

Muscle Perfusion

Its Measurement and Role in Metabolic Regulation

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Methods for measuring muscle blood flow have been evolving over the past 120 years (1,2). Studies of hormonal regulation of muscle flow and metabolism began with the classical work by Andres et al. (3). Numerous diabetes investigators interested in muscle metabolism *in vivo* have estimated the net balance of glucose and other metabolites across a skeletal muscle bed or limb from the product of the arterial-venous concentration difference and the blood flow. In this review, drawing upon early studies, we will emphasize some of the principles and limitations of various techniques for measuring flow to estimate net exchange or rates of production or consumption of metabolites. Table 1 summarizes pertinent strengths and limitations of the most commonly used methods for estimating either muscle blood flow or perfusion. From later studies, we will deal more directly with the issue of how flow is hormonally regulated and the relationship between skeletal muscle flow regulation and metabolic regulation. That discussion will extend beyond flow alone as an important regulated variable, emphasizing instead perfusion, which encompasses both the rate and distribution of blood flow in a tissue. We will highlight some of the new methodologies that have helped clarify further the linkage between the regulation of skeletal muscle perfusion and metabolic function.

LIMB BALANCE MEASUREMENTS IDENTIFY SITES OF INSULIN ACTION AND RESISTANCE

It is appropriate to begin this discussion with the development of the forearm balance technique by investigators at Johns Hopkins in the early 1950s. These investigators put forward the hypothesis that through continuous infusion of a dye “tracer” (in this case Evans blue dye) that binds tightly and rapidly to serum proteins into the brachial artery and sampling from an ipsilateral antecubital vein, blood flow to the forearm could be quantified using simple spectrophotometric methods (3). They pointed out several advantages to the forearm for such studies, including 1) that skeletal muscle makes up the preponderance (~80%) of the tissue mass of the forearm; 2) the forearm’s relatively small mass and slow blood flow allow infusion

of very small amounts of dye, which minimizes the contribution of recirculating dye; and 3) the vascular anatomy of the forearm is well understood and in >80% of individuals bifurcation of the brachial artery occurs below the antecubital crease, and therefore infusion of dye above the elbow should distribute to both the radial and ulnar vessels. Their measurements of flow corresponded well with the plethysmographic measurements that were available at that time. Plethysmography measures blood flow from the time-dependent increase in volume of a segment of a limb after venous outflow occlusion using either a strain gauge or other detection device. The development and application of plethysmographic limb flow measurements have recently been excellently reviewed (4). As there is no gold standard for measuring flow in clinical studies, cross-validation between methods provides needed assurance.

In these dye dilution studies, the issue of dye mixing in the brachial artery was extensively examined (3), as adequate mixing is clearly required for accurate blood flow measurements. Despite the finding that dye streaming occurred at the infusion rate used, it was observed by simultaneously sampling from multiple forearm veins that adequate mixing had occurred in most subjects. Interestingly, use of a jet injector to promote mixing of the infusate at the arterial injection site provoked downstream vasodilation (perhaps secondary to ATP or adenosine released by the endothelium traumatized by the jet shear) and was abandoned. Traction on the arterial catheter also altered downstream arterial resistance and flow, underscoring that care must be taken with this method.

Combining this dye dilution method with arterial-venous (A-V) metabolite sampling allowed estimation of the substrate balance across the forearm (Fig. 1). These “limb balance” studies took advantage of the fact that the forearm receives only approximately one-fiftieth of the cardiac output (5). As a result, infusion of low doses of insulin (e.g., 0.05 mU/min/kg body wt) into the brachial artery provoked physiologically significant increases in plasma insulin concentrations bathing the forearm musculature, but when diluted in the whole-body plasma pool it had minimal or no effect on plasma glucose, potassium, or other metabolite concentrations. The same circumstance does not pertain for infusion of insulin into the femoral artery when leg balance measurements are made. The leg’s greater mass and blood flow require higher rates of insulin infusion, and the insulin recirculates and affects plasma glucose and other metabolites. Using this forearm balance method a decade before they developed the insulin clamp (6), these investigators demonstrated that physiologic doses of insulin stimulated skeletal muscle glucose uptake under euglycemic conditions in humans (5,7) and that this action of insulin was impaired in obese adults (7).

