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ScienceDirect
Microvascular Research 75 (2008) 323–329
Microvascular Research
www.elsevier.com/locate/ymvre

Regular Article

Contrast-enhanced ultrasound measurement of microvascular perfusion relevant to nutrient and hormone delivery in skeletal muscle: A model study *in vitro*

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Received 21 September 2007; accepted 9 November 2007
Available online 22 January 2008

Abstract

Contrast-enhanced ultrasound (CEU) has been used to measure muscle microvascular perfusion *in vivo* in response to exercise and insulin. In the present study we address whether CEU measurement of capillary volume is influenced by bulk flow and if measured capillary filling rate allows discrimination of different flow pattern changes within muscle. Three *in vitro* models were used: (i) bulk flow rate was varied within a single length of capillary tubing; (ii) at constant bulk flow, capillary volume was increased 3-fold by joining lengths of capillary in series, and compared to a single length; and (iii) at constant bulk flow, capillary volume was increased by sharing flow between a number of lengths of identical capillaries in parallel. The contrast medium for CEU was gas-filled albumin microbubbles. Pulsing interval (time) versus acoustic–intensity curves were constructed and from these, capillary volume and capillary filling rate were calculated. CEU estimates of capillary volume were not affected by changes in bulk flow. Furthermore, as CEU estimates of capillary volume increased, measures of capillary filling rate decreased, regardless of whether capillaries were connected in series or parallel. Therefore, CEU can detect a change in filling rate of the microvascular volume under measurement, but it can not be used to discriminate between different flow patterns within muscle that might account for capillary recruitment *in vivo*.

Keywords: Capillaries; Perfusion; Insulin; Blood flow; Vasculature; Skeletal muscle; Exercise

Introduction

Microvascular delivery of nutrients and hormones to skeletal muscle involving changes in microvascular perfusion may be a key regulatory step in insulin action. Impairments associated with vascular dysfunction can contribute to the metabolic syndrome and insulin resistance. Recently, using an ultrasound imaging technique, we reported that insulin action and muscle contraction each resulted in a marked increase in microvascular perfusion (capillary recruitment) of hind leg muscles of the rat (Dawson et al., 2002; Rattigan et al., 1997) and of human forearm (Coggins et al., 2001). We also observed that insulin's control of this process was impaired in insulin resistant states (Vincent et al., 2006; Wallis et al., 2002). The imaging technique is based on contrast-enhanced ultrasound (CEU) where gas-filled microbubbles served as the contrast medium. The microbubbles are infused intravenously to reach a steady-state arterial concentration in the blood and the ultrasound beam is focused on a region of interest with parameters set to both destroy the microbubbles and capture the echoed signal. From these it is then possible to measure both muscle microvascular volume and microvascular filling rate in the region of interest. However, there are two important aspects in the application of CEU for the measurement of capillary recruitment in muscle. First, it is essential that the signal is not influenced by the increase in bulk flow associated with muscle contraction (Shoemaker and Hughson, 1999) or the increase in bulk flow that occurs during hyperinsulinemic clamps (Yki-Jarvinen and

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0026-2862/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.mvr.2007.11.002

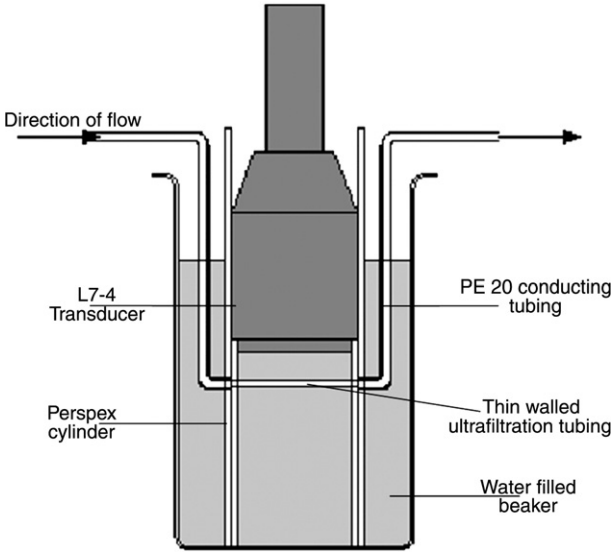

Fig. 1. Single capillary model comprising one piece of capillary tubing with each end inserted into 23-gauge stainless steel needle and in turn joined to a length of non-permeable conducting tubing. A suspension of microbubbles was continuously perfused with the length of capillary tubing (50 mm) held in place in a perspex frame positioned under the ultrasound transducer. To allow conduction of sound, the capillary tubing and head of the transducer were immersed in a beaker of water. Other details are given in the text. The 'in series' tubing or 'in parallel' models (not shown) fitted similarly under the transducer replacing the single capillary tubing model. All capillaries ran horizontally across the field of measurement and multiple capillaries were arranged vertically one above the other.

