Nutritive blood flow affects microdialysis O/I ratio for $^{14}$C-ethanol and $^3$H$_2$O in perfused rat hindlimb

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Newman, John M. B., Carla A. Di Maria, Stephen Rattigan, and Michael G. Clark. Nutritive blood flow affects microdialysis O/I ratio for $^{14}$C-ethanol and $^3$H$_2$O in perfused rat hindlimb. Am J Physiol Heart Circ Physiol 281: H2731–H2737, 2001.—Changes in the microdialysis outflow-to-inflow (O/I) ratio for $^{14}$C-ethanol and $^3$H$_2$O were determined in the perfused rat hindlimb after increases and decreases in nutritive flow mediated by the vasoconstrictors norepinephrine (NE) and serotonin (5-HT), respectively. Microdialysis probes (containing 10 mM $^{14}$C-ethanol and $^3$H$_2$O pumped at 1 or 2 µl/min) were inserted through the calf of the rat. Hindlimb perfusion flow rate was varied from 6 to 56 ml·min$^{-1}$·100 g$^{-1}$ in the presence of NE, 5-HT, or saline vehicle. The O/I ratios for both tracers were determined at each perfusion flow rate, as was perfusion pressure, oxygen uptake (a surrogate indicator of nutritive flow), and lactate release. Both tracers showed a decreased O/I ratio as hindlimb perfusion flow was increased, with $^{14}$C-ethanol being higher than $^3$H$_2$O. NE decreased the O/I ratio compared with vehicle, and 5-HT increased it for both tracers and both microdialysis flow rates. We conclude that the microdialysis O/I ratio, while able to detect changes in total flow, is also sensitive to changes in nutritive and nonnutritive flow, where the latter still extracts tracer, but less than the former.

Microdialysis is a technique used to monitor the concentrations of biologically important compounds in the interstitial fluid of various organs including the brain, skin, adipose tissue, and muscle (for reviews, see Refs. 3 and 16). The technique is also used to monitor blood flow in tissues by the addition of ethanol to the microdialysis solution. As it passes through the probe, ethanol diffuses into the tissue and is removed by the blood. The ratio of the outflow concentration to inflow concentration (O/I ratio) of ethanol has been found to vary inversely with the total blood flow in skeletal muscle (19, 36), which has been mathematically modeled (38). The method has been extended to use $^{14}$C-ethanol as well as $^3$H$_2$O, both of which have been shown to give similar qualitative results (36), with $^3$H$_2$O having a lower O/I ratio, reflecting its ability to diffuse more readily across the dialysis membrane and through the interstitial fluid.

Work in this laboratory using the constant flow perfused rat hindlimb has followed from a number of studies that concluded that there were two vascular pathways in skeletal muscle (1, 15, 21, 27, 32) and has been recently reviewed (7). It has led to the finding that vasoconstrictors can be characterized into two groups based on their effects on muscle metabolism (6). These vasoconstrictor-mediated changes occur without any changes in nutritive flow between individual muscles or other tissues, as indicated by microsphere embolism (7). The first group, type A, to which norepinephrine (NE) belongs, increase oxygen uptake (11), aerobic muscle contraction (31), and release of lactate (17), glycerol (5), uracil, and uric acid (9). Type B vasoconstrictors, to which serotonin (5-HT) belongs, on the other hand, decrease oxygen uptake (12), aerobic muscle contraction (13), insulin-mediated glucose uptake (29), and acute release of lactate (6), glycerol (6), uric acid, and uracil (6). These effects of vasoconstrictors are mediated via the vasculature, because the effects on pressure and metabolism can be reversed by the addition of vasodilators (10, 11, 17, 29). In addition, the stimulatory (or inhibitory) effects of vasoconstrictors on contractility or insulin-mediated glucose uptake seen in perfused muscle are absent when muscles are isolated and incubated (13, 29, 30). Accordingly, we proposed that the two types of vasoconstrictors (A and B) achieve these effects by redistributing flow at the microvascular level, with type A or B increasing or decreasing nutritive flow, respectively (7). To add to this notion, we have shown that type A vasoconstrictors increase blood flow to vessels supplying the muscle cells (7, 25), whereas type B vasoconstrictors redirect flow away from the muscle cells (7, 25) toward vessels of limited exchange capacity probably associated with the interfibrillar septa of muscle and with tendon (26). An inverse relationship between oxygen uptake and tendon vessel flow exists (26), suggesting that, under the conditions of the constant-flow perfused hindlimb, oxygen uptake is a surrogate indicator of nutritive flow.
flow. Further evidence that the nonnutritive route is present in the muscle body comes from our most recent studies (4, 37) where both nutritive and nonnutritive routes were found to be distributed homogeneously in muscle.