Within a few years, other laboratories had begun to apply this limb balance method. In particular, the Cahill

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TABLE 1
Methods for measurement of limb and muscle blood flow

Method	Provides volume flow	Tissue-specific	Subject risk	Cost	Availability	Estimates microvascular function
Plethysmography	Yes	No	Minimal	Minimal	Widespread	No
Dye or thermal dilution	Yes	No	Modest	Modest	Widespread	No
Doppler ultrasound	Yes	No	Minimal	Modest	Widespread	No
PET imaging	Yes	Yes ^a	Moderate	High	Limited	No H ₂ ¹⁵ O, maybe C ¹⁵ O
Contrast ultrasound	No	Yes ^b	Modest	Moderate	Widespread	Yes
Electromagnetic flow probes	Yes	No	High	Modest	Limited	No
Video microscopy (Nail bed; not muscle)	No	Yes ^c	Minimal	Modest	Limited	Yes
Near-infrared spectroscopy	Yes	No	Minimal	Modest	Limited	Uncertain
Arterial spin-labeling magnetic resonance imaging	Yes	Yes ^d	Minimal	High	Limited	Uncertain
Labeled microspheres	Yes	Yes ^e	Not applicable	Moderate	Not for humans	Not capillary but terminal arterioles
Microdialysis	Yes	Yes ^f	Modest	Moderate	Limited	Yes

^aSpatial resolution of ~0.6 cm³ and requires 3–5 min to accumulate signal at usual isotope doses. ^bSpatial resolution 0.5–1.0 cm³ and requires ~5 min for image sequence capture. ^cIndividual capillaries visualized in real time. ^dExcellent spatial resolution; however, not a specific vascular marker but measures rate of water access to tissue. ^eDepending on isotope dose, sections down to 1 mm³ can be measured. ^fSpatial resolution dependent on probe length and 2-mm radius, typically ~0.2 cm³; poor time resolution requiring 20–40 min for sample collection.

laboratory (8–10) at the Joslin Clinic and its diaspora (11–13) used limb balance measurements very effectively to study the actions of glucoregulatory hormones and exercise. Limb blood flow was measured by indocyanine green dye dilution or plethysmography. It is worthwhile noting that while these studies frequently ascribed the observed substrate use or production to muscle, other tissues within the limb can contribute to this measurement by either adding or removing metabolites and by contributing to the flow measurement. The former is perhaps less of an issue for the forearm where the deep antecubital vein cannulated in a retrograde fashion drains predominantly skeletal muscle, while the femoral vein is draining all muscle, adipose, and osseous tissues of the leg. However, to the extent that the A-V difference measurement selectively samples blood draining muscle (as it does in the forearm with retrograde deep forearm vein cannulation), multiplying the A-V difference for a metabolite by total forearm flow may not give an accurate balance if muscle and fat do not handle the metabolite in an identical fashion. It is important to recognize that when either forearm or leg metabolism is studied using the limb balance method, results are typically expressed as millimoles of substrate per

minute per 100 mL of forearm or leg. As sex (14), age, and body habitus (15) influence the relative proportion of skeletal muscle versus adipose tissue present in the limb, caution needs to be used regarding ascribing metabolite exchange across the forearm or leg to a particular tissue type. In this regard, it bears keeping in mind that estimated rates of blood flow to subcutaneous adipose tissue and resting skeletal muscle are comparable (~3–5 mL/min/100 g) (16) and glucose uptake to subcutaneous adipose tissue contributes significantly to body glucose disposal (17,18). Along this line, leg blood flow (milliliters per kilogram of leg) is not different between men and women, despite greater fat-free mass in males (19). These cautions and limitations pertain whether one is studying forearm or leg balances. With exercise, muscle blood flow can increase by >20-fold.

Using the leg balance method combined with the euglycemic insulin clamp, investigators demonstrated that leg glucose uptake could account for ~40% of body glucose disposal and suggested that muscle was principally responsible. Extrapolating from leg muscle mass to total body muscle, they concluded that during steady-state hyperinsulinemia, muscle accounted for 80–90% of glucose

