



# Men exhibit faster skeletal muscle tissue desaturation than women before and after a fatiguing handgrip

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## Abstract

**Purpose** The purpose was to test the hypothesis that sex and fatigue effect of the early phase of skeletal muscle tissue oxygenation (StO<sub>2</sub>, %) desaturation rate as well as that strength matched adults may exhibit similar responses.

**Methods** Twenty-four adults visited the laboratory twice to quantify this early phase of desaturation during vascular occlusion tests (VOT) while in a rested state. The second visit included a sustained handgrip task at 25% of maximal muscular strength until task failure. At failure, a post-task VOT was initiated. Muscle desaturation was defined as StO<sub>2</sub> and collected by a near-infrared spectroscopy device. The muscle size and adipose thickness were determined via ultrasonography. Linear regression was used to quantify the rates of desaturation during the VOTs as well as during the fatiguing handgrip.

**Results** There were sex differences in the rate of desaturation pre- and post-handgrip, such that independent of fatigue, the men ( $p < 0.001$ ) desaturated more rapidly than the women (pre:  $b = -0.208$  vs.  $-0.123\% \cdot s^{-1}$ ; post:  $-0.079$  vs.  $-0.070\% \cdot s^{-1}$ ). During the fatiguing handgrip, the transformed StO<sub>2</sub> values indicated that the males desaturated more rapidly than the females ( $b = -0.070$  vs.  $-0.015$ ). The matched pairs exhibited the same responses as the total sample.

**Conclusion** Overall, muscle size and strength as well as adipose tissue were likely not the primary cause of the differences in rates of muscle desaturation. We hypothesized that differences in fiber type and mitochondria were the principle mechanisms provoking the differences in muscle oxygenation.

**Keywords** Sex differences · Fatigue · Oxygen delivery · Exercise

## Abbreviations

ICC	Intraclass correlation coefficient
MTT	Muscle tissue thickness
MVIC	Maximal voluntary isometric contraction
NIRS	Near-infrared spectroscopy
O <sub>2</sub>	Oxygen
StO <sub>2</sub>	Skeletal muscle tissue oxygenation
TTF	Time to task failure
VOT	Vascular occlusion test

## Introduction

Near-infrared spectroscopy (NIRS) has garnered, significant interest pertaining to its ability to provide both static and dynamic quantifications of microvascular function including measurements of oxygen (O<sub>2</sub>) kinetics during vascular occlusion tests (VOT) (Townsend et al. 2019; Nelson 2020; Rosenberry and Nelson 2020; Horiuchi and Okita 2020; Soares et al. 2021). For example, during a VOT, Townsend et al., (2019) utilized the rate of decline in skeletal muscle tissue oxygenation (StO<sub>2</sub>, %) as an index of aerobic metabolism. Quantifications such as this (Townsend et al. 2019) are based on the theory that myoglobin is typically completely saturated, and thus, acute changes in StO<sub>2</sub> can be attributed to variations in oxyhemoglobin and deoxyhemoglobin (Boushel and Piantadosi 2000; Barstow 2019). This has been applied to investigate a 2-min segment of a total ischemic period to examine differences in StO<sub>2</sub> among groups of young, midlife, and older women (Horiuchi and Okita 2020). However, there still remains a need for additional studies in which sex is included as a factor (Hunter

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2016; Ansdell et al. 2020). Currently, there are limited studies that have used NIRS to investigate potential sex differences in muscle oxygenation characteristics (Beltrame et al. 2017; Mantooth et al. 2018; Ansdell et al. 2019), especially by examining differences in the rate of StO<sub>2</sub> desaturation despite known differences in microvascular and mitochondria function (Miotto et al. 2018; Cardinale et al. 2018, 2019; Skattebo et al. 2020). It has been reported, however, that females exhibited 19% and 22% faster peripheral O<sub>2</sub> extraction and desaturation, respectfully, than males during moderate walking exercise tests (Beltrame et al. 2017). It has been hypothesized, however, that aerobic fitness and adipose tissue thickness likely provoke differences in NIRS-derived outcomes between males and females (Van Beekvelt et al. 2001; Niemeijer et al. 2017; Mantooth et al. 2018; Barstow 2019; Ansdell et al. 2019; Beever et al. 2020). For example, Beever et al. (2020) demonstrated that NIRS-derived muscle oxidative capacity was similar between males and females only when matched for relative VO<sub>2max</sub> (mL O<sub>2</sub> kg lean body mass<sup>-1</sup> min<sup>-1</sup>). This may in part be related to females typically presenting greater quantities of adipose tissue than males, which could be the major factor leading to their lower relative VO<sub>2max</sub> (normalized to total body mass) values. It should also be noted that a relationship ( $r=0.574$ ) between adipose tissue (measured with skin calipers) and resting StO<sub>2</sub> has been reported, and therefore, there is rationale to speculate that males and females of different body composition may present differences in NIRS responses (Niemeijer et al. 2017). While Beever et al. (2020) controlled for sex differences in aerobic fitness, investigations controlling for muscle mass and strength as well as adipose tissue thickness remain warranted. Thus, during VOTs, we intended to address this need by quantifying the early phase of StO<sub>2</sub> desaturation in young, healthy males and females while also determining the possible influence of muscular strength and size as well as adipose tissue thickness.