The redirection of flow within each muscle of the perfused rat hindlimb in the absence of changes in total flow may have important implications for the microdialysis ethanol technique. The addition of a type A vasoconstrictor would be expected to switch flow from nonnutritive to nutritive and thereby improve the removal of ethanol from the probe, leading to a decrease in the O/I ratio. Type B vasoconstrictors would have the opposite effect, switching flow from the nutritive to the nonnutritive, which still extracts tracer, but less than nutritive flow; thus the O/I ratio should increase. In this study, the O/I ratio for [14C]ethanol and 3H2O were determined in the perfused rat hindlimb over a range of total blood flow rates with and without the addition of the vasoconstrictor NE or 5-HT to alter the proportion of nutritive to nonnutritive flow.

METHODS

Animals. Male hooded Wistar rats were cared for in accordance with the principles of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (Australian Government Printing Service; Canberra, Australia). Experimental procedures were approved by the Committee on the Ethical Aspects of Research Involving Animals of the University of Tasmania. Rats (180–200 g) were housed at 22°C and were given free access to water and a commercial rat chow (Gibsons; Hobart, Australia; containing 20.4% protein, 4.6% lipid, 69% carbohydrate, and 6% crude fiber with added vitamins and minerals). Anesthesia was administered by pentobarbitone sodium (6 mg/100 g body wt ip) before all surgical procedures.

Microdialysis probes were linear in construction (Fig. 1A). The point of a 23-gauge Terumo syringe needle was blunted by filing, and the syringe adapter was removed. A short length (25 mm) of microdialysis tubing (Bioanalytical Systems; molecular-weight cutoff, 30 kDa; outer diameter, 320 μm) was inserted into the blunt end of the needle to a depth of ~5 mm and glued using Araldite. The amount of glue was kept to a minimum so that the overall diameter was less than the internal diameter of a 18-gauge needle.

Hindlimb perfusions. Perfusions were conducted in a temperature-controlled cabinet set at 37°C. The perfusion medium was a modified Krebs-Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, and 8.3 mM glucose) containing 2.5 mM CaCl2, 4% bovine serum albumin, and washed bovine erythrocytes (35% hematocrit). Fresh bovine erythrocytes were washed three times in saline (0.9% NaCl) and twice in Krebs-Henseleit buffer and were stored in Krebs-Henseleit buffer at 4°C until use (erythrocytes were never >5 days old when used). Perfusate was gassed via a Silastic tube oxygenator with 95% air-5% CO2 and brought to 37°C by a heat exchanger coil. Both the arterial and venous perfusate passed through an arteriovenous oxygen difference analyzer (A-VOX Systems), which measures the spectral difference of arterial versus venous blood at 660 nm (35). Arterial pressure and the arteriovenous difference in blood oxygen (in ml O2/100 ml blood) were recorded continuously by the data acquisition program Windaq. The arterial and venous perfusate were periodically sampled for the determination of lactate concentration on a Yellow Springs Instrument glucose/lactate analyzer.

Hindlimb surgery was essentially as described in Ref. 34 with modifications as given in Ref. 11. Flow was restricted to the left hindlimb by ligation of the right common iliac artery and vein. Also during the surgery, a small area of skin over the gastrocnemius and tibialis muscles was removed. One or two microdialysis probes were inserted into the muscle with the aid of an 18-gauge syringe needle. This was achieved by initially inserting the 18-gauge steel needle through the muscle at the site where the probe was to be positioned. The steel 23-gauge needle end of the probe was then inserted into the emergent end of the 18-gauge needle, which was then slowly withdrawn, leaving only the microdialysis tubing in contact with the muscle. Other details are given in the text.
min with either one 10-min sample for probes set at 1 μl/min or two 5-min samples for probes set at 2 μl/min. The volume of sample collected was then determined by reweighing the tubes, which were then put into 5-ml vials containing 3 ml of scintillant. Prior testing indicated that the volume of the collected dialysate was as expected from the perfusion rate and time of collection; the consecutive 5-min samples at 2 μl/min were averaged because they were not significantly different. A known volume of the inflow solution was also put into a vial containing 3 ml of scintillant. Vials were then counted for 14C and 3H in a Beckman counter (LS 6500), and the O/I ratios for both [14C]ethanol and 3H2O were determined.