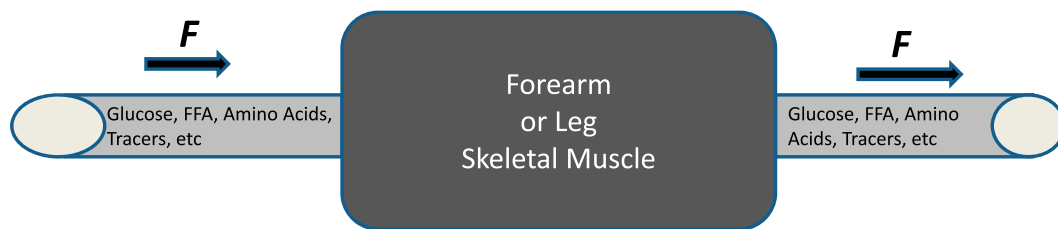


FIG. 1. Measurement of the limb balance for any given substrate or hormone is simply obtained from the measurement of flow (F) to the limb or, preferably, directly to skeletal muscle multiplied by the arterial minus venous concentration difference. It is important to know whether the substrate under study partitions only to the plasma compartment or to the erythrocyte water and how quickly this occurs. For example, in humans the balance for glucose requires use of the product of blood flow and arterial minus venous concentration difference in whole blood, while for FFAs the calculation of limb balance is the product of plasma flow and the plasma FFA concentration difference between the artery and vein. (A high-quality color representation of this figure is available in the online issue.)

disposal (20). Leg glucose uptake was also found to be a major site of insulin resistance in both obesity and diabetes. The combined measurements of bulk blood flow together with the A-V substrate concentration differences proved to be a powerful tool for quantifying the metabolism of carbohydrates (21), fats (22), amino acids (23), and oxygen (24) by the limb tissues. Either dye dilution or plethysmographic methods were used. Whereas only modest data are available in the literature comparing these two methods for measurement of bulk blood flow, they do appear to yield comparable values for bulk limb blood flow (25).

The introduction of simultaneous A-V measurement of radiolabeled substrate with limb blood flow added yet another dimension to the limb balance technique. These methods were initially used to measure fatty acid uptake and oxidation by exercising muscle (26) and later to quantify both the transport and net uptake of glucose (27), free fatty acid (FFA) (28), and amino acids and subsequently to quantify rates of protein synthesis and degradation (29), of lipolysis and lipogenesis (22), and of oxidative and nonoxidative glucose disposal (30). Once again, the quantitative accuracy of these measurements hinges entirely on the measurement of blood flow. In aggregate, these studies demonstrated the great utility of the limb balance method to quantify metabolic events in peripheral tissue and to discover how they are regulated in health and disease.

With improvements in ultrasound methodology, Doppler flow measurements have become a third widely used method for measuring bulk limb blood flow, which has increasingly displaced the more invasive dye dilution technique. Doppler flow measurements were observed to correlate well with plethysmographic measurements (31).

Muscle perfusion can be evaluated by various nuclear magnetic resonance (NMR) techniques. One of the most useful, because of its ability to determine both blood flow and flow distribution noninvasively, is arterial spin labeling (ASL). This technique can be used in humans and experimental animals (32). It has the potential to be directly compared with metabolism determined by other NMR techniques in the same region of tissue but not simultaneously (32). The ASL technique has been shown to correlate reasonably well ($r^2 = 0.85$) with leg blood flow measured by venous plethysmography in humans (33) and well ($r^2 = 0.95$) with limb blood flow in a rat-perfused hindlimb model (34). However, due to the expense of specialized NMR equipment and the substantial complexity of postacquisition data processing required, this technique has not been widely used by physiologists or clinical researchers.

Muscle perfusion has also been measured by microdialysis where a semipermeable membrane is placed in the tissue and perfused with dialysate that contains markers such as ethanol or radiolabeled water. The exchange and recovery of these markers can be used to estimate total blood flow (35) and distribution of nutritive and non-nutritive blood flow (36). Combined with other tracers, the microdialysis technique can also provide information on the interstitial concentrations of metabolic and vasoactive moieties such as glucose, glycerol, lactate, amino acids, insulin, nitric oxide (NO), and adenosine (37). The technique, however, has several limitations that include its invasive nature; lack of spatial resolution, as it is difficult to determine the amount of tissue being sampled; and temporal resolution because of the long sampling times

(minutes) required to collect sufficient dialysate for measurement.

PERFUSION AS A REGULATOR OF MUSCLE METABOLISM

A conceptual shift in the consideration of the role of blood flow in muscle metabolism occurred with studies from the laboratory of Alain Baron. He modified a method first used by Andersen and Saltin (38) for quantifying leg blood flow, which relied on a use of a thermodilution catheter introduced into the femoral vein (39). Thermodilution methods had previously been used extensively to measure blood flow in vascular beds other than skeletal muscle. While invasive, this methodology allowed very frequent measurements of blood flow during the course of prolonged metabolic balance studies. The design of the catheter allowed spraying of the chilled saline infusate to assure good mixing and nonlaminar flow prior to the temperature-sensing probe. With this technique, Baron observed that insulin not only increased glucose uptake by leg muscle in healthy individuals but also simultaneously increased blood flow. Further increasing flow with cholinergic stimulation during hyperinsulinemia significantly augmented leg glucose uptake (40). He and collaborators subsequently demonstrated that insulin increased flow by stimulation of NO production and that this flow increase was inhibited in type 2 and type 1 diabetes and obesity (39) and by experimentally induced insulin resistance (rev. in 41).

