Measuring tibial hemodynamics and metabolism at rest and after exercise using near-infrared spectroscopy

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Novelty bullets: points that summarize the key findings in the work:	<ul style="list-style-type: none"> • NIRS can be used to monitor tibial hemodynamics and metabolism with good reliability., • Short-duration local muscle exercise increased metabolic rate in muscle but not in bone., • High level of loading and exercise volume may be needed to elicit measurable metabolic changes in bone.
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Measuring tibial hemodynamics and metabolism at rest and after
exercise using near-infrared spectroscopy

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Abstract

The bone vascular system is important; yet, evaluation of bone hemodynamics is difficult and expensive. This study evaluated the utility and reliability of near-infrared spectroscopy (NIRS), a portable and relatively inexpensive device, in measuring tibial hemodynamics and metabolic rate. Eleven participants were tested twice using post-occlusive reactive hyperemia technique with the NIRS probes placed on the tibia and the medial gastrocnemius (MG) muscle. Measurements were made at rest and after two levels of plantarflexion exercise. The difference between oxygenated and deoxygenated hemoglobin signal (HbDiff) could be reliably measured with small coefficients of variation (CV; range 5.7 – 9.8%) and high intraclass correlation coefficients (ICC; range 0.73 – 0.91). Deoxygenated hemoglobin rate of change, a potential marker for bone metabolism, also showed good reliability (CV range 7.5 – 9.8%, ICC range 0.90 – 0.93). The tibia was characterized with a much slower metabolic rate compared to MG ($p < 0.001$). While exercise significantly increased MG metabolic rate in a dose-dependent manner (all $p < 0.05$), no changes were observed for the tibia after exercise compared to rest (all $p > 0.05$). NIRS is a suitable tool for monitoring hemodynamics and metabolism in the tibia. However, the local muscle exercise protocol utilized in the current study did not influence bone hemodynamics or metabolic rate.

Keywords: near-infrared spectroscopy; tibia; hemodynamics; metabolism; reliability; exercise

Novelty bullets

- NIRS can be used to monitor tibial hemodynamics and metabolism with good reliability.
- Short-duration local muscle exercise increased metabolic rate in muscle but not in bone.
- High level of loading and exercise volume may be needed to elicit measurable metabolic changes in bone.

Introduction

Bone fragility and its related low-energy fractures are important public health concerns. There is a growing body of evidence to suggest that bone vascular health plays an important role in the development and progression of bone fragility (Stabley et al., 2015, Prisby, 2019). The vascular system in bone is responsible for nutrient supply, waste removal and many other physiological functions. A healthy bone vascular system is crucial to bone remodeling (Barou et al., 2002, Eriksen, 2010). Bone remodeling is a process that repairs bone micro-damage and prevents larger structural damage from happening. Being able to monitor bone tissue hemodynamic alterations due to pathology or intervention could provide earlier biomarkers of bone vascular health changes, and therefore offer insights on how bone mass and quality may be modulated.

There are limited methods available to non-invasively assess human bone blood flow and hemodynamics due to the non-elastic nature of the tissue (Binzoni et al., 2002). While positron emission tomography scanning or dynamic contrast enhanced magnetic resonance imaging are capable of such tasks, the high technical requirement to perform and analyze the scans and the associated high costs have made them difficult to be integrated as part of the clinical routine. In addition, these techniques are not ideal for monitoring tissue oxidative metabolism. The disadvantages of these imaging modalities call for a simple and cost-effective means that can be easily performed to assess bone hemodynamics and metabolism. Near-infrared spectroscopy (NIRS) is a method that's commonly used to assess brain activation (Heinzel et al., 2013, Holtzer et al., 2011, Holper et al., 2012) as well as muscle oxygenation (McCully et al., 1994a, McCully et al., 1994b, van Beekvelt et al., 2001b, Abozguia et al., 2008) and function (Ryan et al., 2013a, Erickson et al., 2013, Lagerwaard et al., 2019). Several studies have used NIRS to assess

hemodynamics and oxygenation at the medial aspect of the tibial shaft, a location where direct access to the bone by NIRS is possible (Binzoni et al., 2003, Binzoni et al., 2006, Draghici et al., 2018, Khakha et al., 2006); however, studies have not assessed the reliability of NIRS in the assessment of bone.

To this end, the purposes of our study were: 1) to determine the day-to-day reliability of NIRS-derived tibial hemodynamic and metabolic parameters at rest and after exercise using the post-occlusive reactive hyperemia (PORH) technique, and 2) to investigate whether the tibia shows changes in hemodynamics and metabolism in response to plantarflexion exercise using the medial gastrocnemius (MG) muscle as a comparison. A comparison between the tibia and the MG muscle, a tissue that is known to respond to exercise, could provide better insight into how tibial metabolism may respond to acute exercise.