In theory, a decline in StO<sub>2</sub> indicates an imbalance between tissue O<sub>2</sub> consumption (i.e., VO<sub>2</sub>) and arterial flow, such that the supply did not match the demand (Hammer et al. 2017, 2020). During sustained handgrips to failure at submaximal loads, it has been shown that StO<sub>2</sub> only declined during tasks above 20% of maximal strength as well as that males and females exhibited similar changes across time (Mantooth et al. 2018). However, these interpretations may have been incomplete due to low temporal resolution (Mantooth et al. 2018). Interestingly, Felici et al. (2009) examined a mixed sample of males and females to report that sustaining a 30 s isometric muscle action at 20% of maximal strength resulted in ~10% decline in StO<sub>2</sub>, but these investigators did not assess sex as a factor. There does, however, appear to be a sex difference in the exercise-induced declines in StO<sub>2</sub> (Ansdell et al. 2019), such that males may exhibit greater decreases across time due to differences in O<sub>2</sub>

availability triggered by differences in vasodilatory (Parker et al. 2007) and strength (Barnes 1980) capacities. Due to these reported sex differences in muscle oxygenation during exercise, there may be differences in VOT responses before and after exercise. This is of interest given NIRS techniques and applications are becoming more accessible, yet there still remains discrepancies in signal processing despite a call for uniformity (Barstow 2019). For example, it has been reported that fully, recently activated mitochondria function differently than the mitochondria in a rested condition (Cardinale et al. 2019). However, various research groups have previously normalized NIRS signals collected during exercise via ischemic calibration (e.g., VOTs) before (Beever et al. 2020) or after (Pethick et al. 2021) the exercise bout. Of note, investigations have even normalized NIRS signals to a 30 s average immediately preceding exercise (Beltrame et al. 2017; Ansdell et al. 2019). Thus, there remains a need to (1) report sex differences in NIRS-derived responses during VOTs and (2) determine if the potential sex differences are maintained in both a rested and a fatigued state. We are currently unaware of any studies that have investigated the possible differences in desaturation rates before and after a fatiguing exercise bout in males and females.

Currently, there is a critical need to determine whether a difference in StO<sub>2</sub> desaturation rate exists between males and females as detected by NIRS as well as if this potential difference may be explained by muscle size and strength as well as adipose tissue thickness. As previously alluded, it remains unknown what effect exercise-induced fatigue has on the desaturation rates. Therefore, the purpose of the current study was to examine the effects of sex and fatigue on the early phase of StO<sub>2</sub> desaturation rate. Second, we aimed to further examine the potential sex differences by comparing a subsample of matched males and females. Based on previous studies (Beltrame et al. 2017; Cardinale et al. 2018, 2019; Mantooth et al. 2018; Ansdell et al. 2019; Beever et al. 2020), we hypothesized that during VOTs, males would exhibit faster rates of StO<sub>2</sub> decline than females as well as that fatigue would attenuate this rate independent of sex, perhaps due to overall O<sub>2</sub> availability. Additionally, we hypothesized that the males would exhibit a greater decline in StO<sub>2</sub> during the sustained task.

## Materials and methods

### Ethics approval

The current study was performed according to the ethics standards established by the Declaration of Helsinki 2013 and was approved by the local Institutional Review Board for Human Subjects at the University of South Alabama (IRB#:20-338/1650333-1) on September 21st, 2020. The

study was not registered in a database, and all participants signed an informed consent document prior to completing any experimental protocols.

## Participant characteristics

Twenty-four college-aged, recreationally active (as defined by the American College of Sports Medicine) males and females with no reported cardiovascular, metabolic, neuromuscular, or renal diseases volunteered to participate in this investigation (Table 1). Based on previous reports (Vranish et al. 2017; Ansdell et al. 2019) of sex differences related to VOT and NIRS data, our power analysis ( $\alpha=0.05$ , power=0.8) recommended a total sample of 22 participants. To ensure an adequate power, we enrolled 12 males and 12 females. All participants were instructed to continue their typical dietary, hydration, and medication habits, but were required to abstain from exercise for 48 h before each visit. Additionally, each participant reported that he or she had not consumed any nutritional supplement with an actual or perceived ergogenic effect within the 2 weeks leading up to the investigation. Each participant scheduled his or her visits at approximately the same time of day ( $\pm 2$  h) and completed both visits within 10 days. Three of the twelve female participants reported the use of a daily oral contraceptive. No additional information was collected related to the menstrual cycle phases of the other nine female participants. Based on previous findings (Limberg et al. 2010; Mattu et al. 2020) as well as the purpose of the current study, it was our interpretation the likely variation in menstrual cycle phases did

not substantially affect the current findings (see the section “[Experimental considerations](#)”). Finally, due to the SARS-CoV-2 pandemic and the resulting university and local state guidelines, all participants as well as the investigators wore a self-provided face covering for the entirety of each visit, which likely had little-to-no effect on the current findings (Chan et al. 2020).

## Experimental design

Before and after a fatiguing, handgrip task, we observed the rate of change in forearm StO<sub>2</sub> during 5 min of transient ischemia in males and females. Additionally, the current investigation quantified the StO<sub>2</sub> responses during a handgrip task to examine potential effects of time and sex. To complete these procedures, the participants reported to the laboratory on two separate occasions (Familiarization and Experimental Visit), which allowed for the calculation of test–retest reliability (Weir 2005). In total, each participant completed three VOTs (one during the familiarization visit and two during the experimental visit). A portable NIRS device was worn over the dominant wrist flexor muscle group during each VOT and during exercise, which provided StO<sub>2</sub> values. All testing was performed in a temperature (21–22 °C) and ambient light-controlled room.