This work strongly suggested that insulin's action to promote the uptake of glucose, amino acids, and other substrates into muscle was aided by the concerted action of insulin on the vasculature to increase blood flow and presumably the delivery of insulin, glucose, and other metabolites to the muscle (41). The observation that this vascular action of insulin was blocked by inhibitors of NO synthase (NOS) (42–44) suggested that the endothelium was the target for insulin's vascular action. Subsequent studies in isolated endothelial cells demonstrated that insulin, acting via the phosphatidylinositol-3 kinase Akt pathway (45,46), specifically enhanced the activity of NOS (Fig. 3).

While these observations of insulin's effect of increasing total limb blood flow were confirmed by a number of laboratories (47,48), concerns were raised and controversy ensued around both the reported need for prolonged, relatively high steady-state insulin concentrations to provoke insulin's vascular action relative to effects on glucose disposal (49,50) and the lack of effect of increasing flow pharmacologically on limb glucose uptake in insulin-resistant (51,52) subjects.

Studies using positron emission tomography (PET) methods by investigators in Turku, Finland, were particularly informative, as PET allowed simultaneous measurements of perfusion, using labeled water ($H_2^{15}O$) and glucose (^{18}F -deoxyglucose) within voxels of muscle, avoiding the issue of tissue heterogeneity and bulk flow distribution to multiple tissues in the limb (51,53).

Radiolabeled or fluorescent microspheres have also been used in a number of animal studies to examine the effect of insulin on blood flow (54–56). As the technique requires tissue removal, it has not been applicable to clinical studies. However, results of published studies confirm that insulin can enhance skeletal muscle blood flow. Inasmuch as the microspheres used typically measure $15 \pm 3 \mu$ in diameter, it is not clear that they have

access to the capillary bed; they are more likely retained within the terminal arterioles. Multiple measurements can be made in a single animal provided different isotopes or fluorescent labels are available whose emissions can be distinguished by differences in energy spectrum. Of interest is the study by Liang et al. (55), which included measurements of the effects of insulin on multiple tissues. Insulin-induced increases in blood flow during euglycemia were most apparent in skeletal and cardiac muscle.

Since the various laboratories examining the relationship between insulin's effect on limb blood flow and metabolism used different methods for measuring flow (e.g., strain gauge plethysmography, dye- or thermodilution, or PET), some of the discordant findings may have arisen from these measurements. This issue was never fully settled. However, additional opportunities became available with the development of techniques to measure the distribution of flow within a tissue rather than simply bulk flow to the tissue. The observation that the volume of muscle microvasculature perfused was regulated dates back to August Krogh (57). Changes in the volume of muscle microvasculature perfused could influence hormone and nutrient exchange within the muscle by altering the endothelial surface area exposed.

As shown in Fig. 2, the muscle microvasculature consists of third- and fourth-order arterioles, the capillary network, and small venules. Each terminal arteriole supplies multiple

capillaries in an orderly arcade. At any given moment, only approximately one-third of the capillaries appear to be actively perfused in resting muscle (58). There is no conclusive evidence for the presence of smooth muscle "sphincters" at the capillary origin. Instead, it appears likely that many capillaries are functionally at least transiently not perfused because of 1) the residual tone limiting flow to the terminal arteriole, 2) the position of a particular capillary relative to the inflow into the arteriole, 3) the interstitial pressure around the capillary, 4) the intrinsic resistance of the capillary, and 5) the residual pressure within the lumen of the draining venule. These factors in aggregate likely determine whether an individual capillary will be perfused at a particular time. Relaxation of the terminal arteriole, with consequent increases in precapillary pressure, will lead to perfusion of previously under- or unperfused capillaries. Time variable perfusion of terminal arterioles, a process termed "vasomotion" or "flowmotion," appears to be due to the action of sympathetic nervous input, intrinsic myogenic responses in the vascular smooth muscle, and autoregulation by the endothelium within terminal aspects of the microvasculature conducting signals retrograde through gap junctions to signal to feed arterioles when some relaxation is needed to at least transiently restore perfusion (59). While intermittent perfusion of muscle microvasculature has been difficult to demonstrate in intravital microscopy studies of surgically exposed thin muscles (60),