Methods

Participants

A convenient sample of twelve healthy participants between the ages of 18 and 35 years participated in this study. The inclusion criteria included generally healthy young adults with no known cardiovascular or peripheral vascular diseases, no lower extremity musculoskeletal injuries within the past year, and not currently taking medications that affect vascular function or musculoskeletal health. The Institutional Review Board of University of Georgia approved this study, and all participants provided written informed consent. All procedures were carried out in accordance with the Declaration of Helsinki. Participants were tested twice within a 10-day period and were instructed to refrain from performing exercise or consume alcohol 24 hours prior to each testing session. Participants were also instructed not to consume caffeinated drinks on the day of each testing session.

88 *Anthropometrics and adipose tissue thickness (ATT)*

89 Height was measured with a stadiometer (Seca GmbH & Co. KG., Hamburg, Germany) to
90 the nearest 0.1 cm. Weight was measured with a digital weight scale (Tanita Corp., Tokyo,
91 Japan) to the nearest 0.1 kg. Body mass index was calculated according to the measured height
92 and weight. Adipose tissue thickness (ATT) measurements were made at the anatomical
93 locations where the NIRS measurements were made. ATT was measured using B-mode
94 ultrasound imaging (LOGIQe; GE Healthcare, Chicago, Illinois) and a multifrequency (8-12
95 MHz) linear probe. ATT was determined as a previous study showed that ATT influences NIRS
96 signal (van Beekvelt et al., 2001a).

97 *Experimental set-up*

98 All NIRS testing was carried out in a temperature-controlled (22 °C) laboratory. All data
99 were collected on the right leg with participants lying supine on a bed. For NIRS placement at
100 the tibia, the distance between the tibial plateau and medial malleolus was first determined by
101 manually palpating the relative bony landmarks, and the mid-point was subsequently determined.
102 A continuous wavelength NIRS probe (58 x 28 x 6mm, Portalite, Artinis Medical Systems B.V.,
103 Einsteinweg, The Netherlands) was placed at the mid-point using double-sided tape, and care
104 was taken to ensure that the center of the probe overlapped with the mid-point, and that the probe
105 covered as much bone area as possible with minimal interference from other surrounding tissues.
106 A second NIRS probe was placed at the most prominent part of the MG muscle to serve as a
107 comparison. Both NIRS devices were loosely secured in place using self-adhesive wraps. The
108 sampling frequency for the NIRS devices was set to 10 Hz. The NIRS devices have one light
109 source and three detectors, which enables three separation distances of 3, 3.5 and 4 cm. To
110 ensure the utmost signal contribution from the tissue being measured, for tibia measurements the

signal from the minimal separation distance (3 cm) was used, which gave a mean penetration depth of approximately 1.5 cm (Hamaoka et al., 2007, Chance et al., 1992). For MG muscle the longest separation distance (4 cm) was used, which gave a mean penetration depth of approximately 2 cm. The choice of different separation distances is consistent with previous studies, which generally choose long separation distances (or channels) for muscle, but short separation distances for bone (Draghici et al., 2018, Zhang et al., 2020, Malagoni et al., 2013, Binzoni et al., 2006). The longer separation distance for muscle is in part due to the greater amount of ATT over the muscle compared to the tibial bone. In addition, due to a lack of available differential pathlength factor (DPF) for bone, DPF for both tissues was set to 5.51, the assumed value for calf muscle (Duncan et al., 1995).

Limb ischemia was produced using a blood flow restriction training cuff (Delphi Medical Innovations Inc., Vancouver, BC, Canada) placed above the knee to provide arterial occlusion. The cuff was connected to a rapid cuff inflation system (Hokanson E20, Bellevue, WA). The participant's right foot was placed against a pedal in a custom-built pneumatic-powered plantarflexion exercise device with range of motion between 90° (neutral position) to 115° (plantarflexion) degrees that connected to an air compressor, providing a 25° range of motion (Ryan et al., 2013b). At the beginning of each test session, the participant rested his/her foot at the 115° angle. Once the air compressor was turned on, the pedal was moved to the neutral position (90°). The level of resistance provided by the device was be adjusted by changing the outflow pressure of the air compressor, which was read from a dial and expressed in units of pounds per square inch (PSI). Prior to the beginning of each testing session and with the participant lying down in position, a 3-5 second arterial occlusion was induced by turning on the rapid cuff inflation system, with the purpose to familiarize the participant with the sensation of

occlusion. Afterwards, the air compressor connected to the foot pedal was turned on, and the participant performed 2-3 plantarflexions at each resistance level to become familiar with the exercise. The compressor was then turned off, and the pedal returned to the 115° angle.