A separate set of experiments was conducted to assess the effect of a vasodilator on the O/I ratio of [14C]ethanol and 3H2O. Perfusions were equilibrated with a single probe in the calf muscle set at 2 mM nitroprusside (NP), 70 nM NE, 70 nM NE + 0.5 mM NP, 300 nM 5-HT, or 300 nM 5-HT + 0.5 mM NP were infused for 40 min. During the last 10 min of the equilibration and treatment periods, two 5-min samples of microdialysate were collected. The O/I ratios for both [14C]ethanol and 3H2O were determined in the same way as the previous set of experiments.

Statistics. One-way or two-way repeated-measures ANOVA was performed using SigmaStat (SPSS Science; Chicago, IL), with comparisons made between conditions using the Student-Newman-Keuls post hoc test. Significance was assumed at the level of P < 0.05. Data are presented as means ± SE; if error bars are not visible, they are within the symbol.

RESULTS

Figure 2 shows changes in hindlimb perfusion pressure, oxygen uptake, and lactate efflux of the perfused hindlimb in response to increasing total flow. At the doses chosen, NE and 5-HT produced similar increases in pressure, which were significantly above that of the vehicle (Fig. 2A). Figure 2B shows that NE significantly increased oxygen uptake compared with vehicle at all flow rates except 1 ml/min, whereas 5-HT significantly decreased oxygen uptake at the higher three flow rates. Lactate efflux was significantly different to vehicle in the presence of NE at the three higher flow rates but was unaffected by 5-HT (Fig. 2C).

The effect of increasing hindlimb perfusion flow rates on the O/I ratio for [14C]ethanol is shown in Fig. 3. Figure 3A shows data from probes set at 1 μl/min, and Fig. 3B shows data from probes set at 2 μl/min. Increasing the perfusion flow rate decreased the O/I ratio for [14C]ethanol under all three conditions, as indicated by a significant difference compared with values at 1 ml/min. NE significantly decreased the O/I ratio compared with vehicle at perfusion flow rates of 2, 6, and 9 ml/min for probes set at 1 μl/min (Fig. 3A) and at all perfusion flow rates for probes set at 2 μl/min (Fig. 3B). 5-HT, on the other hand, significantly increased the O/I ratio at perfusion flow rates of 4, 6, and 9 ml/min for probes set at 1 μl/min (Fig. 3A) and at 6 and 9 ml/min for probes set at 2 μl/min (Fig. 3B).

The effect of increasing hindlimb perfusion flow rates on the O/I ratio for 3H2O is shown in Fig. 4. Increasing perfusion flow rate led to decreasing O/I ratios for 3H2O under all three conditions (shown by a significant difference compared with values at 1 ml/min). NE significantly decreased the O/I ratio for 3H2O at all perfusion flow rates for both probe flow rates compared with vehicle. 5-HT significantly increased the O/I ratio compared with basal at all perfusion flow rates except 1 ml/min for probes set at 1 μl/min (Fig. 4A) and perfusion flow rates of 4–9 ml/min for probes set at 2 μl/min (Fig. 4B).

Figure 5 shows the effect of the vasodilator NP as well as NE and 5-HT on the oxygen uptake, perfusion pressure, and O/I ratios for [14C]ethanol and 3H2O. NP blocked all of the increase in pressure due to NE or 5-HT. There was a slight dilation below basal, although this was not significant. The effect of NP on oxygen

![Fig. 2. Effect of vehicle, norepinephrine (NE), or serotonin (5-HT) on perfusion pressure (A), oxygen uptake (B), and lactate efflux (C) in the perfused rat hindlimb at varying total flow rates. The conditions were: • vehicle, ■ NE, and ● 5-HT; n = 6 for all conditions at flow rates of 1 and 2 ml/min and n = 10 for the higher flow rates. Statistical significant was assessed by two-way repeated-measures ANOVA, with comparisons using the Student-Newman-Keuls multiple-comparison procedure. Data were compared with 1 ml/min within each condition (*P < 0.05) or to vehicle values at corresponding perfusion flow rates (**P < 0.05).](http://ajpheart.physiology.org/)