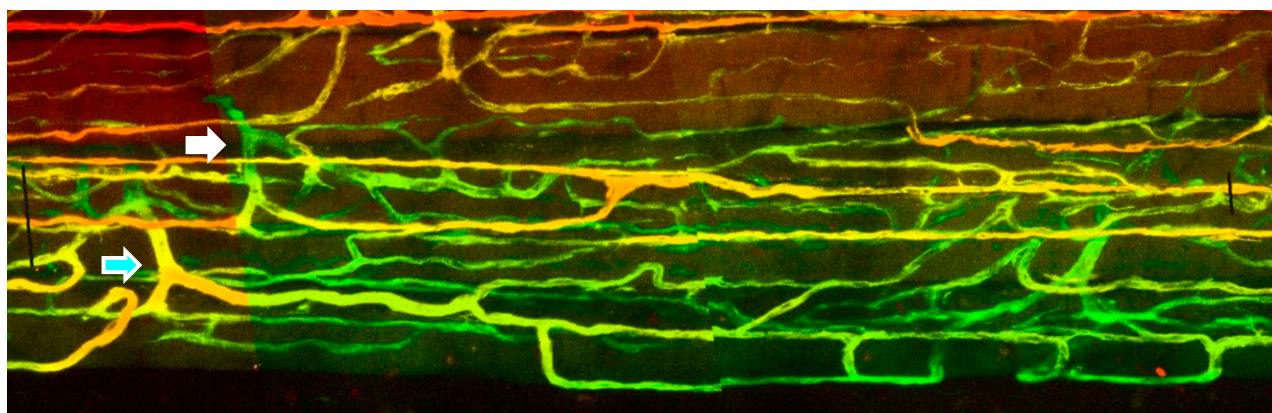
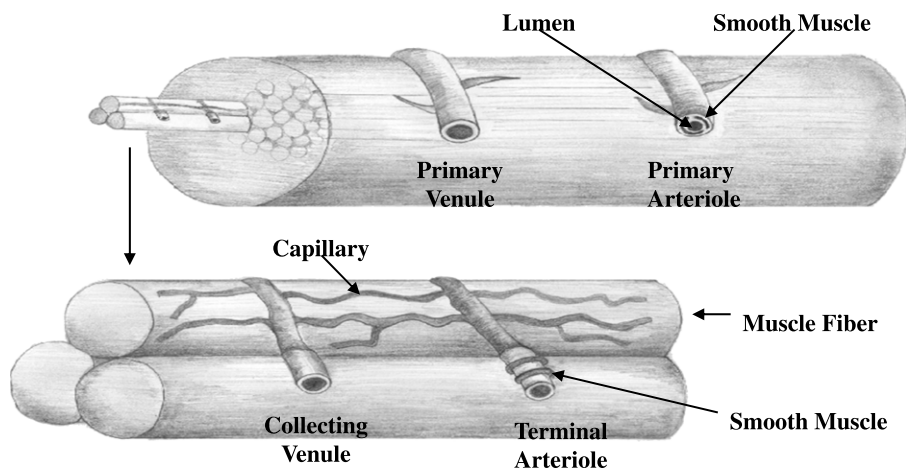


FIG. 2. A schematic (*upper panel*) and example of immunohistochemical-stained microcirculation of the spinotrapezius muscle of the rat. The capillaries line up longitudinally along the muscle fibers. Larger terminal arterioles (blue arrow, *lower panel*) cross the fiber from top to bottom, as do the draining venules (white arrows). Multiple capillaries branch off each arteriole and venule. (A high-quality digital representation of this figure is available in the online issue.)

the use of noninvasive contrast ultrasound (see below) appears to have resolved this issue in larger muscle groups in both human and animal studies.

Just over a decade ago, our laboratories began collaborating to develop and apply two methodologies for assessing the volume of vasculature perfused within muscle. The first relied on measurement of the A-V concentration difference of intravenously infused 1-methyl-xanthine (1-MX). Xanthine oxidase in the endothelial cell converts 1-MX to 1-methylurate, and the extent of metabolism provides an index of the endothelial surface perfused. Insulin was found in rats to increase 1-MX metabolism (61), and this was inhibited by factors that acutely provoked metabolic insulin resistance, including α -methyl serotonin (62), tumor necrosis factor- α (63), elevated FFA concentrations (64), and NOS inhibition (65), and with chronic obesity (66). This method, however, was not found to be useful in humans because of the substantially lower extraction ratio for 1-MX across the skeletal muscle capillary bed.