Experimental procedure

NIRS testing was performed on two separate days within a 10-day period, and at least 48 hours apart. The tests were carried out at similar times of day (± 1 hour) on the two testing days for the same participant. On each testing day, participants were tested under three different conditions: resting, low-resistance exercise (LR) and high-resistance exercise (HR). The resting condition was always tested first, with LR and HR tested in a randomized cross-over order. Specifically, if a participant was tested on the LR condition first (after resting) on day 1, the same participant is tested on HR condition first (after resting) on day 2.

For both male and female participants, the LR condition was carried out with PSI level set to 10. For male participants, the HR condition was carried out with PSI level set to 20. For female participants, HR condition was carried out with PSI level set to 15. For both LR and HR conditions, participants followed the rhythm of a metronome and performed plantarflexion exercise once every 5 seconds for 3 minutes. The participants were instructed to push as far down as possible (plantarflexion) using half a second, and relax so the pedal could return to a neutral position using another half second, then wait for about four seconds until the next push. The resistance level and the intensity of the exercise were set based on pilot measurements (data not shown). The higher level resistance exercise was set to 15 PSI for females because one female participant was unable to perform the exercise at 20 PSI properly for 3 minutes.

Each condition started with a 5-minute rest period. For the resting condition, the participant's right foot was placed against the foot pedal which was set to a 115° angle. As soon as the resting period finished, the cuff was rapidly inflated to a super-systolic pressure (270-300 mm Hg) to provide full arterial occlusion. The occlusion was on for exactly five minutes, and then was rapidly deflated with the participant lying still for another five minutes. For the LR and HR conditions, after the 5-minute rest period, the pneumatic pedal was brought upright to 90° by turning on the air compressor. The participant then performed 3-minutes of exercise as described above. As soon as the exercise was finished, the air compressor was turned off so that the pneumatic pedal returned to its original position (115°). The cuff was then rapidly inflated for five minutes, and then deflated while the participant kept still for another five minutes. The NIRS signals were continuously monitored throughout the procedures until the post-exercise five minutes was finished. Therefore, the NIRS evaluation for each condition was divided into four stages: resting, exercise (not for the resting condition), occlusion and cuff release (reactive hyperemia).

Data analysis

All NIRS data were processed using a custom-written Matlab script (version 2020a, The Mathworks, Natick, MA) script. The NIRS devices measured the concentration changes of deoxygenated (HHb) and oxygenated (HbO_2) Hb relative to an arbitrary baseline within the tissue, and therefore elicited four measured or derived parameters, including HHb, HbO_2 , the difference between HbO_2 and HHb (HbDiff), and total Hb (tHb) concentrations. We measured the range of change during occlusion and post-occlusion, as well as half-time recovery ($1/2$ recovery, the time it takes for the magnitude of signal to reach half of the maximal changes during PORH), a commonly used index to indicate microvascular reperfusion (Willingham et al.,

2016) for all parameters during post-occlusion. For most of our participants, we observed a noticeable HHb signal increase right after cuff release in the tibia (Figure 1A and 1B). Therefore, the 1/2 recovery for tibial HHb signal was calculated starting from when HHb signal reached its maximal value, which was typically 5-30 seconds after cuff release.

Metabolic rate for the tibia was measured during occlusion in a method analogous to the method for measuring muscle metabolic rate with NIRS (Zhang et al., 2020). Due to the irregular signal shape of HbO₂ and HbDiff after exercise (Figure 1B), it was only possible to consistently determine HHb signal rate of change during occlusion. HHb showed clear linearity during most of the occlusion period. Therefore, the rate of HHb increase was calculated over a 20s window of the slope of the HHb signal during occlusion using simple linear regression. For muscle at rest, the HHb increase was also linear, and the rate of HHb increase was calculated over a 20s window of the slope using simple linear regression during occlusion similarly to that of the tibia. The 20s window was chosen in accordance with previous studies aimed at measuring oxidative metabolic rate that generally used 20s -30s arterial occlusion in the muscle (Zhang et al., 2020, Southern et al., 2014). However, the muscle HHb signal changed most quickly during the initial part of the occlusion period (Figure 1D and 1H). Therefore, the initial HHb rate of increase was calculated as the rate of HHb change over a 3-second window immediately after cuff occlusion using simple linear regression. We believe these time windows represent the influence of previous exercise on HHb signal change, based on the linearity of the data (Figure 1).

Statistics

All statistical analyses were performed with SPSS (version 24 IBM, Armonk, NY). Data are presented as mean and SE in both tables and figures. Coefficient of variation (CV) for reliability (Shechtman, 2013), intraclass correlation (ICC) using two-way mixed model for

absolute agreement, as well as paired t-test were carried out to assess reliability and agreement of the measurements in the tibia (de Vet et al., 2006). ANCOVA analysis was performed with the order of exercise as a covariate to determine if exercise order influenced any post-exercise measurements.

