The Vascular Contribution to Insulin Resistance: Promise, Proof, and Pitfalls

Eugene J. Barrett[1] and Etto C. Eringa[2]

Insulin resistance is a potent and highly prevalent risk factor for diabetes and cardiovascular disease. A landmark compartmental analysis of human insulin kinetics (that led to the development of the euglycemic insulin clamp) identified insulin's slow transit from plasma to muscle as a rate-limiting step for insulin-mediated glucose disposal (1). This first step of insulin-stimulated glucose uptake, i.e., insulin's crossing from plasma to muscle interstitium, is governed by vascular endothelium. Accumulating evidence supports a contribution of endothelial insulin transport to insulin resistance (2). The insulin receptor can mediate transendothelial insulin transport (3), and mice lacking insulin receptor substrate 2 specifically in vascular endothelium are insulin resistant. Nevertheless, the regulation of muscle transendothelial insulin transfer, especially in humans, is poorly understood (2) (Fig. 1).

Findings from previous studies using cultured endothelial cells (3–5) have demonstrated a transfer process involving insulin binding to the insulin or (at high concentrations) the IGF-I receptor. Insulin uptake requires intact insulin signaling to endothelial nitric oxide synthase within the endothelial cell (6), and transendothelial insulin transport appears to involve a complex vesicular trafficking process (2). In vivo, the endothelial cells in rat muscle accumulate insulin and its transport is a saturable process, indicating a role for the insulin receptor in the transendothelial insulin transport (5,7) in muscle.

In humans, the contribution of impaired transendothelial insulin transport to insulin resistance can potentially be quantified by measurement of interstitial insulin concentrations in insulin-sensitive and -resistant conditions, as is done using microdialysis by Szendroedi et al. (8) in this issue of *Diabetes*. In the context of their data, it is important to consider both the strengths and limitations of current experimental approaches to the assessment of insulin access to muscle interstitium.

One approach uses arterial/venous (A/V) sampling coupled with measurements of limb plasma flow. Such balance measurements are widely used to study glucose, amino acid, and fat metabolism. Surprisingly, although this can provide direct continuous sampling of muscle insulin uptake, a careful kinetic study in control versus insulin-resistant subjects has not been done. Both older and more recent data suggest that in healthy individuals the single pass extraction ratio of insulin across forearm skeletal muscle is 10–15% (9,10). The clearance of insulin declines when the plasma insulin concentration is raised, suggesting that the transfer process is saturable (9). Limitations to using A/V sampling include that it requires *1*) excellent assay precision as the A/V differences are small and *2*) invasive arterial cannulation. An important caveat to the interpretation of A/V differences is that the limb plasma flow measurement includes flow to nonmuscle tissues. Finally, because the metabolic clearance rate of insulin within muscle is unknown, A/V insulin measurements do not allow construction of a time course for changing interstitial insulin concentration.

Lymphatic insulin sampling, pioneered in canine studies by the Bergman laboratory, has demonstrated a two- to threefold steady-state plasma to interstitial insulin gradient and a much tighter temporal correlation for glucose disposal with lymphatic than with plasma insulin in animals (11) and humans (12). This suggests that lymph insulin is a reasonable surrogate for interstitial insulin. However, lymphatic sampling is uncommonly used in clinical metabolic studies. The technique is invasive and technically demanding and is limited by the slow rate of lymph flow, which introduces a delay beyond that due to transendothelial insulin movement. Encouraging lymph flow by limb heating or compression maneuvers may itself affect insulin transfer (12). Beyond that, the lymph vessels that have been sampled in humans were in the ankle and drain mixed tissues without a significant muscle volume (12).

Several groups have used microdialysis to study the regulation of muscle interstitial insulin (13,14). A critical untested assumption of microdialysis is that the microdialysis catheter itself does not influence the interstitial insulin concentration by affecting either local flow or vascular permeability (15). Beyond that, a significant limitation is insulin's inefficient transfer to the dialysate. Careful studies put this at only ~3% (16). Consequently, the insulin concentration in the dialysate is extremely low, and assay variance and small changes of transfer efficiency will be multiplied substantially. In addition, because dialysate flow must be slow to allow even this minimal equilibration, there is a delay between insulin concentration changes in interstitial fluid and dialysate.