We subsequently applied the technique of contrast-enhanced ultrasound (CEU) to the measurement of microvascular blood volume in both rodent and human studies. CEU was developed initially to image myocardial perfusion. The method relies on the insonation of intravenously infused, perfluorocarbon-filled lipid microbubbles, which enhance the video intensity of ultrasound images. These microbubbles are typically 1–4 μ in diameter, remain within the vasculature, and have rheologic properties similar to erythrocytes. Most importantly, the microbubbles oscillate or rupture (depending on the energy of the ultrasound signal) when exposed to the ultrasound beam. This behavior is responsible for an intense reflected sound wave that enhances the image intensity.

Unlike Doppler, plethysmography, PET, or tracer dilution methods, CEU does not measure volume flow, i.e., milliliters per minute per 100 mL tissue. Therefore, CEU cannot be used to provide an estimate of blood flow that could be coupled with A-V difference measurements to obtain a limb balance. Rather, it provides a sensitive index of the volume of microvasculature (and therefore the microvascular surface area available for nutrient exchange) that is perfused. It is most useful for comparing acute responses to interventions like increasing plasma insulin, exercise, feeding, etc. In this regard, it has proven quite useful for examining the action of insulin on the microvasculature. Similar to PET, CEU allows study of a region of interest within the muscle or adipose tissue. However, while PET signal intensity in muscle can be calibrated to the activity in blood measured simultaneously yielding a flow measurement, this is not available with CEU. However, CEU affords several advantages over PET scanning for clinical studies. It is less expensive, involves no radioisotopes, is portable, and the image analysis is substantially simpler. Perhaps most importantly, it allows separation of signals arising from larger conduit and feed arteries from signals arising from the microcirculation. This separation is based on capturing a “replenishment curve,” i.e., a time series of images that is initiated by instantaneously destroying all microbubbles in the volume being studied and following the microbubble replenishment and recovery of videointensity until it reaches a plateau. The plateau signal intensity is reached when all vessels in the tissue that are being perfused have again filled with blood containing microbubbles. Since flow in conduit and feed arteries is rapid, these fill quickly, while the capillaries and venules refill slowly. The image

intensity created by vessels that fill rapidly can straightforwardly be subtracted from that seen at the plateau, with the difference being due to microvasculature containing microbubbles. The flow velocity for the replenishment curve is also provided by the image analysis, and flow is the product of flow velocity and volume perfused. However, both velocity and volume are measured in video-intensity units, and their product is not readily converted to units of volume flow.

In a series of studies using either contrast ultrasound or the A-V concentration difference for 1-MX as an index of the endothelial surface perfused, we observed that insulin at physiologic concentrations enhanced the microvascular volume perfused within either human forearm (67) or rat hind leg muscle. This occurs within 15 min of onset of hyperinsulinemia (68,69) at modest physiologic insulin concentrations (70) and is blocked by inhibition of NOS (65). Importantly, loss of insulin’s ability to enhance microvascular volume either by a blockade of NOS or as a result of endogenous insulin resistance secondary to obesity (71), diabetes (72), or experimental conditions (e.g., elevating FFA [73] or tumor necrosis factor- α concentrations) impedes skeletal muscle glucose disposal. This recruitment process is illustrated in Fig. 3.

The simplest explanation for this apparent relationship between the microvascular volume perfused within skeletal muscle and metabolic insulin resistance arises from understanding that the delivery of glucose and insulin to skeletal muscle appears to be a limiting process for insulin action within skeletal muscle (6,74). Increasing the endothelial surface area and thereby enhancing insulin delivery could facilitate both the time of onset and the magnitude of the metabolic response to insulin. There may in addition be factors within the endothelium that relate to insulin transendothelial transport that are adversely affected by insulin resistance and contribute further to the metabolic disarray.

There are two other potential techniques that could be used to measure the volume of vasculature perfused within skeletal muscle. The first is an adaptation of PET in which ^{15}O -labeled carbon monoxide is inhaled and binds rapidly to hemoglobin. In this manner, the PET tracer becomes a vascular tracer. With this technique, investigators have observed that insulin increases the intensity of the PET signal consistent with an increase in the volume occupied by vasculature within a given voxel. While statistically significant effects were seen, the magnitude of the effect is substantially less than is seen with CEU. This may relate to the fact that much of the PET signal appears to arise from larger blood vessels and there is no straightforward way to subtract out this component. Additionally, in order to have sufficient signal, the PET image is collected over a substantially longer period of time, and this exposure is longer than the mean transit time for blood traversing skeletal muscle. As a result, flowmotion within the tissue slice may lead to capturing signal from vessels that are only open a portion of the time. Perhaps because of these difficulties, very little has been done with this technique to examine microvascular perfusion within skeletal muscle using carbon monoxide-labeled tracers.