For comparisons between the tibia and MG, data were averaged for the two days for the ease of comparison. Mixed-ANCOVA analyses performed with tissue (tibia or MG) as the between subject factor, condition (rest, LR or HR) as the within subject factor, and order of the exercise as the covariate. Bonferroni adjustment was applied for post-hoc analysis when applicable. Alpha value was set at 0.05.

Results

Of the 12 participants, one participant showed involuntary contraction during occlusion following high level of exercise, and t was excluded from the final analysis. Among the remaining 11 participants, 5 were males. The physical characteristics of the participants are summarized in Table 1. Reliability of NIRS-measured tibia parameters are presented in Table 2. For tibial hemodynamics, HbDiff signal showed robust day-to-day reliability, as indicated by small CV (range 5.7 – 9.8%) and high ICC (range 0.73 – 0.91) across all conditions. Further analysis also indicated that HHb slope, a potential biomarker for bone metabolism, can be measured with good reproducibility (CV range 7.5 – 9.8%, ICC range 0.90 – 0.93). None of the measures showed a significant day-day difference, as indicated by the results of paired t-tests (all $p > 0.05$). No order effect was observed for HbDiff measurements or HHb slope (all $p > 0.05$) except for HbDiff during recovery ($p = 0.041$) at LR.

Further comparisons between the tibia and MG were done using HbDiff parameters and HHb slope. For HbDiff magnitude during occlusion, there was no tissue type by condition effect ($p = 0.147$) or main effect for condition ($p = 0.804$), but there was a main effect for tissue ($p < 0.001$; Figure 2A). The tibia showed a smaller change compared to MG at each condition. Exercise did not significantly alter HbDiff magnitude of change in either type of tissue. For HbDiff magnitude after cuff release, there was a significant interaction between tissue and condition ($p = 0.001$). Post-hoc analysis revealed that both LR and HR exercise increased HbDiff magnitude for the tibia ($p = 0.009$ and 0.005 , respectively). However, no differences were found between LR and HR exercise ($p = 0.611$). On the other hand, there were small, but significant, dose-dependent increases for HbDiff magnitude for MG ($p < 0.05$ for all pairwise comparisons; Figure 2B). For HbDiff 1/2 recovery, there was a significant interaction between tissue and condition ($p = 0.022$). Post-hoc analysis revealed that HR exercise significantly increased MG HbDiff 1/2 recovery compared to rest and LR ($p = 0.020$ and 0.022 , respectively) before adjustment; however, the significance was nullified after Bonferroni adjustment (both $p > 0.05$; Figure 2C). For HHb slope, there was a significant interaction between tissue and condition ($p < 0.001$). Post-hoc analysis suggested that exercise significantly increased HHb slope in a dose-dependent manner for MG ($p < 0.05$ for all pairwise comparisons) but not for the tibia ($p > 0.05$ for all pairwise comparisons; Figure 3).

Discussion

To our knowledge, this is the first study that systematically investigated the day-to-day reliability of NIRS for measuring hemodynamics and metabolism of the human tibia. Using the PORH technique, we found HbDiff to be a robust hemodynamic parameter across conditions (resting or post-exercise) for both absolute changes (during occlusion and after cuff release) as

well as the speed of recovery (1/2 recovery) following cuff release. We also found that HHb could be a good indicator of tibial oxidative metabolism as it showed a consistent pattern of increase during occlusion at both resting and post-exercise conditions. In addition, the comparisons between the tibia and MG suggest that: 1) the metabolic rate in bone is much slower compared to muscle, and 2) while the exercise protocol employed in the current study successfully increased MG metabolism by 7-12 fold, no changes in metabolic rate was found for the tibia. Our results are consistent with a previous study which indicated that oxidative metabolic rate is much slower in bone than in muscle, and that isometric exercise significantly increased local muscle oxygen consumption rate but elicited no significant changes in bone as measured using NIRS (Klasing and Zange, 2003).

