Point:Counterpoint: Glucose phosphorylation is/is not a significant barrier to muscle glucose uptake by the working muscle

POINT: GLUCOSE PHOSPHORYLATION IS A SIGNIFICANT BARRIER TO MUSCLE GLUCOSE UPTAKE BY THE WORKING MUSCLE

The assertion that membrane transport of glucose is rate limiting for muscle glucose uptake (MGU) appears throughout the scientific literature (2, 18, 20, 21, 25, 28–30). There is no debate that facilitated glucose transport is essential to MGU. In particular, the quantity of glucose transport protein, GLUT4, in the sarcolemma is closely related to MGU (11). Glucose phosphorylation, as the first committed step, is also essential to MGU. This step is catalyzed by hexokinase (HK) I and HK II. The issue is whether the ability to phosphorylate glucose is always adequate to handle flux across the sarcolemma or whether there are times of high glucose flux, like exercise, when it is not. This has not been a simple issue to resolve because of the close coupling of glucose transport and phosphorylation and the existence of glucose compartmentalization and spatial gradients in the muscle. Our contention is that exercise is a condition where the sarcolemma is sufficiently permeable to permit glucose entry at rates high enough to challenge phosphorylation capacity.

Muscle membranes of sedentary subjects have a low permeability to glucose. In response to exercise, the sarcolemma becomes considerably more permeable to glucose as GLUT4 translocation to it is accelerated (1, 3, 26). There is evidence from our laboratory that the working muscle becomes freely permeable to glucose and therefore offers no barrier to MGU under these conditions (5, 14). In contrast, it is difficult to know whether the capacity of muscle to phosphorylate glucose is increased with exercise. An increase in soluble HK II activity has been reported (19). However, the only identified allosteric regulator of HK II, glucose 6-phosphate, is inhibitory. This inhibitor can be increased under conditions such as exercise where muscle glycogenolysis is high. Thus exercise creates a situation where glucose should easily pass through the sarcolemma, whereas the ability to phosphorylate it may change very little or be inhibited. These cellular events predict that the site of resistance is shifted from membrane transport to glucose phosphorylation.

The paradigm above could be validated if intracellular glucose, which is the product of membrane glucose transport and the substrate for glucose phosphorylation, were known. However, intracellular glucose can neither be directly measured nor realistically calculated. This is because it requires the difference between two comparatively large numbers (total muscle glucose and interstitial glucose), each requiring inherent measurement errors and assumptions. These create an insurmountable signal-to-noise ratio. The problem gets more complicated when one considers that there is likely to be compartmentalization or spatial gradients of glucose in interstitial and intracellular space. A change could occur in one part of the cell (e.g., inner membrane surface), which may be missed or underestimated because measurements reflect the entire tissue water.

We used two independent rodent models to test the functional control of MGU. The first approach uses isotopic glucose analogs to obtain a surrogate for intracellular glucose in catheterized conscious rats. This is accomplished by applying the isotopic glucose analogs 3-0-[3H]methylglucose (3-0-[3H]MG), U-[14C]mannitol (U-[14C]MN), and 2-deoxy[3H]glucose (2-[3H]DG) to principles of glucose countertransport (22–24, 23). Countertransport is defined as the difference in the steady-state distribution of one sugar between intracellular and extracellular water induced by a transmembrane gradient of a second sugar (22). With this technique, the distribution of trace 3-0-[3H]MG between intracellular and extracellular water is determined at steady state to assess the trans-sarcolemmal glucose gradient (TSGG). Because 3-0-[3H]MG is not metabolized, its plasma and interstitial concentrations are equal. Thus plasma 3-0-[3H]MG is combined with tissue measurements to calculate intracellular 3-0-[3H]MG. U-[14C]MN is a membrane-impermeable marker used to calculate the ratio of extracellular to intracellular water. The glucose concentration of the outer ([G]om) and inner ([G]im) surfaces of the sarcolemma can be calculated from this approach and used to determine the TSGG and intracellular glucose available for phosphorylation ([G]im). Muscle glucose influx (Rg) or a rate constant for the process (Kg) is calculated from the accumulation rate of phosphorylated 2-[3H]DG. Measurements of TSGG and [G]im can be combined with Rg to assess resistances to glucose flux through the sarcolemma and intracellular metabolism using a variation of Ohm’s law for electrical circuits where TSGG and Rg are analogous to voltage gradient and current. TSGG decreases and Rg increases in working muscle, demonstrating that resistance to membrane glucose transport decreases (i.e., TSGG/Rg decreases) precisely as one would expect from accelerated GLUT4 translocation. In contrast, [G]im increases in the presence of the increase in Rg and resistance at intracellular phosphorylation increases. Results obtained using the countertransport method clearly demonstrate a shift in the barriers to MGU during exercise so that phosphorylation is the chief site of resistance.

