Mixed meal and light exercise each recruit muscle capillaries in healthy humans


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The first technique is based on the metabolism of exogenously infused 1-methylxanthine (1-MX) to 1-methylurate by capillary endothelial xanthine oxidase (18, 29). This has worked well in rodent models but has not been adapted to human studies. The second technique, contrast-enhanced ultrasound (CEU), relies on the contrast introduced by intravenously infused microbubbles (27). To measure microvascular volume, CEU takes advantage of the fact that during their continuous infusion it is possible to disrupt microbubbles by a single pulse of high-energy ultrasound and acquire an image from the ultrasound energy released. From the measured video intensity observed when sequential images with variable delay times are collected, the rate and extent of microbubble replenishment within the vasculature can be detected. Because flow through arteriole and arteriolar vessels is rapid relative to flow through capillaries, images acquired with short delay times (50–1,000 ms) after microbubbles are disrupted and enriched with signals from these larger vessels (27). With time, the microvascular contribution to image intensity increases, and by subtracting images obtained with short delays from those acquired later the contrast enhancement from postarteriolar microvessels can be quantified.

Using CEU, we have shown that physiological hyperinsulinemia increases microvascular blood volume ~1.6-fold in rat hindlimb muscle within 10 min (24), whereas total limb blood flow increases required >90 min of insulin infusion (25). Recent insulin dose-response studies have shown that muscle capillary recruitment is more sensitive than total muscle blood flow to small increases of plasma insulin (29). The potential importance of insulin’s microvascular action is underscored by the finding that inhibiting nitric oxide synthase blocks insulin-induced capillary recruitment and concomitantly inhibits glucose disposal (23).

Most studies of insulin’s actions on resistance, conduit, and microvascular vessels have used the insulin clamp method with several hours of steady-state euglycemic hyperinsulinemia. Although this method allows excellent assessment of responsiveness to physiological hyperinsulinemia, it does not replicate the physiological responses that accompany feeding, which also involves changes in circulating concentrations of glucose, amino acids, gut and pancreatic peptides, changes in parasympathetic and sympathetic tone, and other factors. There have been no studies of muscle microvascular flow regulation following a meal and only a few studies of total limb flow. Some studies report significant forearm blood flow increases...
following a mixed meal (9), whereas others report no effect (12). These differences may relate to techniques used to measure flow, to the types of meals used, or to differences among the subjects studied. However, in each of these clinical studies investigators have measured only total limb flow, not capillary flow. It is the latter that more directly mediates nutrient exchange in muscle (19, 20), and, as we have shown previously for insulin, hemodynamic responses of resistance vessels and preterminal arterioles may differ markedly (24, 29).

We have observed that, in experimental animals, insulin’s vascular actions are qualitatively similar (although quantitatively less dramatic) to those of exercise. Using CEU, we have shown that high-frequency electrical stimulation (2 Hz) of rat hindleg muscle increases both muscle capillary recruitment and total muscle blood flow (8). In ongoing studies (Vincent MA, Ingard A, Clerk LH, and Barrett EJ, unpublished observations), we have seen, using CEU, that low-frequency stimulation increases muscle microvascular volume even in the absence of changes in total flow (analogous to the effect of low-dose insulin). Honig et al. (13), using histological techniques, demonstrated over 20 years ago that electrically stimulated, denervated canine gracilis muscle vasculature exhibits a graded response to stimulation frequency, with capillary recruitment being evident at low-frequency stimulation, whereas higher frequency stimulation both recruited microvasculature and increased total flow. Others had shown that contraction of even a single muscle fiber could selectively dilate arterioles feeding that microvascular unit (11). The invasiveness of the methods used in these animal studies precluded the study of voluntary exercise in either animals or humans. As a result, whether exercise differentially affects resistance and terminal arterioles in humans, as is generally considered to be the case, has not been assessed experimentally.

In the present work, we used Doppler ultrasound and capacitance plethysmography to study total forearm blood flow and CEU to quantify microvascular volume and microvascular flow velocity responses of forearm muscle in healthy adults in response to each of two simple physiological stimuli, i.e., a mixed meal and a brief bout of exercise of low or high intensity. We hypothesized that each would increase microvascular volume whether or not total blood flow increased.