![Fig. 3A](http://ajpheart.physiology.org/)
uptake was inhibitory and so did not block the 5-HT-mediated decrease in oxygen uptake. NP, however, was able to block the increase in oxygen uptake due to NE. NP entirely blocked the change in the O/I ratios for both tracers due to NE and 5-HT. A tendency for NP to increase the O/I ratio under basal conditions was not significant.

**DISCUSSION**

The major finding arising from this study is that, whereas the O/I ratio of [14C]ethanol and 3H2O is inversely related to the total flow in the perfused rat hindlimb, the ratio can also be affected by the addition of the vasoconstrictors NE and 5-HT. The inverse relationship between the O/I ratio and total flow supports data by other researchers (19, 36). The important new data are that two vasoconstrictors that have similar effects on pressure, but opposing effects on muscle oxygen uptake and hence nutritive flow, can also have opposing effects on the O/I ratios of [14C]ethanol and 3H2O. This is despite the proportion of total flow to individual muscles and muscle as a whole, as indicated by microsphere entrapment being unchanged by NE or 5-HT (5).

Explanations for this must, therefore, concentrate on how NE and 5-HT can affect the removal of [14C]ethanol and 3H2O from the microdialysis probe. For the tracers to be removed from the system, they must first diffuse into the interstitial fluid and from there into the capillaries to be carried away by the blood flow. Diffusion into the capillaries can be affected by two things: one is the blood flow rate through capillaries with direct access to the interstitial fluid and the other is the number of capillaries through which the blood flow is passing at any time. The total blood flow, as assessed by 15-μm microsphere distribution, to individual muscles was unaffected by NE or 5-HT (5). It is thus the second component which the vasoconstrictors are likely to have altered. That is, we would envisage that NE and 5-HT have changed the effective tissue blood flow at a level of vessels smaller than 15 μm that are in contact with the interstitial fluid around the probe. Therefore, because NE decreased the O/I ratio, it is

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*Fig. 3. Effect of vehicle, NE, or 5-HT on the outflow-to-inflow (O/I) ratio of [14C]ethanol in the perfused rat hindlimb. Microdialysis probes were set at 1 μl/min (A) or 2 μl/min (B). Conditions and symbols were the same as in Fig. 2; n = 8 for all conditions at all perfusion flow rates in A and the same as for Fig. 2 in B. Data were compared with 1 ml/min within each condition (§P < 0.05) or to vehicle values at corresponding perfusion flow rates (*P < 0.05).*

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*Fig. 4. Effect of vehicle, NE, or 5-HT on the O/I ratio of 3H2O in the perfused rat hindlimb. Microdialysis probes were set at 1 μl/min (A) or 2 μl/min (B). Conditions and symbols were the same as in Figs. 2 and 3; n = 8 for all conditions at all perfusion flow rates in A and the same as for Fig. 2 in B. Data were compared with 1 ml/min within each condition (§P < 0.05) or to vehicle values at corresponding perfusion flow rates (*P < 0.05).*

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*Fig. 5. Effect of vehicle, NE, or 5-HT on the O/I ratio of 3H2O in the perfused rat hindlimb. Microdialysis probes were set at 1 μl/min (A) or 2 μl/min (B). Conditions and symbols were the same as in Figs. 2 and 3; n = 8 for all conditions at all perfusion flow rates in A and the same as for Fig. 2 in B. Data were compared with 1 ml/min within each condition (§P < 0.05) or to vehicle values at corresponding perfusion flow rates (*P < 0.05).*
likely to have increased the local tissue blood flow adjacent to the probe by increasing the number of capillaries and/or the flow rate through the capillaries near the probe. Because total flow to each muscle remained constant, the increase in flow around the probe must come at the expense of flow to another part of the muscle. The concept of increased number of capillaries being perfused is consistent with previous studies showing that NE accesses a new vascular space in muscle (25) at the expense of flow in the nonnutritive route, which may be located nearby in muscle (4) or in connective tissue (26).

