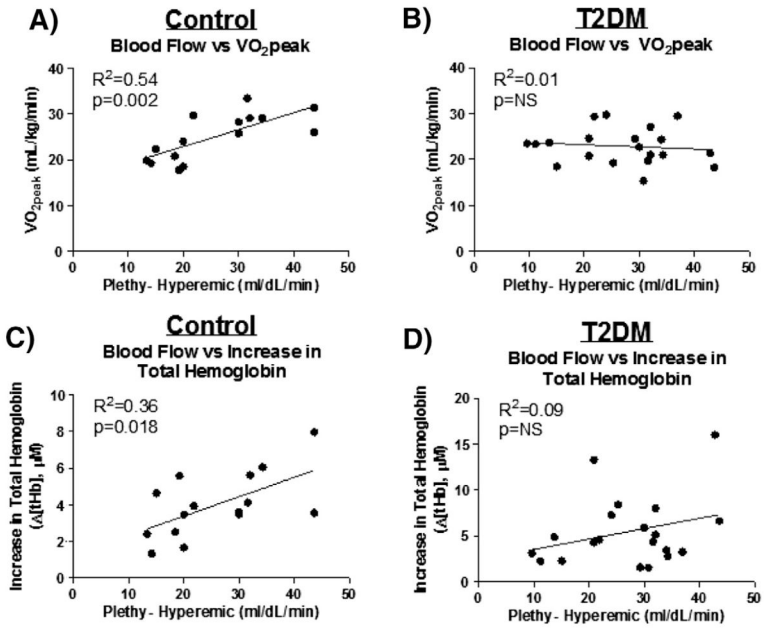

Fig. 4.

Mutual correlations between RH blood flow, increase in total hemoglobin, and VO_2peak are abolished in type 2 diabetes. (A) RH blood flow and VO_2peak are significantly correlated (p = 0.002) in control subjects. (B) RH blood flow and VO_2peak are not correlated (p = NS) in type 2 diabetes. (C) RH blood flow and increase in total hemoglobin are significantly correlated (p = 0.018) in control subjects. (D) RH blood flow and increase in total hemoglobin are not correlated (p = NS) in type 2 diabetes.

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 $p < 0.05$.