METHODS

The study protocols were approved by the Human Investigations Committee of the University of Virginia. Healthy, nonsmoking adult volunteers on no chronic medications were studied after a 12-h overnight fast.

Protocol 1: mixed meal. Eighteen (9 M, 9 F) volunteers (age 29 ± 2 yr, BMI 23 ± 1 kg/m²) were admitted to the University of Virginia General Clinical Research Center the evening before the study. On the morning of the study, catheters were placed in the antecubital veins of both arms. The catheter in the nondominant arm was used for blood sampling of glucose and lactate, and the contralateral arm was used for the infusion of microbubbles. Before the beginning of exercise, measurements were made of total forearm blood flow (capacitance plethysmography), forearm muscle microvascular volume and microvascular flow velocity, and plasma glucose and lactate. Brachial artery Doppler flow measurements were not made, as the microbubble infusion interferes with Doppler flow measurements. Subjects then exercised at 25 or 80% (order of study randomized) of maximal handgrip (sequentially), using the handgrip ergometer following the same exercise protocol employed during brachial artery Doppler studies. At 6 min after beginning the exercise protocol, while contractions were occurring every 20 s, microvascular volume and microvascular flow velocity were again measured using CEU. CEU data were collected within 3 min, after which total forearm blood flow was again measured using capacitance plethysmography and venous blood was sampled from the exercising arm. Because both the capacitance measurements and CEU measurements are sensitive to movement, data collection for each was done during the 20-s rest intervals between successive forearm contractions.

Doppler ultrasound. For the two-dimensional (2-D) and Doppler ultrasound measurements, an ultrasound system (HD1 5000; Philips Medical Systems, Bothell, WA) with a linear array transducer was used with a transmit frequency of 12 MHz. Two-dimensional imaging of the brachial artery was performed in the long axis ~5 cm proximal to the antecubital fold. Images were triggered to the R wave of the cardiac cycle, and the brachial artery diameter was measured using online video calipers. A pulsed-wave Doppler sample volume was placed at the same location in the center of the artery, and the mean blood velocity was measured using online angle correction and analysis software. Brachial artery mean blood flow was calculated from 2-D and Doppler ultrasound data using the equation: $Q =$
using either a two-tailed Student’s paired test or repeated-measures ANOVA, as appropriate. For the latter, when a significant difference was found, pairwise comparisons by Student-Newman-Keuls test were used to assess treatment differences. Individual values in the results are presented as the means ± SE.

RESULTS

Mixed-meal studies. Figure 1 shows the time course of plasma glucose and insulin levels following the ingestion of the mixed meal. By 30 min, plasma glucose levels were elevated above basal (4.9 ± 0.06 vs. 6.7 ± 0.2 mM, *P < 0.05), and over the course of the next 90 min plasma glucose levels declined towards basal. Likewise, plasma insulin levels rose by 30 min (24 ± 6 vs. 264 ± 30 pM, *P < 0.05) and remained elevated for the additional 90 min of the study.

As shown in Fig. 2, compared with basal, brachial artery diameter significantly increased 60 min after the mixed meal (3.3 ± 0.1 vs. 3.5 ± 0.1 mm, *P < 0.01). Mean brachial artery flow velocity also trended higher following the meal; however, this was not statistically significant (9.5 ± 1.2 vs. 12.4 ± 2.0 cm/s, *P = 0.10). Brachial artery blood flow increased markedly (48.4 ± 6.2 vs. 72.3 ± 11.3 ml/min, *P < 0.05) by 60 min following the meal.

Figure 3 shows the effect of the mixed meal on forearm microvascular responses. At 60 min following the mixed meal, capillaries within the muscle bed were recruited, as shown by an increase in microvascular volume (13.3 ± 1.7 vs. 19.0 ± 2.5 VI units, *P < 0.01). Although not statistically significant, microvascular flow velocity trended lower following the meal (0.18 ± 0.02 vs. 0.15 ± 0.02 s⁻¹). Capillary flow in the designated region of interest (given by the product of microvascular volume and microvascular flow velocity) rose modestly but not significantly (2.3 ± 0.3 vs. 2.8 ± 0.4 units) with the meal.