Near-infrared spectroscopy provides yet another potential methodology. This technique relies on the deeper penetration of near-infrared electromagnetic radiation into tissue compared with visible or ultraviolet light. It has been used extensively with pulse oximetry, and it can

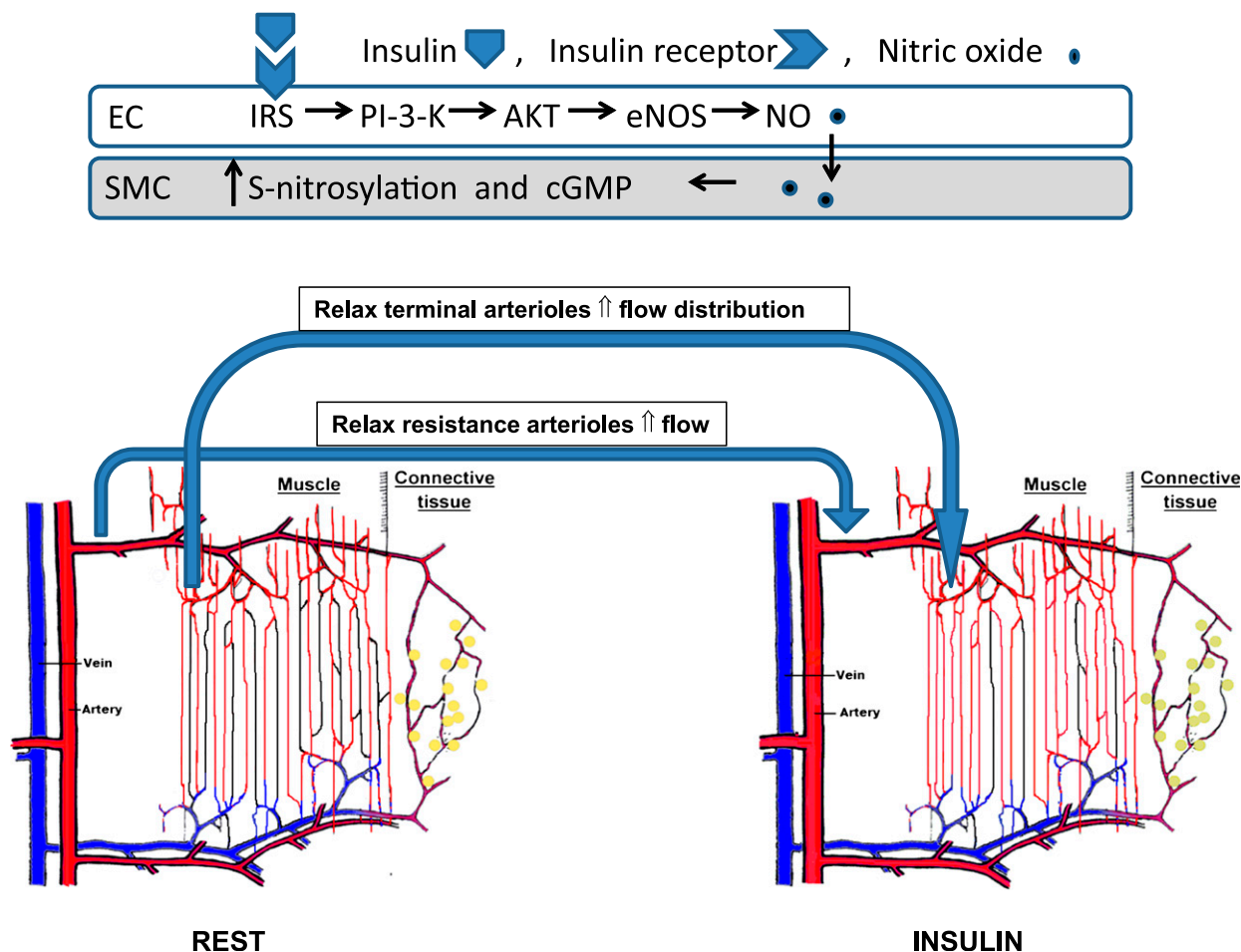


FIG. 3. *Upper panel:* Summary of the pathway by which insulin binds to and activates its receptor, thereby enhancing signaling through the phosphatidylinositol-3 kinase (PI-3-K) Akt pathway to phosphorylate endothelial NOS (eNOS) and increase its enzymatic activity, augmenting the production of NO. NO can readily diffuse to nearby smooth muscle cells (SMCs) and act both to increase the production of cyclic GMP and to directly nitrosylate specific proteins to promote smooth muscle relaxation. *Lower panel:* Effect of this relaxation on resistance and terminal arterioles is illustrated. Relaxing the former can increase overall blood flow, while relaxing the latter increases the number of capillaries perfused within the tissue (indicated by the increased numbers of red capillaries in the *right panel*) at any one time. It should be noted that this is a dynamic process, and because of flowmotion, the particular capillaries that are perfused at any time will vary over time scales from 2 to 40 s. IRS, insulin receptor substrate.

measure the relative amounts of oxyhemoglobin and desoxyhemoglobin. When coupled with venous occlusion, it can be used to determine the total rate of inflow of oxygenated blood to a muscle and potentially the microvascular volume filled. To our knowledge, it has not been applied to studies of insulin or low-intensity exercise-mediated capillary recruitment within muscle. There is concern regarding how much contribution skin and subcutaneous adipose tissue make to the near-infrared spectroscopy signal, and this would be a particular issue in obese individuals and females.

PARALLEL EFFECTS OF INSULIN AND CONTRACTION ON MICROVASCULAR RECRUITMENT

It has long been known that exercise acts synergistically with hyperinsulinemia to enhance glucose disposal. Multiple mechanisms within muscle account for this including increased glycolytic activity, recruitment of GLUT4 transporters to the plasma membrane, and increased tissue perfusion. The impact of expanding microvascular volume by recruitment of previously unperfused or underperfused capillaries on delivery of insulin to muscle tissue can be

appreciated from studies looking at the effect of microvascular recruitment induced by muscular contraction. We recently demonstrated that in the rat hind limb, even very modest contraction enhances the delivery of both insulin and albumin to the muscle interstitium (75). We were particularly intrigued by the parallel between low-dose insulin and very light exercise, which both stimulated microvascular recruitment within muscle without affecting total blood flow to the tissue. In contrast, more intense exercise (75,76) and higher insulin concentrations increase both microvascular recruitment and total blood flow. This suggests that there is an orderly, staged vascular response to either stimulus, with the microvasculature being more sensitive to either stimulus than larger resistance vessels, which regulate total flow.