Utriainen, 1998). Second, there is the issue of whether CEU has the potential to discriminate between increased microvascular perfusion resulting from either flow redistribution from short (possibly non-nutritive) to tortuous (nutritive) capillaries, where capillary blood flow rate would not be expected to change and/or by flow sharing into capillaries of similar properties, where capillary blood flow rate might be expected to decrease. These studies can not be conducted *in vivo* and only one previous modeling study of this kind has been reported (Wei et al., 1998) where bulk flow was shown to correlate well with measured filling rate, but capillary volume was not determined. Accordingly, in the present study, CEU is used with constructed capillary tubing models *in vitro* to assess the effect of bulk flow and its potential for discriminating between different flow patterns within muscle that might explain capillary recruitment reported *in vivo* (Dawson et al., 2002; Rattigan et al., 1997; Vincent et al., 2006).

Methods

Capillary models

In preliminary experiments a number of different commercially available capillary tubing were tested. Wall thickness was found to contribute significantly to the reflected ultrasound signal and thus masked the signal from the microbubbles. Accordingly, we chose to use thin-walled microdialysis tubing (Ultrafiltration Probes, Catalogue No. MF-7023/UF-3-12, BAS Bioanalytical Systems, Inc., West Lafayette, IN, USA). This had minimal reflectance and proved to be suitable for construction of the models. Each ultrafiltration probe consists of three 14-mm loops of capillary tubing (320 μm external diameter and 280 μm internal diameter) with the six ends inserted into a single larger non-permeable length of conducting tubing. For the construction of the single tube model 60 mm of one of the capillary loops was cut from the probe and one end inserted into 23-gauge stainless steel needle (10 mm in length, that had been blunted and removed from a hypodermic needle adapter). This was in turn joined to a length of non-permeable conducting tubing (PE 20, Becton Dickinson, Parsipanny, NJ, USA), by a short sleeve (1.5 cm) of PE 50. All junctions

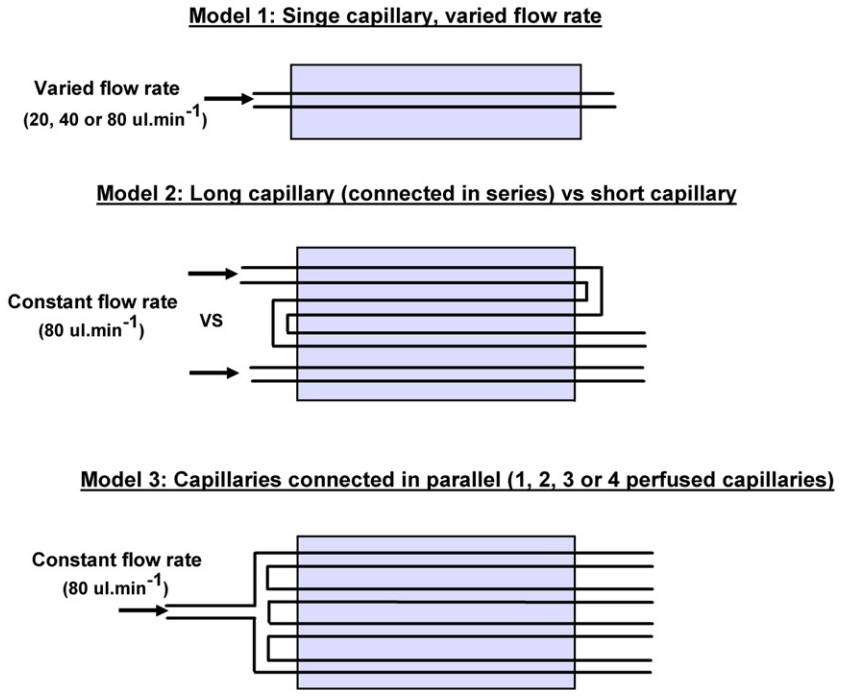

Fig. 2. The three capillary tubing arrangements. Model 1 depicts the single capillary model where delivery rate was varied. Model 2 depicts an 'in series' model, where the flow through a long tortuous capillary, which crosses the region of interest three times is compared to a short capillary. Model 3 depicts the 'in parallel' tubing model, where one, two, three or four capillaries were compared at a constant flow rate. The region of interest is shown by the colored box around each model.