Forearm graded-intensity exercise. With the first exercise protocol, in five healthy volunteers forearm flow was measured...
in triplicate using capacitance plethysmography at baseline and when the forearm was exercised at 25, 50, and 80% of maximal handgrip. Blood flow was measured between 5 and 20 s following a contraction during the last 4 min of a 12-min exercise period with handgrips performed every 20 s. As expected, contraction induced a significant increase in forearm flow (P < 0.05 repeated-measures ANOVA). There was no significant rise in mean forearm flow above basal when the forearm was exercised at 25% of maximal handgrip (5.3 ± 1.7 vs. 5.4 ± 2.5 ml·min⁻¹·100 ml⁻¹). Flow increased when isometric grip strength was 50% (11 ± 5.8 ml·min⁻¹·100 ml⁻¹) and further increased (29 ± 14 ml·min⁻¹·100 ml⁻¹) with the 80% maximal handgrip protocol (P < 0.05). Figure 4 gives the responses of brachial artery flow (2-D Doppler ultrasound) to the graded exercise protocols. With Doppler ultrasound, we measured brachial artery flow every 5 s during the 20-s intervals between contractions, thus the time course of flow over the 20-s rest period could be assessed. At each level of exercise intensity, brachial artery flow followed a similar pattern where flow transiently increased following the contraction and then decayed back toward basal. The amplitude of these changes was greatest during higher exercise intensity. At the 25% maximum handgrip, mean brachial artery flow was no different from baseline, consistent with the responses in using capacitance plethysmography, which measures the integrated response over the 20-s interval between contractions. Within the first 5 s, there was a borderline significant increase in blood flow at 25% maximal contraction. Studying a larger group of subjects, Brock et al. (6) observed a transient increase in flow after a single contraction of 1 s duration at 20% maximal intensity.

With CEU we observed that low-intensity exercise (25% maximum handgrip) significantly increased muscle microvascular volume, as indicated by the increase in the steady-state VI plateau seen with CEU (Fig. 5). The approximately threefold rise suggests that this modest level of repetitive contraction effected a marked recruitment of microvascular units. Total forearm flow was again measured by plethysmography during that study immediately after the CEU measurements while handgrips continued every 20 s, and again we observed no increase in total forearm flow compared with baseline (4.8 ± 2.4 vs. 6.3 ± 4.1, P = not significant (NS)). Likewise, there was no significant change from basal in the concentration of glucose in venous blood draining the forearm (4.4 ± 0.6 vs. 4.4 ± 0.6 mM), and plasma lactate declined slightly (1.1 ± 0.6 vs. 1.0 ± 0.6 mM, P < 0.01).

The highest-intensity (80% maximum) handgrip likewise increased plateau image VI observed during microbubble infusion, indicating an increase in microvascular volume (Fig. 5). Total forearm blood flow also increased markedly (Fig. 4). With this exercise there was again no change from basal in venous glucose concentration draining the exercised arm, but lactate rose slightly (0.9 ± 0.2 vs. 1.1 ± 0.4 mM, P = 0.08). Compared with low-intensity exercise, the high-intensity exercise plateau VI increased only modestly (P = 0.05; Fig. 5).

The microvascular flow velocity (the time constant for the rise in VI) was unchanged from basal in the low-intensity exercise group, whereas with high-intensity exercise microvascular flow velocity increased approximately twofold (P < 0.05). The value of microvascular flow velocity during low-intensity exercise was significantly less (P < 0.01) than during the high-intensity exercise. The product of microvascular volume and microvascular flow velocity increased marginally

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Fig. 3. Microvascular blood volume (MBV), microvascular flow velocity (MFV), and microvascular blood flow (MBV × MFV) measured by contrast-enhanced ultrasound at basal and 60 min following ingestion of a mixed meal. #P < 0.01 compared with basal; paired t-test.