In this issue of *Diabetes*, Szendroedi et al. measured muscle interstitial insulin using microdialysis during both an oral glucose tolerance test and an insulin clamp in healthy humans who also received either a lipid or glycerol infusion. Lipid impaired insulin action but did not affect interstitial insulin concentrations, supporting an effect of lipid primarily on the myocyte and not on transfer of insulin from plasma to interstitium. However, surprisingly, during the oral glucose tolerance test there was no increase whatsoever in muscle interstitial insulin concentration

From the [1]Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia, Charlottesville, Virginia; and the [2]Department of Physiology, Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, the Netherlands.
Corresponding author: Eugene J. Barrett, ejb8x@virginia.edu.
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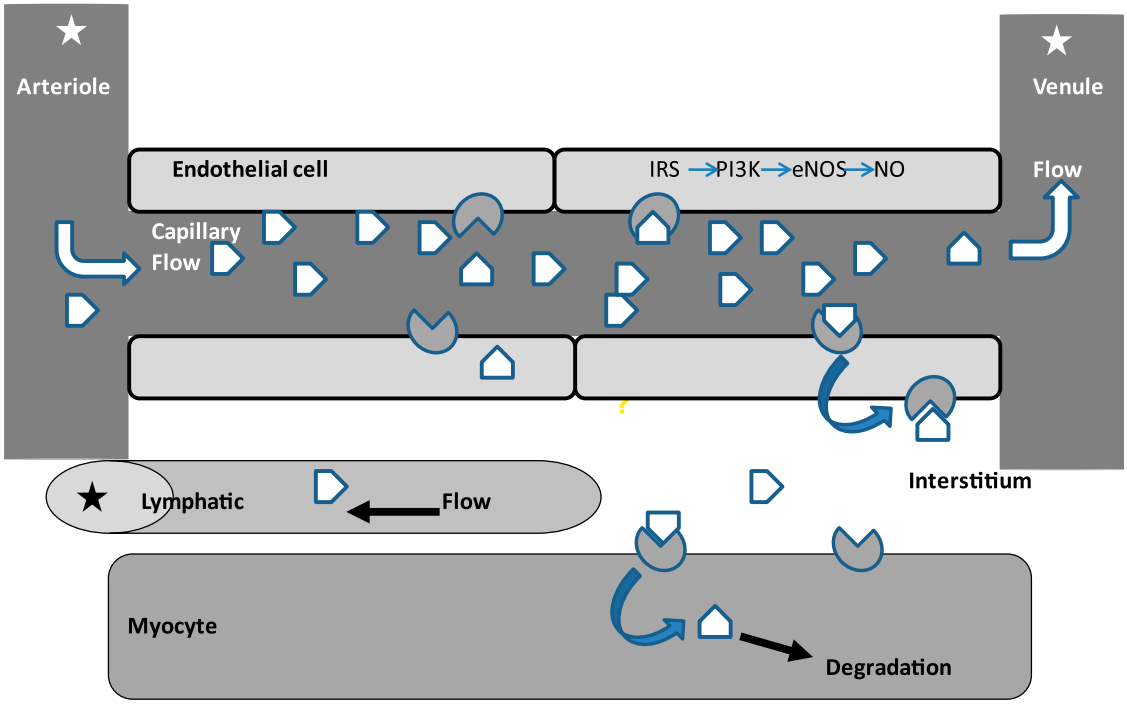

FIG. 1. Insulin (pentagons) enters the skeletal muscle capillary microvasculature where it can exchange between the plasma and the interstitium. This can be assessed by sampling A/V insulin and quantifying blood flow (white stars) or by sampling lymphatic insulin (black star). Alternatively, a microdialysis catheter introduced into muscle is used for sampling (a typical catheter is ~30× larger than the capillary displayed). Plasma insulin concentrations both fasting and during steady-state hyperinsulinemia are estimated two- to threefold higher than interstitial. Insulin transits to the interstitium from plasma by binding with the insulin receptor on the endothelial cell, activating a signaling cascade that increases nitric oxide (NO) formation. Insulin crosses the vascular endothelium by a vesicular transport pathway and accesses the interstitium where most is removed by muscle through receptor-mediated endocytosis and subsequent degradation. Small amounts of insulin return via lymphatic drainage because flow through the muscle lymphatic system is only approximately 1/100th that of blood flow to muscle. eNOS, endothelial nitric oxide synthase; IRS, insulin receptor substrate; PI3K, 1-phosphatidylinositol 3-kinase.

measured by microdialysis. Likewise, with the insulin clamp there was little increase during lipid and none during glycerol infusion, despite robust increases in plasma insulin and glucose disposal. Such findings are perplexing and again underscore the technical difficulties of assessing interstitial insulin concentrations.

Although the study of Szendroedi et al. does not definitively answer whether insulin's access to interstitium contributes to insulin resistance in muscle, it underscores the need for studies to advance our understanding of the cell biology and clinical physiology of transendothelial insulin movement. For future studies in humans, a noninvasive method, perhaps involving positron emission tomography or other quantitative imaging technologies, may allow quantification of the insulin transfer rate into muscle on a real-time basis. Meanwhile, improvements in optical imaging techniques such as multiphoton and total internal reflection fluorescence microscopy may permit us to address in vivo (at least in animal models) the cellular pathways involved in insulin transfer. Such studies will be important to our understanding of how impairments in insulin transfer in muscle or other tissues with continuous endothelium impact body metabolism in states of insulin resistance. Clearly, much remains to be done, but progress will increase our understanding of both the metabolic and vascular dysfunction seen with diabetes and metabolic syndrome.

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