## Familiarization visit

During the familiarization visit, the dominant arm (based on throwing preference), height, and body weight were

**Table 1** Mean ( $\pm$ SD) values of participant characteristics and fatiguing task data by sex

	Males ( <i>n</i> =12)	Females ( <i>n</i> =12)	<i>p</i> value ( <i>d</i> )
Age (years)	21.8 $\pm$ 3.0	21.1 $\pm$ 0.9	0.42 (0.316)
Height (cm)	184.4 $\pm$ 7.7	165.2 $\pm$ 3.6	< <b>0.001</b> ( <b>3.19</b> )
Body mass (kg)	85.5 $\pm$ 12.0	71.7 $\pm$ 14.0	<b>0.02</b> ( <b>1.06</b> )
Adipose tissue thickness (cm)	0.47 $\pm$ 0.1	0.66 $\pm$ 0.2	<b>0.02</b> ( <b>1.20</b> )
Muscle tissue thickness (cm)	2.05 $\pm$ 0.2	1.71 $\pm$ 0.2	<b>0.01</b> ( <b>1.70</b> )
Resting StO <sub>2</sub> (%)	68.0 $\pm$ 4.7	70.6 $\pm$ 3.1	0.13 (0.653)
Time to task failure (s)	248.4 $\pm$ 81.2	257.7 $\pm$ 85.8	0.79 (0.111)
Fatiguing task absolute load (kg)	11.7 $\pm$ 2.4	6.5 $\pm$ 1.8	< <b>0.001</b> ( <b>2.45</b> )
	Strength matched		<i>p</i> value ( <i>d</i> )
	Males ( <i>n</i> =6)	Females ( <i>n</i> =6)	
Fatiguing task absolute load (kg)	9.6 $\pm$ 1.6	7.9 $\pm$ 1.3	0.06 (1.16)
Muscle tissue thickness (cm)	1.96 $\pm$ 0.3	1.81 $\pm$ 0.2	0.27 (0.667)
Adipose tissue thickness (cm)	0.48 $\pm$ 0.1	0.65 $\pm$ 0.2	0.06 (1.07)
Time to task failure (s)	276.7 $\pm$ 97.3	276.0 $\pm$ 116.4	0.99 (0.01)
Workload (kg•s)	2166.6 $\pm$ 954.1	2714.3 $\pm$ 1312.1	0.43 (0.48)
Pre MVIC (kg)	38.5 $\pm$ 6.5	31.4 $\pm$ 5.0	0.06 (1.22)

Bolded *p* values indicate significance at the level of  $\alpha=0.05$

recorded. Subsequently, the participants were instructed to quietly rest for 5 min while seated upright on an isokinetic dynamometer (Biodex Systems 3, Biodex Medical Systems, Inc., Shirley, NY, USA) with their dominant arm supported by a commercially available arm attachment. During this quiet rest, a digital ultrasound device (Logiq E R7-Next Gen, GE Healthcare, USA) was used to quantify adipose tissue thickness and muscle tissue thickness (MTT) at the NIRS device attachment site. The NIRS device was fixed to the dominant flexor digitorum superficialis 2 cm distal to the medial epicondyle. Two blood pressure cuffs were then placed on this limb for the execution of the VOT: 1 around the most proximal portion of the upper arm and 1 around the wrist. After the initial 5 min of quiet rest, the participants were instructed to practice generating force with a handgrip dynamometer (microFET Handgrip, Hoggan Scientific, LLC, Salt Lake City, UT, USA) at various estimated relative intensities (e.g., 50% and 75% of maximum) while still seated and their arm supported. Once adequate practice and warm-up had been accomplished, the participants were instructed to complete 2–3, 6 s maximal voluntary isometric contractions (MVICs) trials. A third trial was only used if the maximal force value resulting from trials 1 and 2 differed by  $\geq 5\%$ . One minute of rest was provided between trials and strong verbal encouragement was given during each of the MVIC trials. Following the MVIC trials, a VOT was conducted.

## Experimental visit

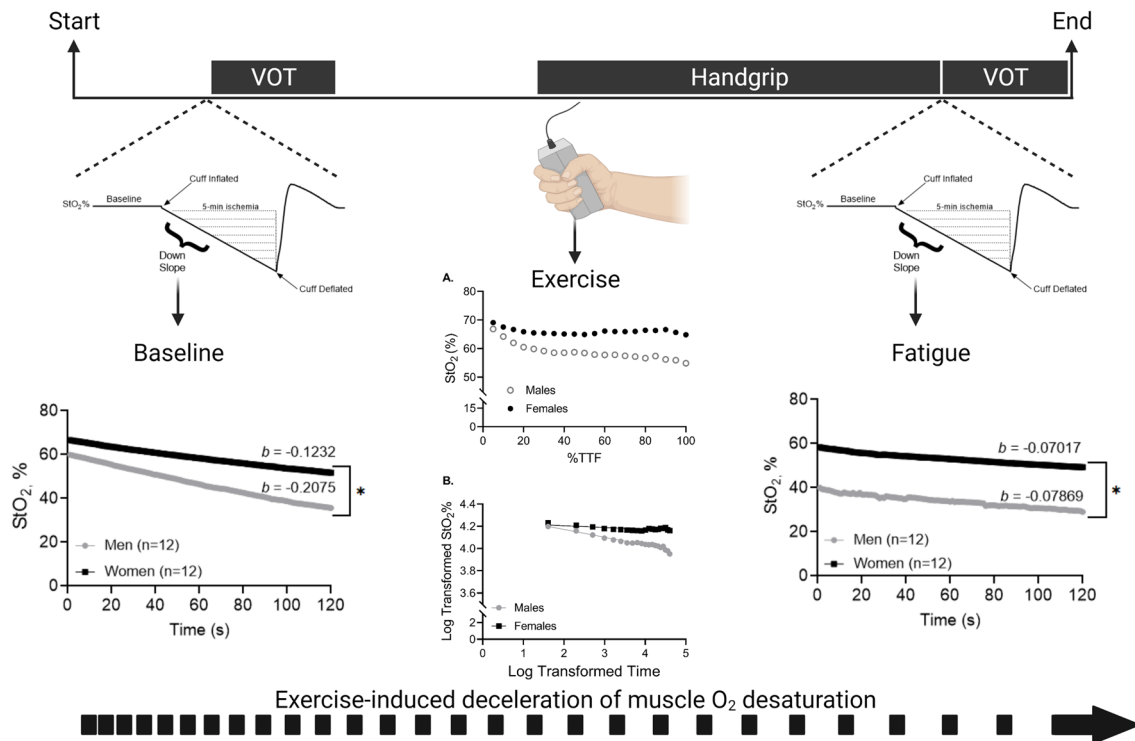
Upon arrival to the laboratory, the participants were again instructed to sit on the isokinetic dynamometer to start the 5 min of quiet rest. Adipose tissue thickness and MTT were again quantified before attaching the NIRS device and cuffs to the dominant limb. After completing the imaging, setup, and rest, the participants completed 2–3 MVIC trials (same procedure as familiarization). Once the MVIC trials were completed, the highest recorded force value was used to define the intensity of the fatiguing handgrip task. This pre-task MVIC was also used to define muscular strength (Table 1). Before starting the handgrip task, a pre-exercise VOT was completed. Following the VOT, the participants rested without either cuff inflated for 3 min prior to starting the handgrip task. The handgrip task was characterized by requiring the participants to generate force at a consistent level of 25% MVIC until volitional failure ( $\geq 5\%$  decline in force for 3 consecutive seconds). Strong verbal encouragement was provided as needed throughout the task. Time to task failure (TTF) was defined as the start of force production to volitional failure. As soon as failure was identified, a post-exercise VOT was immediately initiated.