Non-invasive monitoring of bone vascular health is of potentially great clinical importance, as many studies and reviews have pointed out the crucial role of the bone vascular system on bone development and fragility (Prisby, 2019, Prisby et al., 2007, Prisby, 2014, Ramasamy et al., 2016). In the current study, the relatively small CV values and moderate to high ICC values suggest that most parameters can be measured with acceptable day-to-day reliability. The results are encouraging as they indicate that NIRS, a relatively inexpensive and portable device can potentially be used for the purpose of assessing tibial hemodynamics and oxidative metabolism. Here we presented the reliability for all the parameters we measured; however, the choice of NIRS parameter(s) to present in a research study should be based on the specific research question. For example, HbDiff is a robust hemodynamic measure across all conditions and could be a good indicator of oxygenation in the microvasculature; on the other hand, HHb was found to be a reproducible NIRS variable which could potentially represent bone oxidative metabolism. Our results are supported by previous studies that used HHb signal to

indicate muscle metabolic rate in the calf (Pethick et al., 2019, Wang et al., 2006). Clearly more research is needed to confirm this inference. Nevertheless, this study opens up new possibilities for research in the direction of *in vivo* muscle-bone interactions, as few studies of this kind have examined such a relationship from a hemodynamic perspective. In addition, studies are needed to identify the relationship between NIRS-measured parameters and bone strength and quality measurements for better understanding the role of vascular health in bone development (Zhang and McCully, 2020).

It should be noted that there was a consistent blood volume decrease during occlusion in the tibia in the current study, as shown by the declined values of tHb (Figure 1A and 1B), which could potentially confound the metabolic measurement calculated using HHb slope of change. Blood volume changes during short-term occlusion following exercise, as measured using continuous wavelength NIRS, have been previously reported in the muscle (Ryan et al., 2012), and it was postulated that this could be due to either the change in shape of the muscle, which causes change in scattering, or the blood volume redistribution between small and big vessels. Since bone is a non-elastic organ that does not change in shape, the most likely explanation for the changes in blood volume during occlusion in the tibia is the blood volume shift from small vessels to big vessels, instead of changes in scattering. Since NIRS cannot measure hemoglobin concentration changes in large vessels ($> \sim 1$ mm diameter) (McCully and Hamaoka, 2000), this shift could result in the smaller total blood volume remaining in small vessels, and is reflected by the declined tHb during occlusion in the present study, although more studies are needed to confirm this inference. However, if this is the case, then using HHb rate of change would be the best indicator to represent bone oxidative metabolism. HbO_2 , and consequently HbDiff, will be heavily confounded by blood volume shifts, at least at the initial phase of occlusion when tissue

oxygen level is high, while HHb maintains a constant linear pattern regardless of prior condition (rest or exercise). Some studies have used tissue saturation index (TSI) change to indicate tissue oxygenation and metabolic changes (Adami et al., 2017, Dipla et al., 2017). TSI is a parameter that could offer insights into the oxygenation status of the bone as well and may be worth further exploration. However, TSI is potentially influenced by blood volume changes similar to that of HbO₂ and HbDiff.

The current study found that the tibia is characterized by a much slower metabolic rate compared to MG muscle at rest. We also discovered that the differences in metabolic rate between the tissues were further augmented by exercise. The exercise protocols in the current study successfully increased the initial metabolic rate in MG by 7-12 fold, as indicated by the HHb rate of change following occlusion. However, no change in HHb slope during occlusion was observed for the tibia following exercise. Despite the increasing realization that muscle contraction is a major stimulus for bone growth and development (Goodman et al., 2015), it is possible that the lack of change in the tibia compared to the substantial increase in MG for metabolic rate following exercise may indicate that simple muscle contraction exercise is not able to cause bone microvascular and metabolic demand alterations. In addition, the NIRS probe was placed at the mid-tibia in the current study, a location that consists primarily of cortical bone. There is evidence that maximal cortical bone formation is induced by high loading rather than more repetitions (Rubin and Lanyon, 1984). It is therefore plausible to infer that more bone-specific exercise, such as weight-bearing exercise, may be needed to produce measurable changes in metabolism in the tibia, although more research is needed. The result of this study is inconsistent with a previous study showing that able-bodied individuals increased HHb concentration in the tibia after a 5-minute rowing exercise at 70% peak workload (Draghici et al.,

2018). Compared to this study, our exercise protocols were shorter (3-minute) and had longer intervals between repetitions (plantarflexion once every five seconds). Taken together, it seems that more bone-specific loading exercise and larger exercise volume may be needed to elicit measurable changes in bone compared with muscle. Future studies that manipulate different exercise parameters (type, duration, resistance levels and intensity) are needed in order to better understand the effect of exercise on bone hemodynamics.

