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Is near-infrared spectroscopy a valid method for measuring skeletal muscle microvascular blood flow?

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Skeletal muscle microvascular blood flow (MBF) plays an important role in the delivery and exchange of nutrients between the circulation and the muscle. One method for measuring MBF is contrast enhanced ultrasound (CEU) which provides real-time in-vivo assessment of microvascular blood flow. However, it is invasive, requires expensive equipment and is restrictive in the number of measurements that can be obtained in a single session. Near-infrared spectroscopy (NIRS) may be a non-invasive alternative that can indirectly estimate MBF via changes in haemoglobin concentration in the muscle. However, NIRS is limited in its penetration depth, and it is unclear if it solely measures blood flow in the muscle, or if other tissues can interfere with the signal. The aim of this study was to determine whether the estimation of blood flow using NIRS aligns with CEU, and thus determine if NIRS could be used as a surrogate for CEU. Sixteen participants (29 $\pm$ 7.4, years $\pm$ SD) had microvascular blood flow measured in the vastus lateralis muscle using CEU and NIRS under four different blood flow conditions: rest, skin heating (to increase skin and subcutaneous MBF) and single leg knee extensions at 25% and 50% of 1 repetition maximum (1-RM) (to increase skeletal muscle MBF in a stepwise fashion). For NIRS measurements, participants underwent 4 venous thigh cuff occlusions (80 mmHg), 45 seconds apart during each condition to calculate microvascular blood flow via the change in haemoglobin. The data from all occlusions for each condition were then averaged together. Participants also underwent an infusion of contrast agent (Definity) during each condition to measure microvascular blood flow using CEU, where three measurements were acquired and averaged together. During contractions, CEU demonstrated an increase in MBF from rest to 25% 1-RM (50-fold increase, $p < 0.001$) and a further increase at 50% 1-RM (69-fold increase, $p < 0.001$). MBF when assessed by NIRS revealed a moderate increase from rest to 25% 1-RM (10-fold increase, $p = 0.01$) and 50% 1-RM (12-fold increase, $p < 0.01$. Contraction-mediated MBF was significantly lower with NIRS compared to CEU for both contraction conditions ($p<0.001$). For the skin heating conditions, MBF measured via NIRS was significantly higher than MBF measured by CEU ($p<0.001$). Linear regression analysis indicated that NIRS and CEU measures were not significantly correlated (all $p > 0.74$) and the two techniques were not in agreement using the Bland Altman plot. The results from the skin heating condition suggest that NIRS is greatly influenced by skin or subcutaneous blood flow and does not differentiate this from muscle blood flow. The larger difference between CEU and NIRS measures for each contraction condition and lack of correlation suggests that NIRS is not an acceptable alternative to measuring skeletal muscle MBF.