## Vascular occlusion test

Utilizing the previously mentioned cuff positions, the cuffs were rapidly inflated to a supra-systolic value ( $\sim 200$  mmHg) for 5 min to induce transient ischemia. Cuff inflation time has previously been shown to affect values, and thus, it is important to note that both cuffs were controlled by a rapid ( $< 0.3$  s) inflator/deflator device (E20 Rapid Cuff Inflator, Hokanson Inc., WA, USA). This methodology allowed for isolation the forearm tissue by preventing blood from pooling in the vasculature of the hand. This protocol is well accepted and was chosen to mimic the reactive hyperemia protocol used in investigations of various endothelial-derived vasodilatory pathways (Townsend et al. 2019; Rosenberry and Nelson 2020; Horiuchi and Okita 2020).

## Near-infrared spectroscopy

A portable, dual-wavelength (760 and 850 nm) continuous wave NIRS device (Portamon, Artinis Medical Systems, Elst, Netherlands) captured the  $StO_2$  responses during the VOTs and handgrip task. Derived from the modified Beer-Lambert law, the device provided relative changes in concentrations with respect to the initial baseline value (Barstow 2019). Specifically,  $StO_2$  was calculated via spatially resolved spectroscopy and the equation  $StO_2 = \frac{oxy[heme]}{oxy[heme]+deoxy[heme]} \times 100$  (Barstow 2019). In theory,  $StO_2$  reflected the dynamic balance between  $O_2$  supply and consumption. The NIRS device was attached to the same location as previously described for ultrasound imaging. It was wrapped and secured with self-adhesive black Coban™ to the forearm, and reasonable efforts were made to minimize exogenous light for reaching the photodiode. During the VOTs and handgrip task, signal quality was continuously assessed by viewing the percentage of light reaching the photodiode and the signal fit factor. These data were collected at a sampling rate of 10 Hz with a differential path-length factor (DPF) of 4.0, which has been shown to reflect the average DPF in the human forearm (Van Beekvelt et al. 2001). During each VOT, the rate of desaturation (downslope) was assessed (Fig. 1) and was calculated across 120 s (30–150 s) during the 5 min of ischemia (Soares et al. 2019, 2021; Horiuchi and Okita 2020). To ensure similar baseline  $StO_2$  values for the males and females, the resting  $StO_2$  was quantified as the average of the last 30 s of rest immediately prior to the cuff inflation. As for the  $StO_2$  values during the handgrip task, responses were reported in standardized segments of 5% TTF yielding 20 time points across time due to the varying TTF.



**Fig. 1** The schematic presents the timeline of the experimental visit that included two vascular occlusion tests (VOT) separated by a sustained handgrip at a constant load of 25% maximal strength. During the first VOT, the males demonstrated a significantly ( $p < 0.05$ ) faster rate of decline in skeletal muscle tissue oxygenation ( $\text{StO}_2$ , %) than the females ( $b = -0.2075$  vs.  $-0.1232\% \cdot \text{s}^{-1}$ ). The center panel depicts the  $\text{StO}_2$  responses across relative time (A) and the log-transformed responses (B). The A graph indicated that across time,

the pattern of  $\text{StO}_2$  was biphasic for both the males and females, but the log-transformed values (B) indicated that the males demonstrated a significantly faster mean rate of decline than the females ( $b = -0.070$  vs.  $-0.015\% \cdot \text{s}^{-1}$ ). During the second VOT, the previously seen sex difference in desaturation rate was preserved, such that the males exhibited a faster decline across time ( $b = -0.07869$  vs.  $-0.07017\% \cdot \text{s}^{-1}$ ) in a fatigued condition

## Ultrasonography

Muscle tissue and adipose tissue thickness were assessed via ultrasonography prior to attaching the NIRS device to the forearm. Ultrasound images of the dominant flexor digitorum superficialis were obtained using the portable brightness mode function with a multi-frequency linear array probe (12L-Rs; 5–13 MHz; 38.4 mm field-of-view). All measurements were performed at a sampling rate of 10 MHz at a gain of 58 dB. Ultrasound images were analyzed using ImageJ software (Version 1.47v., National Institutes of Health, Bethesda, MD, USA), and prior to all analyses, images were scaled from pixels to centimeters using the straight-line function. Muscle thickness was determined as the distance from the adipose tissue–muscle interface to the muscle–bone interface. The thickest portion of the adipose tissue was used to calculate the length. Two images were taken each day and the resulting lengths were averaged for subsequent analyses. Great care was taken to ensure that consistent, minimal pressure was applied to limit compression of either tissue. To enhance acoustic coupling and

reduce artifact, a generous amount of water-soluble transmission gel was applied to the skin prior to imaging.