Using HbDiff, we found that the tibia was characterized with a much slower reperfusion rate, as indicated by a slower 1/2 recovery following cuff release compared to MG at rest. This is consistent with other studies that tested both the tibia and tibialis anterior with the PORH technique and found a much slower reperfusion rate for the tibia after occlusion at rest (Binzoni et al., 2002, Binzoni et al., 2003). Physiologically, HbDiff, as measured using a continuous wavelength device, is a representation of the change in oxygen bioavailability of the tissue relative to an arbitrary baseline (Hamaoka and McCully, 2019), which potentially determines the extent to which the tissue could conduct cellular respiration and is therefore an important biomarker. However, it should be recognized that the measurement of HbDiff related markers could be confounded by other physiological factors in this study. As previously discussed, we observed tHb changes during occlusion that could be due to blood volume shift. Therefore, the changes seen for the tibia are a combination oxygen consumption and hemoglobin redistribution. On the other hand, during the reactive hyperemia phase, HbDiff signal change reflects the balance of several factors, mainly the blood influx and possibly redistribution, as well as venous drainage. This, combined with the fact that NIRS only measures microvascular hemoglobin concentrations, may indicate that 1/2 recovery following cuff release could reflect the overall speed at which the blood fills the microvascular system, and consequently can be used as a

marker for microvascular function for the tibia. Though, more research is needed to determine the link between this biomarker and the bone vascular system, as well as overall bone health.

Interestingly, although not a focus of this study, we found that HbDiff 1/2 recovery tends to be slower after HR exercise compared to rest or LR exercise for MG, although the results were marginally significant. This could be due to the higher level of oxygen debt accumulated during HR exercise compared to other conditions; therefore, it takes a longer time for tissue reoxygenation. Yet, a similar phenomenon was not found for the tibia, which reaffirms the observation that bone metabolism was not altered by the exercise protocols employed in the current study, and more bone-specific exercise and larger exercise volume may be needed to cause metabolic changes in bone. Nevertheless, together with results for HHb rate of change during occlusion in the current study and results from other studies (Meertens et al., 2016), it appears that although bone is a highly vascularized organ, its overall microvascular response and oxidative metabolism are slower compared to muscle. Importantly, NIRS measured blood flow kinetics may be used to identify those at risk of bone fragility. One study suggested that it takes a longer time for tibia post-occlusive re-oxygenation in individuals with spinal cord injury, a population with known bone fragility (Modlesky et al., 2004, Modlesky et al., 2005, Dudley-Javoroski and Shields, 2012), compared to their able-bodied peers (Khakha et al., 2006). The demonstration that the NIRS method can be applied to clinical populations is of great value, although more research is needed to determine the efficacy of using blood flow kinetics to differentiate fragility risks.

Following the release of occlusion, we observed a noticeable initial increase in HHb signal in the tibia before the decrease, which was expected (Figure 1A and C). The mechanism underlying the initial rise is not clear, and we did not observe the same pattern for MG. There are

several possibilities for this phenomenon. The first is that the sudden increase in HHb signal comes from the HHb of the new blood entering the previously occluded segment after cuff release. Due to the relatively small amount of HHb existing in the tibia prior to cuff release, the sudden increase in HHb results in a prominent change of post-occlusive HHb signal magnitude. On the other hand, the abundant amount of HHb in the MG prior to cuff release makes the new incoming HHb negligible and therefore does not show an observable initial increase. In addition, a recent study showed that DPF is altered in the muscle during occlusion and reperfusion phases compared to baseline (Hammer et al., 2019). It is currently unclear whether a similar phenomenon occurs in bone, which needs to be confirmed with devices that are capable of measuring scattering as it may partly account for the initial increase in HHb.

There are limitations associated with this study. First, it only involved healthy young participants. Whether the same level of reproducibility of the NIRS measured bone parameters can be achieved in aging and clinical populations who may present with bone fragility needs to be further investigated. Second, as previously mentioned, we used a continuous wavelength device and a fixed DPF which may have led to the initial signal change in HHb following cuff release at the tibia. In addition, the DPF for the tibia in the current study was set the same as that for the MG. Due to the lack of an available DPF for bone, it is unknown how using a muscle-specific DPF can affect the calculation of absolute concentrations of the parameters measured in the tibia in this study. Therefore, development of a bone-specific DPF is needed to reaffirm our findings. Third, the placement of the NIRS probe was set at the mid-point between the tibial plateau and the medial malleolus. Because of the high inhomogeneity in the structure and composition of bone, different placement locations will likely result in different patterns for NIRS measurement. More studies are needed to investigate the influence of NIRS probe

placement, for example the proximal end vs. the mid-point of the tibia, on NIRS measurements (Zhang and McCully, 2020). Lastly, the superficial and surrounding tissues are likely to also contribute to the NIRS measurement of the tibia. Specially, ATT has been shown to affect continuous wavelength NIRS measurement in the muscle (van Beekvelt et al., 2001a); therefore, ATT could also contribute to the NIRS signal measured in the tibia. The wide range of ATT (0.42 – 0.94 cm in the current study) among participants makes it impossible to determine how much NIRS signal comes from cortical bone or bone marrow.