Both microvascular recruitment and enhanced blood flow could increase the delivery of insulin, glucose, and other metabolites to skeletal muscle, but there may be some advantage to having a staged response with very modest increases in insulin or exercise. Capillary recruitment would not require simultaneous increases in cardiac output to prevent a fall in systemic arterial pressure. For insulin, this may be helpful particularly in the

postprandial setting when there is already a demand for increased blood flow to splanchnic tissues. However, when the stimulus is particularly strong (as occurs with high concentrations of insulin or intense exercise), other systemic vascular regulatory responses must be called into play to maintain vascular homeostasis.

Increases in microvascular volume secondary to microvascular recruitment are not restricted to skeletal muscle. With use of contrast ultrasound, insulin has been observed to increase microvascular volume within cardiac muscle (77) and subcutaneous adipose tissue of the leg (78). Using an entirely different approach, investigators in the Netherlands have demonstrated increased numbers of perfused capillaries within the nail fold as captured by video microscopy in human skin (79). This method is unique in allowing direct quantitation of the number of perfused capillaries. Insulin can regulate this recruitment, but its impact is diminished in obesity and hypertension and by insulin resistance (80).

Closing remarks. Accurate measurements of blood flow are critical for in vivo quantitation of skeletal muscle fuel metabolism and how it is affected by insulin resistance, diabetes, or other metabolic disorders. Multiple methodologies are currently available for use by clinical and basic scientists performing studies of muscle metabolism (Table 1). Plethysmography, Doppler ultrasound, and dye dilution measurements are widely available, inexpensive, portable and not technically demanding and are consequently most commonly used. Other methods such as PET and magnetic resonance imaging, while more complex and expensive and not portable, give more detailed information about specific blood flow within a region of interest of tissue. This latter information is not available with the more readily performed methods. Doubtless, with improving technology these types of measurements will be made more often. Contrast ultrasound can provide unique information with regard to the perfusion of the microvasculature and a measurement of microvascular volume. However, CEU does not provide a measure of bulk flow. Using a variety of different techniques, investigators have clearly realized the intimate relationship between regulation of perfusion and metabolic regulation within muscle. In coming years, more will be learned with regard to the chemical signals responsible for the coordination of these two functions.

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