## Statistical analysis

Test–retest reliability (Table 2) was calculated utilizing the 2,1 intraclass correlation coefficient model ( $\text{ICC}_{2,1}$ ). Independent  $t$ -tests were used to identify mean sex differences in participant characteristics (Table 1). Cohen's  $d$  ( $d$ ) was used as a measure of effect size. Simple linear regression was used to examine the rate of desaturation across Time. The slopes of the males and females resulting from the simple linear analyses were tested for mean differences (Zar 2017; Chapt 18). During sustained tasks,  $\text{StO}_2$  generally presents a biphasic response across time, thus rendering linear regression inappropriate. To circumvent this, the  $\text{StO}_2$  values during the sustained handgrip were natural log-transformed to examine potential mean differences in the linear slope coefficients of the males and females. To assess our secondary aim, the slopes resulting from  $\text{StO}_2$  across time relationships were again assessed with the strength matched pairs

**Table 2** Reliability (2,1 model; ICCs), systematic error (repeated-measures ANOVA)

<i>n</i> = 24	Familiarization	Pre-exercise	ICC	<i>p</i> value	CV
Resting StO <sub>2</sub> (%)	69.3 ± 4.1	68.4 ± 6.5	0.78	0.33	4.76
Downslope (% s <sup>-1</sup> )	- 0.15 ± 0.1	- 0.17 ± 0.1	0.90	0.10	15.9
Adipose tissue thickness (cm)	0.56 ± 0.21	0.57 ± 0.20	0.98	0.80	7.84
Muscle tissue thickness (cm)	1.91 ± 0.30	1.86 ± 0.32	0.90	0.16	6.6
Handgrip strength (kg)	35.8 ± 13.2	36.3 ± 13.4	0.98	0.33	5.20

Resting = average of last 30 s of 3 min rest; physiological range = peak—min resulting from 5 min occlusion; Downslope = slope of 30–150 s interval during occlusion; coefficient of variation (CV) = (SEM/grand mean) × 100

(*n* = 6). As noted by our power analysis, the strength matched comparisons were theoretically underpowered; therefore, a supporting ANCOVA (covariate: strength [Pre MVIC]) was used to determine adjusted mean downslope differences between the males and females. All calculations and statistical analyses were conducted utilizing Statistical Package for the Social Sciences software (version 26.0. IBM Inc. Chicago, Ill, USA). A *p* value ≤ 0.05 was considered statistically significant, and all data were reported as mean ± SD.

## Results

### Test–retest reliability

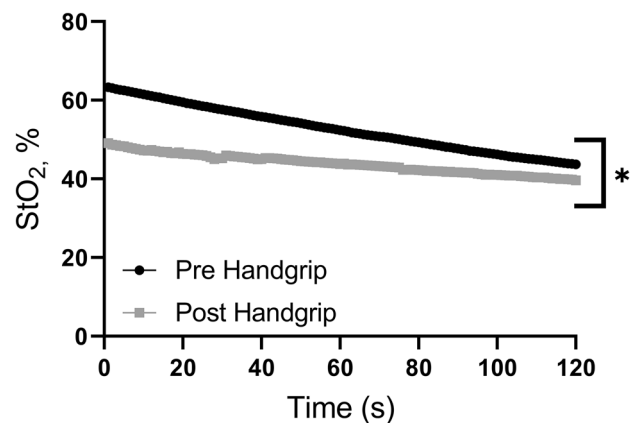
The repeated-measures ANOVAs used for test–retest reliability indicated moderate-to-strong ICCs (range 0.78–0.98) and no significant (*p* > 0.05) systematic error. Exact *p* values are presented in Table 2.

### Participant characteristics

The males exhibited significantly (*p* < 0.05) greater height, body mass, MTT, and absolute load used during the task than the females, whereas the females exhibited significantly (*p* < 0.05) greater adipose tissue thickness than the males (Table 1). The strength matched pairs exhibited no mean differences (*p* > 0.05) in task load, adipose tissue, or MTT.

### Effects of sex and fatigue during the early phase desaturation phase

Prior to the handgrip task, the men and women exhibited significant (*p* < 0.01; *r*<sup>2</sup> = 0.99) negative, linear StO<sub>2</sub> vs. Time relationships (Fig. 1). The males and females exhibited similar relationships following the handgrip task (*p* < 0.01; *r*<sup>2</sup> = 0.98). Independent of sex, the rate of the early phase StO<sub>2</sub> tissue desaturation was significantly (*p* < 0.001) greater pre-handgrip (*b* = - 0.165% s<sup>-1</sup>) compared to immediately following the handgrip (*b* = - 0.070% s<sup>-1</sup>) task (Fig. 2). There were also sex differences in this desaturation rate



**Fig. 2** Collapsed across sex, the rate of the early phase of muscle desaturation was significantly (*p* < 0.001) greater pre-handgrip (*b* = - 0.165% s<sup>-1</sup>) compared to immediately following the handgrip (*b* = - 0.070% s<sup>-1</sup>) task

pre- and post-handgrip, such that, independent of fatigue, the males consistently, significantly (*p* < 0.001) declined faster than the females (pre: *b* = - 0.208 vs. - 0.123% s<sup>-1</sup>; post: - 0.079 vs. - 0.070% s<sup>-1</sup>, Fig. 1).