Fig. 4. Forearm blood flow measured by Doppler ultrasound during study 2. Blood flow was measured at rest and during the last 4 min of a 12-min exercise protocol with handgrip performed every 20 s. Measurements were made in 5-s blocks during the 20-s interval following a contraction. *P < 0.05 compared with rest flow (0%); repeated-measures ANOVA.
increase the delivery of nutrients or hormones to muscle in direct proportion to the surface area available for diffusion as predicted by Fick’s law, $J_s = P \times A(C_p - C_i)$, where $J_s$ is flux, $P$ is permeability, $A$ is area and $C_p$ and $C_i$ are the plasma and interstitial concentrations, respectively. In this regard, the changes in microvascular volume seen with both the mixed meal and modest exercise take on particular significance.

Second, within the muscle, microvascular recruitment affords the opportunity to enhance nutrient exchange between the vasculature and muscle interstitium within local environments when there is a local increase in demand. So, in the case of light exercise to small groups of muscle, as occurs with the handgrip exercise used here, we would expect that the finger flexors will have an increased metabolic demand, however modest, relative to the wrist extensors and flexors or the finger extensors. Selective recruitment of microvasculature in the finger flexor muscles might occur either with no flow change to other forearm muscle groups or even a decrease (a steal phenomenon).

Our findings in the current study provide a first demonstration that each of two very physiological stimuli provokes significant microvascular recruitment within muscle in healthy humans. In the case of exercise, we observed that microvascular recruitment increased markedly with the lowest level of exercise selected and that this occurred in the absence of any sustained increase of total forearm blood flow, measured either by plethysmography or by brachial artery Doppler. In studies of a single 20% maximum-intensity forearm contraction, Brock et al. (6) were able to demonstrate a transient rise in brachial artery flow that was maximal within 5 s and returned to basal by $\approx 15$ s. Perhaps corresponding to this, we observed a modest increase in local flow to the lightly exercised muscle, as indicated by the modest increase of product of microvascular volume and microvascular flow velocity. But again, the most striking change was the approximately threefold increased microvascular volume, which corresponds to an expanded endothelial surface available for nutrient exchange. Because total forearm flow was not increased, flow to other areas of forearm muscle, skin, or bone may have declined. The early work from Honig et al. (13) showed that, even within denervated canine gracilis muscle, electrical stimulation of some fibers could recruit capillaries feeding the stimulated fibers in the absence of changes of flow to the entire muscle. Our results using voluntary exercise of varying intensity parallel findings reported by Honig et al. for varying frequency of electrical stimulation (with contraction intensity held constant). We interpret our findings as indicating that muscle can regulate flow in a local and graded manner to specifically facilitate nutrient supply/exchange. This regulation appears to be first at the level of terminal arterioles that regulate flow distribution and with more intense exercise involves resistance arterioles and changes in total flow. It is possible that there are some changes in total forearm flow that do occur even with light exercise, and these were not picked up with the Doppler and plethysmographic methods used here. We cannot totally exclude this, and the small changes seen within the first 5 s following individual contractions may support such an effect (Fig. 4). However, such changes, if they occur, are small compared with the 2.5- to 3-fold increase in microvascular volume that was seen, and only the latter effect was sustained throughout the 20-s intervals between contractions.

Fig. 5. MBV, MFV, and MBV $\times$ MFV were measured at baseline and during the 20-s interval between contractions during modest (left, 25% maximum handgrip) and more intense (right, 80% maximum handgrip) forearm exercise. $^* P < 0.05$ and $^# P < 0.01$ compared with basal; $^\dagger P < 0.05$ and $^\ddagger P < 0.01$ compared with 25% maximum grip; two-tailed Student’s $t$-test.

(1.9-fold, $P = 0.06$) with low-intensity handgrip and strongly (7.7-fold, $P < 0.01$) with high-intensity handgrip.