5-HT, on the other hand, probably decreased the number of capillaries and/or the flow rate through capillaries in contact with interstitial fluid around the probe. In a complementary fashion to NE, 5-HT is likely to have increased flow through vessels smaller than 15 μm that are less involved with nutrient exchange but still within the muscle for the total flow to the muscle to remain constant. Again, this is consistent with previous studies, where 5-HT was found to deny access to a vascular space (25), increase nonnutritive flow (4), and increase connective tissue vessel flow (26).

It is important to note that the effects of either NE or 5-HT to change the O/I ratios were blocked by the nitrovasodilator sodium NP in association with blockade of the pressure increases. This confirms that the effects of NE and 5-HT are the result of the vasoconstrictor activity and are not the result of receptor-mediated metabolic effects in the skeletal muscle. Because the perfused rat hindlimb is essentially fully dilated under basal conditions, NP has no significant pressure-lowering effect unless the hindlimb vasculature is preconstricted by either NE or 5-HT (Fig. 5A). Thus NP prevents flow redistribution by relaxing the sites constricted by either agonist.

At present, there are no methods that allow a quantitative assessment of the proportion of nutritive to nonnutritive flow either under basal conditions (no additions) or after NE or 5-HT addition. Thus the interventions serve simply to alter the proportion of flow, and, at best, only approximations can be made. For example, Fig. 4A shows that 5-HT addition at the total flow rate of 9 ml/min increased the O/I ratio of 3H2O to a value of nutritive flow comparable with that of NE at 1 ml/min. From that, the nutritive flow after 5-HT is approximately one-ninth the total flow, providing NE has fully recruited nutritive flow at 1 ml/min. However, the precise proportion is unknown, and the extent of change induced by each agonist is dependent on dose and bulk perfusion flow rate. While this may account for the similar O/I ratios, such absolute values for relative flow in the two routes must be treated with caution.

The O/I ratio for ethanol has been used in animals to monitor blood flow changes during insulin infusion (14, 20, 22) as well as in humans during hyperinsulinemia (33), glucose ingestion (23), or exercise (18). A recent study (28) on exercising humans showed that exercise, which increased femoral blood flow, decreased the O/I ratio of ethanol but that the O/I ratio was unaffected by the subsequent infusion of adenosine despite further increases in femoral blood flow. The authors concluded that the O/I ratio of ethanol was due to changes in the probe recovery during exercise rather than the increase in flow to the muscles. This apparent discrepancy between flow and O/I ratio may be explained by the notion of two vascular routes through muscle. The infusion of vasodilators such as adenosine, while increasing total blood flow, would not necessarily increase the flow to capillaries in intimate contact with muscle cells. This has indeed been shown to be the case for isoproterenol, which specifically dilates the transverse arterioles of the rabbit tenuissimus muscle to increase flow to the connective tissue at the expense of

Fig. 5. Effect of vehicle, nitroprusside (NP), NE, and 5-HT on perfusion pressure (A), oxygen uptake (B), O/I ratio for [14C]ethanol (C), and O/I ratio for 3H2O (D). Perfusion flow rate was 4 ml/min and probe flow rate was 2 μl/min. Data are shown as a percentage of the corresponding basal for each condition: vehicle (open bars; n = 5), 0.5 mM NP (solid bars; n = 4), 70 nM NE (hatched bars; n = 5), 70 nM NE + 0.5 mM NP (crosshatched bars; n = 4), 300 nM 5-HT (horizontally lined bars; n = 4), and 300 nM 5-HT + 0.5 mM NP (horizontally and vertically lined bars; n = 4). Statistical significance was assessed by two-way repeated-measures ANOVA, with comparison using the Student-Newman-Keuls multiple-comparison test. Data were compared with their corresponding basal values (§P < 0.05) or to corresponding data without NP (∗P < 0.05).
flow to muscle (2). Data from this study also suggest that the vasodilator NP does not enhance nutritive flow in that it decreased oxygen uptake and had a tendency to increase the O/I ratio for both $[^{14}C]$ethanol and $^{3}H_{2}O$ under basal (vehicle only) conditions (Fig. 5).