### Effects of the sustained handgrip task on muscle tissue saturation

As depicted in Fig. 1B, the StO<sub>2</sub> values were natural log-transformed to produce a linear relationship across time for the males and females. The males (*b* = - 0.070, *r*<sup>2</sup> = 0.95) exhibited a significantly (*p* < 0.001) faster rate of decline than the females (*b* = - 0.015, *r*<sup>2</sup> = 0.53).

### Strength matched pairs

Six strength matched pairs were identified (Table 1), and these pairs exhibited the same rate of microvascular tissue desaturation results as the total sample. That is, there were significant (*p* < 0.001) differences between the males (*b* = - 0.215 and - 0.124% s<sup>-1</sup>) and females (*b* = - 0.113 and - 0.059% s<sup>-1</sup>) during the pre- and post-exercise VOTs,

which also demonstrated a deceleration following the handgrip task (Fig. 3).

The supporting mixed-model ANCOVA (Time = within factor; Sex = between factor; Strength [Pre MVIC] = covariate) was used to evaluate mean differences in the downslope responses across Time. The adjusted marginal means (evaluated at covariate = 36.3) indicated that the males desaturated at a faster rate (collapsed across Time) than the females ( $-0.171$  [ $CI_{95\%} = -0.119$  to  $-0.144$ ] vs.  $-0.080$  [ $CI_{95\%} = -0.107$  to  $-0.052$ ], respectively).

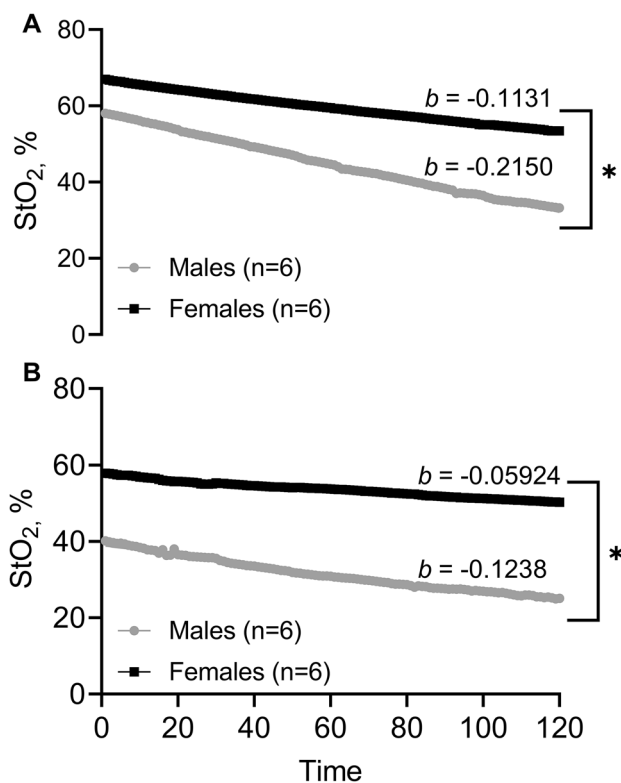
## Discussion

The main objective of the current investigation was to examine the effects of sex and a handgrip task on the early phase of  $StO_2$  desaturation rate as well as to further examine the potential differences with a subsample of strength matched males and females. Here, we presented evidence via the strength matched pairs and ANCOVA to suggest that males desaturated faster than females and that differences in muscle mass and strength as well as adipose tissue were likely not the primary cause of the observed sex differences. Our

main findings also included that the handgrip task to failure induced a deceleration of the rate of the decline in microvascular tissue  $O_2$  saturation independent of sex.

## Early phase skeletal muscle tissue desaturation rate

The early phase of the  $StO_2$  downslope during transient ischemia has routinely been used as an index of changes in tissue saturation (Horiuchi and Okita 2020; Soares et al. 2021). We used this measurement to present a sex difference, such that the males desaturated  $\sim 40\%$  faster than the females. Fellahi et al. (2014) have also shown that males desaturated  $\sim 55\%$  faster than females during a VOT within the calf musculature. However, we are the first known study to present findings of muscle strength and size-matched males and females with the intent to provide further mechanistic insight into this phenomenon. Sex differences as triggered by discrepancies in skeletal muscle mass and strength as well as adipose tissue have previously presented as strong, probable candidates to explain differences in NIRS-derived oxygenation parameters. In contrast to this notion, our six matched pairs reflected the same responses as the total, unmatched, sample. It should be noted, however, that our comparison of 6 men versus 6 women was underpowered (see [Materials and methods](#)), but our supporting ANCOVA (covariate: strength) provided additional rationale to conclude muscular strength was not the primary cause of the observed sex difference. Based on these findings and previous recommendations (Ansdell et al. 2020), future investigations should focus on alternative hypotheses including the influence of skeletal muscle fiber type and/or mitochondrial properties. For instance, it has been shown that females express a greater proportional area of type I fibers compared to males (Roepstorff et al. 2006; Haizlip et al. 2015; Miotto et al. 2018). Despite presenting a lower  $O_2$  consumption capacity, it is well accepted that type II fibers exhibit greater fractional  $O_2$  extraction with faster kinetics as well as superior muscle  $O_2$  diffusion (McDonough et al. 2005; Paradis-Deschênes et al. 2017). That is, sex-specific differences in the proportional area of skeletal muscle fiber types may have contributed to our observed sex difference in the early phase of  $StO_2$  desaturation. Moreover, evidence has been provided to indicate females exhibit a higher density of capillaries per unit of skeletal muscle compared to males (Roepstorff et al. 2006), likely affiliated with the proportional fiber type differences. Thus, during the transient ischemia, it is possible that the females were able to maintain skeletal muscle perfusion to a greater extent than the males (i.e., females did not require additional  $O_2$  extraction). Alternatively, or perhaps in conjunction with the differences in fiber type and capillarization, a possible explanation to the observed sex differences during the downslope phase of ischemia includes differences in factors related to mitochondrial respiration (Miotto et al.