**DISCUSSION**

We have for several years examined how skeletal muscle microvascular recruitment is regulated in response to insulin or exercise in rat hindlimb. This work was predicated on two considerations. First, in the case of substrates like glucose or for the hormone insulin, microvascular recruitment affords the opportunity to enhance the endothelial surface area available for exchange between plasma and muscle interstitium. Expansion of the exchange surface is particularly important when the single-pass extraction fraction ($A-V)/A$ for a particular chemical species is small, as it is for glucose and insulin under fasting conditions (5). As pointed out by Renkin (19) and Renkin and Crone (20), increasing total flow to muscle alone would minimally augment tissue exchange for such substrates. In contrast, expanding the capillary exchange surface can...
We did not attempt to carefully study the timing of the onset or termination of the microvascular recruitment seen during light exercise. We view the major import of the exercise studies to the current work to be twofold. First, inasmuch as exercise would be expected to recruit microvasculature, the increase in microvascular volume measured by CEU at all three levels of exercise serves to further validate CEU as a noninvasive method to study the microvasculature and its regulation and secondarily to confirm what has been reported in animal studies, i.e., that the flow responses within the microvasculature appear to occur at lower levels of exercise or contraction stimulation than is required for sustained changes in blood flow.

The microvascular recruitment responses to meal ingestion suggest that this process contributes to nutrient delivery to muscle postprandially in humans. Previous studies of limb blood flow in humans in response to meal ingestion have yielded divergent findings. None had previously examined muscle microvascular responses, and, as pointed out previously, it is the expansion of capillary surface area that most facilitates nutrient exchange, not increases in total blood flow. Fugmann et al. (9) used venous capacitance plethysmography to measure forearm blood flow in response to a mixed meal. They reported a substantial increase in blood flow and suggested that this vascular response in total limb blood flow was an important determinant of forearm glucose uptake. In contrast, others have failed to see a significant enhancement of limb blood flow following mixed-meal ingestion (12). Likewise, with oral glucose ingestion some studies suggest an increase in muscle blood flow (3), whereas others do not (15). With a pure protein meal, early studies did not report any increase in limb blood flow (26), whereas later work indicated that protein-rich and carbohydrate-rich meals do stimulate limb blood flow but that a high-fat meal is not accompanied by any change in limb blood flow (14). A variety of technical differences in the way blood flow was measured (e.g., capacitance plethysmography, dye- or thermodilution, or nuclear scintigraphy) or whether the meal was liquid or solid may have influenced these findings. Interestingly, our findings, as well as those from a number of feeding studies, suggest that the hemodynamic response to feeding is quite distinct from that with euglycemic hyperinsulinemia. The insulin clamp method has been extensively used to probe the vascular actions of insulin (2, 16, 17). The more complex input that derives from meal ingestion, however, results in more rapid and quantitatively greater changes in plasma catecholamines, cardiac output, and limb blood flow (14) than what is seen with euglycemic hyperinsulinemia. In the present study, the rise in plasma insulin overall was quite modest (~40 mU/l). This magnitude of midphysiological range increments of insulin when achieved by steady-state intravenous insulin infusion have generally not been accompanied by changes in muscle blood flow (22). In addition, in studies using mixed-meal or oral glucose ingestion, where flow to skeletal muscle has changed, the changes typically occur within 30–60 min. This is much more prompt than what is seen with intravenous insulin at similar concentrations (28).

It is of interest that in several studies hyperglycemia has been observed to diminish blood flow and vascular responsiveness (4, 10). However, the hyperglycemia, albeit mild, that accompanied meal ingestion did not appear to block either the changes in total blood flow or those seen in microvascular recruitment.

In summary, our findings suggest that a moderate-sized liquid mixed meal and light exercise are each potent stimuli for microvascular recruitment within skeletal muscle. Although the natures of the two interventions differ markedly, rendering comparisons difficult, we were struck that the magnitude of the exercise response was substantially greater. Inasmuch as microvascular recruitment provides an effective method to enhance nutrient delivery by expanding the endothelial transport surface area, we conclude that these vascular changes are likely important for nutrient exchange during exercise or feeding. We also conclude that exercise provokes a differential response between microvascular recruitment (which is seen at low levels of exercise) and changes in total blood flow, which require a more intense stimulus. These differential effects of exercise intensity in humans are reminiscent of what we have previously observed in rat leg muscle in dose-response studies of insulin. The microvasculature appears more responsive to both low-level exercise and insulin than is the case for resistance arterioles.

REFERENCES


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