In conclusion, our results suggest that NIRS is a suitable tool for monitoring tibial hemodynamics and metabolism. HHb signal slope of change during occlusion showed good reproducibility, which could be used to represent bone metabolism. Good reproducibility was also found for the change in blood signal during cuff inflation and release and the rate of reperfusion after cuff release. These are representative markers of metabolic rate and microvascular responses; however, these are not direct measurements and may be confounded by other physiological factors, such as ATT. While exercise produced large changes in muscle metabolic rate consistent with the literature, minimal changes in the bone metabolism and hemodynamics associated with exercise were observed. A more bone-specific exercise along with a greater exercise volume may be required to elicit observable metabolic rate changes in the tibia compared to MG.

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Text to figures

Figure 1. Figures in the left column show oxygenated hemoglobin (HbO₂), deoxygenated Hb (HHb), the difference between HbO₂ and HHb (HbDiff) and total Hb (tHb) concentration changes for the NIRS testing protocols. The full resting period is not shown in some figures. Panels A and B show resting and exercise conditions for the tibia, respectively. Panels C and D show resting and exercise conditions, respectively, for the medial gastrocnemius (MG). Lower case letters represent different phases of the protocol: a = resting, b = occlusion, c = reactive hyperemia, and d = exercise. Figures in the right column show HHb signal change around the point of occlusion. Panels E and F show resting and exercise conditions, respectively, for the tibia. Panels G and H show resting and exercise conditions, respectively, for the MG. HHb signal showed linearity shortly after point of occlusion for E, F and G. However, HHb signal changed quickly for H after occlusion started.

Figure 2. Comparisons between the tibia and medial gastrocnemius (MG) for the difference in oxygenated hemoglobin and deoxygenated hemoglobin (HbDiff). A) The magnitude of HbDiff change during occlusion, B) the magnitude of HbDiff change following cuff release, and C) microvascular reperfusion measured as the half time recovery for HbDiff following cuff release. LR, low-resistance exercise; HR, high-resistance exercise; 1/2 HbDiff, half time recovery for HbDiff magnitude following cuff release. Data are presented as Mean $\pm$ SE. * Significant difference between the tibia and MG for the same condition. ^a Significant difference compared to rest. ^b Significant difference compared to LR.

586
587 Figure 3. Comparisons between the tibia and medial gastrocnemius (MG) for NIRS metabolism
588 measurement as determined by deoxygenated hemoglobin (HHb) concentration rate of change.
589 LR, low-resistance exercise; HR, high-resistance exercise. There were no differences in HHb
590 slope among conditions for the tibia ($0.010 \pm 0.002 \mu\text{M/s}$, $0.009 \pm 0.001 \mu\text{M/s}$ and 0.010 ± 0.001
591 $\mu\text{M/s}$ for rest, LR and HR conditions, respectively; all $p > 0.05$). Data are presented as Mean $\pm$
592 SE. * Significant difference between the tibia and MG for the same condition. ^a Significant
593 difference compared to rest. ^b Significant difference compared to LR.

Draft

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255x401mm (300 x 300 DPI)

Figure 2. Comparisons between the tibia and medial gastrocnemius (MG) for the difference in oxygenated hemoglobin and deoxygenated hemoglobin (HbDiff). A) The magnitude of HbDiff change during occlusion, B) the magnitude of HbDiff change following cuff release, and C) microvascular reperfusion measured as the half time recovery for HbDiff following cuff release. LR, low-resistance exercise; HR, high-resistance exercise; 1/2 HbDiff, half time recovery for HbDiff magnitude following cuff release. Data are presented as Mean ± SE. * Significant difference between the tibia and MG for the same condition. a Significant difference compared to rest. b Significant difference compared to LR.

118x258mm (300 x 300 DPI)

Figure 3. Comparisons between the tibia and medial gastrocnemius (MG) for NIRS metabolism measurement as determined by deoxygenated hemoglobin (HHb) concentration rate of change. LR, low-resistance exercise; HR, high-resistance exercise. There were no differences in HHb slope among conditions for the tibia ($0.010 \pm 0.002 \mu\text{M/s}$, $0.009 \pm 0.001 \mu\text{M/s}$ and $0.010 \pm 0.001 \mu\text{M/s}$ for rest, LR and HR conditions, respectively; all $p > 0.05$). Data are presented as Mean $\pm$ SE. * Significant difference between the tibia and MG for the same condition. a Significant difference compared to rest. b Significant difference compared to LR.

96x69mm (300 x 300 DPI)

Table 1. Physical characteristics of all participants. Data are presented as Mean ± SE.