**Fig. 3** The six matched pairs indicated that the males ( $b = -0.215$  and  $-0.124\% \cdot s^{-1}$ ) demonstrated significantly ( $p < 0.001$ ) faster rates of  $StO_2$  decline across time than the females ( $b = -0.113$  and  $-0.059\% \cdot s^{-1}$ ) before (Panel A) and after (Panel B) the sustained handgrip task to failure

2018; Cardinale et al. 2018, 2019). Cardinale et al. (2018) have demonstrated that females exhibited upwards of ~33% greater intrinsic mitochondrial respiration (i.e., respiration per unit of mitochondrial protein) than males. This research team (Cardinale et al. 2018, 2019) has also shown that mitochondrial  $O_2$  affinity is lower in females compared to males as well as that females exhibited a lower metabolic rate per unit of body mass. As previously theorized, a trade-off exists between affinity and efficiency, suggesting that the lower  $O_2$  affinity of females is likely accompanied by greater mitochondrial efficiency (Cardinale et al. 2018). Consistent with the current results, we suspect that the lower metabolic rate and lower  $O_2$  affinity of females was reflected as a slower rate of  $O_2$  extraction as compared to the males during a period of arterial occlusion. In support of our NIRS-derived mitochondrial-related hypothesis, it has recently been demonstrated that NIRS measurements were capable of providing indices of mitochondria capacity in females (Lagerwaard et al. 2021). Therefore, the current results provide an extension to previous findings (Fellahi et al. 2014; Beltrame et al. 2017; Miotto et al. 2018; Cardinale et al. 2018, 2019; Ansdell et al. 2019; Lagerwaard et al. 2021), such that females have slower  $O_2$  extraction rates compared to males likely prompted by differences in the proportional areas of fiber types as well as mitochondrial function.

### Sustained handgrip task

During the handgrip task (Fig. 1), the participants exhibited declines in  $StO_2$  across time. As shown in Fig. 1A, there was an initial, rapid decline in  $StO_2$ , previously termed “fast phase” (Felici et al. 2009), followed by a slower rate of decay. Similarly, Hammer et al. (2020) presented a precipitous early decline (i.e., fast phase) in  $StO_2$  during their sustained handgrip task at ~25% MVIC but with a frequency-domain NIRS device, which lends additional support to the current results. Following the fast-phase decline, qualitative assessment of the patterns of  $StO_2$  responses during various exercises suggests that a steady state/plateau was achieved as demarcated by a slope coefficient equivalent to zero or only slightly negative. The fast phase of decline has been said to be directly related to the intensity of the task and to be compatible with the Henneman size principle (Felici et al. 2009). That is, the rate of decline in  $StO_2$  may be dependent on the type/size of recruited motor units whose activation directly corresponds to the target force. Moreover, to evaluate this rate of decline, the  $StO_2$  values during the sustained task were transformed to yield a linear pattern across time. This led to the depiction of the males desaturating nearly 5× faster than females (−0.070 vs. −0.015; Fig. 1B) during the exercise. It is possible that this was a direct consequence of the males demonstrating ~45% greater strength than the females. Despite the tasks being anchored to the same

relative intensity, the sex differences in pre-task MVICs naturally prompted the use of markedly different absolute loads during the handgrip (11.7 vs. 6.5 kg, respectively). However, regardless of differences in the absolute loads, there was no difference between the males and females in the time required to reach volitional failure. In agreement, Mantooth et al. (2018) also reported similar times between males and females during a handgrip task at 20% MVIC (TTF: 255.9 s vs. 260.3 s, respectively). Contrary to the current findings, however, Mantooth et al. (2018) did not find any differences in  $StO_2$  across time during their sustained handgrip at 20% MVIC, but this investigation only examined three time points (e.g., start, middle, and end). Our methodology offered greater temporal resolution (20 time points) that indicated a decline in  $StO_2$  at a similar relative load, albeit 5% greater. Although our experimental protocol utilized a slightly greater relative load, this should not have elicited a significant difference in microvascular responses given the similar TTFs. Ultimately, we hypothesized that sex differences in muscle fiber type (Roepstorff et al. 2006; Hainzlip et al. 2015), and consequentially motor unit activation strategies (Parra et al. 2020), prompted the markedly different rates of desaturation during the fatiguing exercise. It was interesting that despite the sex difference in rate of decline in  $StO_2$ , there was not a difference in TTF. This was interpreted as muscle oxygenation not being the primary factor eliciting volitional fatigue, unless the males and females varied in tolerance to changes in  $StO_2$ . Future studies should continue to investigate and evaluate the presented hypotheses with surface electromyography with motor unit decomposition software as well as continuous monitoring of conduit artery flow and constituent signals of  $StO_2$ .