The use of the microdialysis O/I ratio as a putative indicator of total blood flow in skeletal muscle would seem to be of use only if the proportion of nutritive to nonnutritive flow remains constant. The presence of two vascular pathways in muscle may have also led to discrepancies between total flow and laser Doppler flowmetry (LDF). A study (24) using LDF on rat skeletal muscle during ganglionic blockade found that changes in LDF signal were dissociated with changes in total muscle blood flow, as measured by microspheres during the infusion of vasoactive drugs. In a recent study (4) from our laboratory, the LDF signal from implantable microprobes inserted into muscles of the perfused rat hindlimb was investigated. The data showed there were three characteristic responses to the infusion of NE and 5-HT indicative of discrete regions of nutritive and nonnutritive flow in muscle. Larger surface probes showed only one type of response, that of increasing nutritive flow in response to NE (4).

This study, therefore, provides further evidence for the existence of two flow pathways within muscle, the nutritive and nonnutritive vessels (7, 8). The nutritive vessels, which are probably distributed throughout the muscle in the interfibrillar septa, are readily visible in the tendon region (26). They would probably be of short length and larger diameter, but still be capillary in nature and therefore, unable to pass 15-$\mu$m microspheres and with a low capacity for nutrient exchange. The model most likely is one where there are both nutritive and nonnutritive routes in the immediate vicinity of the probe, so that the two vasoconstrictors have simply acted by switching flow from the nutritive route, which can pick up the $^{3}H_{2}O$ or $[^{14}C]$ethanol to the nonnutritive route, which still extracts tracer but less than nutritive flow. Given that the probe is 320-$\mu$m in diameter, the region within the muscle tissue sensed by the probe is likely to be a cylinder of 640–1,120 $\mu$m [i.e., 2- to 3.5-fold the diameter of the probe (38)]. If this is so, then the region of sensing would contain both nutritive and nonnutritive routes, as recently detected using a randomly placed 260-$\mu$m-diameter LDF microprobe (4) and predicted by the findings of Vincent et al. (37).

The O/I ratio of ethanol in skeletal muscle has been mathematically modeled (38). The model described how the O/I ratio changed as the flow rate through the microdialysis probe and the blood flow rate changed. A further study (19) showed consistency between the model and experimental data. However, there was no contingency for the distribution of flow within muscle. Clearly, this requires a reconsideration of the model, (38) particularly in view of our present findings and the presence of two alternative flow routes in muscle.

It is assumed that the vasoconstrictors do not affect the metabolism of ethanol, which would then affect the O/I ratio. Metabolism of ethanol would seem very unlikely because the O/I ratios of both tracers, $[^{14}C]$ethanol and $^{3}H_{2}O$, show very similar patterns (Figs. 3 and 4) and muscle does not appreciably metabolize ethanol because it contains no alcohol dehydrogenase. Metabolic effects of the vasoconstrictors are also unlikely because the addition of the vasodilator NP blocked the effect of NE and 5-HT on the O/I ratios.

There appears to be little advantage between either of the tracers used in this study due to the similarity of the results gained by them. It may be, however, that $^{3}H_{2}O$ is a more useful tracer because it is more freely diffusible. Also, ethanol is more prone to evaporation in the collection tube than water and thus can lead to greater errors in measurement. Indeed, if a proportion of the ethanol evaporated, this would not make an appreciable difference to the volume collected but would mean that the actual concentration of $[^{14}C]$ethanol was underestimated. In the case of $^{3}H_{2}O$, any loss by evaporation would be matched by a loss of total volume without a net effect on tracer concentration. This may be reflected in the fact that, whereas the O/I ratio of $[^{14}C]$ethanol was above that for $^{3}H_{2}O$, the difference is not as great as has been reported previously (36).

In conclusion, the current study demonstrates that the O/I ratios of $[^{14}C]$ethanol and $^{3}H_{2}O$ reflect both the total flow to muscle and the distribution of flow in pathways within muscle. Therefore, the distribution of flow within muscle, specifically the proportion of total flow that is nutritive, must be taken into account in the interpretation of data obtained with this method. The findings reinforce the notion that the nonnutritive route carries flow but is unavailable for nutrient as well as $[^{14}C]$ethanol and $^{3}H_{2}O$ exchange. Finally, providing bulk blood flow to muscle remains constant, microdialysis using the O/I ratio of $[^{14}C]$ethanol or $^{3}H_{2}O$ has the potential to be a qualitative measure of the proportion of nutritive to nonnutritive flow.

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