	Total	Male	Female
N	11	5	6
Age (years)	24.4 (1.1)	24.5 (1.5)	24.4 (1.8)
Height (cm)	172 (3.4)	180.1 (4.9)	164.5 (2.0)
Weight (kg)	72.3 (5.3)	86.5 (3.8)	60.4 (5.5)
Body Mass Index (kg/m ²)	24.3 (1.4)	26.8 (1.4)	22.3 (2.0)
Tibia Adipose Tissue Thickness (cm)	0.64 (0.05)	0.56 (0.04)	0.70 (0.07)
MG Adipose Tissue Thickness (cm)	0.78 (0.07)	0.73 (0.10)	0.82 (0.05)

MG, medial gastrocnemius.

Draft

Table 2. Reliability of NIRS measured tibial hemodynamic and metabolic parameters between day1 and day2.

		Occlusion (μM)				Cuff release (μM)				1/2 recovery (s)				Slope (μM/s)
Rest		HbDiff	HHb	HbO ₂	tHb	HbDiff	HHb	HbO ₂	tHb	HbDiff	HHb	HbO ₂	tHb	HHb
p		0.71	0.42	0.88	0.54	0.35	0.76	0.23	0.34	0.74	0.06	0.62	0.28	0.15
	ICC (95%CI)	0.89 (0.63-0.97)	0.89 (0.67-0.97)	0.79 (0.39-0.94)	0.73 (0.28-0.92)	0.73 (0.28-0.92)	0.83 (0.47-0.95)	0.56 (0.26-0.86)	0.59 (0.02-0.86)	0.80 (0.40-0.94)	0.35 (-0.16-0.75)	0.76 (0.31-0.93)	0.74 (0.32-0.92)	0.9 (0.68-0.98)
	CV% (95%CI))	5.7 (3.2-8.30)	12.1 (4.0-20.2)	5.4 (2.5-8.4)	12.0 (7.6-16.4)	7.9 (3.1-12.6)	9.2 (5.5-13.0)	10.3 (2.6-18.0)	15.8 (4.5-27.2)	9.8 (5.5-14.1)	11.7 (4.6-18.8)	14.6 (8.2-21.0)	15.2 (6.4-23.9)	8.7 (1.3-16.1)
LR														
p		0.5	0.14	0.9	0.76	0.25	0.04	0.92	0.67	0.72	0.08	0.87	0.86	0.38
	ICC (95%CI)	0.78 (0.38-0.93)	0.73 (0.29-0.92)	0.67 (0.13-0.90)	0.55 (-0.08-0.86)	0.75 (0.34-0.93)	0.73 (0.22-0.92)	0.62 (0.03-0.89)	0.47 (-0.19-0.83)	0.91 (0.69-0.97)	0.68 (0.20-0.90)	0.89 (0.63-0.97)	0.84 (0.50-0.95)	0.92 (0.73-0.98)
	CV% (95%CI))	7.8 (4.2-11.3)	14.1 (7.7-20.6)	6.9 (2.1-11.7)	12.6 (6.2-18.9)	5.3 (2.2-8.3)	10.1 (6.6-13.7)	6.3 (1.8-10.8)	12.0 (3.5-20.5)	7.5 (3.6-11.6)	10.7 (4.7-16.6)	9.3 (4.0-14.5)	9.5 (4.9-14.0)	7.5 (3.9-11.2)
HR														
p		0.51	0.7	0.61	0.74	0.17	0.28	0.51	0.83	0.45	0.16	0.67	0.81	0.6
	ICC (95%CI)	0.78 (0.39-0.94)	0.62 (0.03-0.89)	0.73 (0.25-0.92)	0.54 (-0.09-0.85)	0.84 (0.25-0.95)	0.55 (0.0-0.85)	0.81 (0.46-0.94)	0.65 (0.09-0.89)	0.87 (0.60-0.96)	0.69 (0.22-0.90)	0.77 (0.34-0.93)	0.7 (0.18-0.91)	0.93 (0.76-0.98)
	CV% (95%CI))	7.8 (4.1-11.5)	16.4 (7.6-25.3)	6.6 (2.0-11.2)	20.3 (10.0-30.6)	5.2 (1.9-8.5)	12.2 (6.2-18.2)	4.6 (2.2-7.1)	11.4 (3.6-19.2)	8.5 (4.8-12.3)	10.4 (4.8-15.9)	13.9 (6.0-21.9)	17.5 (9.7-25.3)	9.8 (5.7-13.9)

Occlusion, magnitude of change during cuff occlusion; Cuff release, magnitude of change after cuff release; ½ recovery, half time recovery following cuff release; Slope, rate of change during cuff occlusion; LR, low resistance exercise; HR, high resistance exercise; p, p value for paired t-test results between day1 and day2; ICC, intraclass correlation coefficient; CV (%) , coefficient of variation; 95%CI, 95% confidence interval.