### Fatigue-induce deceleration of the rate of skeletal muscle tissue desaturation

The present study reported that immediately following the handgrip task, there was a ~60% decline in the rate of  $StO_2$  desaturation, which was independent of sex. One possible explanation includes that the handgrip task may have fully activated mitochondrial respiration in an attempt to prolong task failure, which consequentially impaired future  $O_2$  extraction and diffusion from the microvasculature to cytochrome-c-oxidase (Boushel et al. 2011; Cardinale et al. 2019; Skattebo et al. 2020). Consistent with this notion, it has been hypothesized that during tasks requiring small amounts of recruited skeletal muscle,  $O_2$  extraction/utilization becomes more limited by diffusion as opposed to perfusion (Cardinale et al. 2019; Skattebo et al. 2020). Cardinale et al., (2019) stated that compensatory mechanisms at the level of the muscle set the limits of  $O_2$  consumption in response to variations in  $O_2$  delivery. In the current study, by design, there was no  $O_2$  delivery during the VOTs (complete



arterial occlusion), and immediately before the initiation of the post-task VOT, there was a ~20% handgrip-induced decline in  $StO_2$ . That is, the post-task VOT was initiated at a lower  $StO_2$  compared to the pre-task VOT. Mitochondrial respiration is, at least in part, regulated by cellular  $PO_2$ , and it has been shown that exercise to failure reduces  $PO_2$  by ~90% and consequently, mitochondrial respiration (Cardinale et al. 2019). To be specific, mitochondrial  $O_2$  consumption presents a hyperbolic decline in the presence of decreasing  $PO_2$  (Cardinale et al. 2019). During small muscle mass activity (e.g., handgrip) and at peak exercise (e.g., resisting task-failure), it has been demonstrated that mitochondrial capacity is fully activated, which lowers the  $O_2$  affinity and decreases the  $O_2$  diffusion gradient, which cooperatively attenuates  $O_2$  extraction (Cardinale et al. 2019). Interestingly, there was no association reported between red blood cell transit time during peak exercise and  $O_2$  extraction/diffusion, but rather  $O_2$  affinity completely accounted for the lower  $O_2$  extraction (Cardinale et al. 2019). Therefore, aligned with the results and interpretations of Cardinale et al. (2018, 2019), we hypothesized that the current handgrip-induced reduction in the rate of microvascular tissue desaturation was largely due to the prior full activation of mitochondrial respiration as well as the reduced  $O_2$  availability.

### Test reliability

A strength of the current experimental design was that it permitted the determination of test–retest reliability given VOTs were performed in a rested, pre-exercise state during the familiarization and experimental visits. Previous investigations have reported NIRS-related ICCs ranging from 0.26 to 0.98 (Ryan et al. 2012; Southern et al. 2013; McLay et al. 2016; Iannetta et al. 2019; Zhang et al. 2020), yet there are currently no known studies that have examined the reliability of young, healthy males and females. Our measurement of the rate of early phase  $StO_2$  desaturation presented a strong  $ICC_{2,1}$ , which supports that the observed differences were likely due to sex and/or fatigue, not systematic error.

### Experimental considerations

The current study did not quantify brachial artery characteristics such as wall thickness and baseline diameter, which have been identified as important factors to consider when interpreting VOT results. Additionally, we did not collect total lean body mass, which may have provided additional understanding (Cardinale et al. 2019; Beever et al. 2020). In relation to our female participants, we did not control for the menstrual cycle and 25% (3/12) of the females reported use of an oral contraceptive. It is acknowledged that this may have influenced our results. However, Mattu et al. (2020) have demonstrated that NIRS-derived reperfusion and total

hyperemia did not vary across the menstrual cycle or oral contraceptive cycles as well as that there were no differences between the experimental groups (contraceptive vs. non-contraceptive). It has also been shown that menstrual cycle had no effect on post-exercise arterial pressure or vascular conductance (Lynn et al. 2007; Limberg et al. 2010). While not exhaustive, the last methodological consideration is our use of the Portamon NIRS device as opposed to a frequency-domain NIRS device. Investigators/readers should use caution when applying the current interpretations to investigations utilizing different NIRS devices (Rosenberry and Nelson 2020). With great interest, we intend to examine the effects of muscle blood flow (supply), lean body mass, and different NIRS devices as well as the other NIRS-derived variables (e.g., constituent parts of  $StO_2$ ) in our future investigations.

### Conclusions

In summary, the results of the current study suggested that males exhibited a faster rate of  $StO_2$  desaturation than females during the early phase of a cuff-induced period of transient ischemia. It was also determined that our fatiguing handgrip task attenuated the rate of desaturation in males and females. Our analyses of the matched pairs as well as an ANCOVA model provided evidence that differences in muscular strength and size as well as adipose tissue were not the key factors underlying the observed sex differences. Based on these results and previous reports (Cardinale et al. 2018, 2019; Ansdell et al. 2019, 2020; Skattebo et al. 2020), we hypothesized that the most likely explanation of the current findings included sex-specific differences in muscle fiber type and mitochondrial function. Despite these physiological sex differences, we reported that the males and females resulted in similar TTF in the sustained handgrip task, yet the males desaturated at a much faster rate during the fatiguing task. Future studies should continue to explore potential sex differences in physiological mechanisms associated with the prolongation of exercise failure such as metabolic efficiency and stress.

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**Author contributions** All experiments were conducted in the USA Integrated Laboratory of Exercise and Applied Physiology (iLEAP). All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed. The following indicates specific author

contributions: (1) conception or design of the work (JLK and KGK); (2) acquisition, analysis, or interpretation of data for work (JLK and KGK); (3) drafting of the work or revising it critically for importance intellectual content (JLK and KGK); and (4) read and approved the final version of this manuscript (JLK and KGK).

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**Data availability** The data relating to the findings of present manuscript are available from the corresponding author upon reasonable request.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** The authors have no competing interests related to these data or their respective interpretations to declare.

**Ethics approval** The current study was performed according to the ethics standards established by the Declaration of Helsinki 2013 and was approved by the local Institutional Review Board for Human Subjects at the University of South Alabama (IRB#:20-338/1650333-1) on September 21st, 2020.

**Consent to participate** The study was not registered in a database, and all participants signed an informed consent document prior to completing any experimental protocols.

**Consent to publication** All person listed as authors exceeded the minimum requirements to warrant authorship and all listed authors have approved of the final version of the manuscript.

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