The second approach used to dissect control of MGU was to genetically increase muscle GLUT4 and HK II. The hypothesis is that HK II overexpression would increase the ability of working muscle to consume glucose, whereas GLUT4 overexpression would have no effect were it tested (5, 15). Mice overexpressing GLUT4 (GLUT4Tg) or HK II (HKII) were catheterized chronically, and Rg was measured isotopically during treadmill exercise. Consistent with predictions of the countertransport approach, HKII increased exercise-stimulated Rg, whereas GLUT4Tg did not. A corollary to these findings is that reduced HK II would have a diminished exercise-stimulated MGU. This was the case as the Rg response to exercise in mice with a heterozygous HK II deletion was diminished (6).

Figure 1 is a compilation of studies (4, 5, 8, 10) conducted in mice with varying degrees of muscle GLUT4 and HK II expression. Several significant points can be garnered from this figure: 1) HK II overexpression has no effect on Kg in sedentary mice, whereas it increases with GLUT4 overexpression; 2) 50% of normal GLUT4 expression is adequate for the stimulatory effect of exercise on Kg, and GLUT4 overexpression...
causes no added stimulation, and 3) HK II overexpression, on the other hand, causes a nearly twofold increase in the $V_{\text{max}}$ for $K_g$ of working muscle.

In understanding the control of MGU, it is important to recognize that the same shift in control from transport to phosphorylation seen with exercise is also a characteristic of insulin stimulation determined using countertransport and the same mouse models described above (4, 7, 9, 13–15). Insulin-stimulated GLUT4 translocation to the sarcolemma decreases resistance at the transport step, shifting control to phosphorylation. Does the shift in control evident in rodent exercise models also occur in exercising humans? It is difficult to apply the tests used in rodents to humans, and tools to assess control of MGU in humans (e.g., positron emission tomography) cannot currently be applied to working muscle. Biopsy techniques have been used to show that resting human muscle was 2.1 ± 0.3 mmol/kg and rises to 3.3 ± 0.4 mmol/kg after 10 min of moderate exercise and 11.6 ± 0.7 mmol/kg at exhaustive high-intensity exercise (16). Although these measurements are whole tissue and are not intracellular, the increased concentrations are still consistent with a shift in control from membrane transport to phosphorylation. Factors such as muscle fiber type (12, 24), nutritional state (e.g., glycogen stored; 15, 27), and exercise duration (17) may also influence the control of MGU by transport and phosphorylation due to effects on muscle glucose 6-phosphate and, possibly, fatty acid availability.

REFERENCES


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COUNTERPOINT: GLUCOSE PHOSPHORYLATION IS NOT A SIGNIFICANT BARRIER TO GLUCOSE UPTAKE BY THE WORKING MUSCLE

Glucose uptake in skeletal muscle may be increased more than 15- to 20-fold above basal level in response to stimulation with either insulin or muscle contractions (exercise; Refs. 1, 16). This occurs concomitant with a translocation of the GLUT4 glucose transporter to the muscle plasma membrane (including the T-tubule membrane), resulting in an increased flux of glucose into the muscle cell (3). The core of the present debate is whether the activity of the hexokinase is sufficient to prevent accumulation of any significant amount of free intracellular glucose.

As far as insulin-stimulated muscle glucose uptake is concerned this appears to be the case. A very early observation concerning glucose uptake in skeletal muscle was that glucose uptake is a saturable process and that during insulin stimulation the major rate-limiting step is transport through the plasma membrane. Thus, in 1939, on the basis of a series of experiments on eviscerated, hindlimb perfused cats, Einar Lunds-gaard (9) showed that the dose-dependent uptake of glucose approached saturation at high concentrations and that even then, the intracellular glucose concentration in muscle in response to insulin was essentially zero. Today, 65 years later, these initial observations have withstood the scrutiny of numerous studies. Thus it appears fair to say that at least during physiological insulin-stimulated glucose utilization in skeletal muscle, glucose transport is the rate-limiting step. Furthermore, it has recently been shown that reduced insulin-stimulated glucose transport activity is the major step responsible for muscle insulin resistance in type 2 diabetes (7, 10).

As far as exercise-stimulated muscle glucose uptake is concerned, the actual magnitude in glucose uptake may be rather variable because it is influenced by a complex mixture of factors including type (e.g., dynamic (concentric, eccentric), static), intensity and duration of exercise, training status, energy status (e.g., high vs. low muscle glycogen level), availability of other substrates (e.g., FFA), species, and choice of experimental system (reviewed in Refs. 12, 15).

The diversity in maximum glucose uptake capacity between muscles with different fiber type composition is considered to be due to their different content of GLUT4 protein (4), suggesting that glucose transport is rate limiting. Furthermore, in perfused rat hindquarter muscle, subjected to maximum stimulating muscle contractions for 20 min, it took approximately 5 min from the onset of contractions to achieve maximum permeability of the plasma membrane for glucose (19). This was most likely the time needed to complete the GLUT4 translocation process. For the subsequent 15 min of continuous contractions, uptake of the phosphorylable glucose analog 2-deoxyglucose was constant over time in three different muscles with markedly different fiber type composition. This was in contrast to the nonmetabolizable analog 3-O-methylglucose, where apparent uptake rates rapidly decreased in a monoeponential fashion due to backflow out of the muscle cells of the nontrapped 3-O-methylglucose analog. This clearly shows that the hexokinase has the capacity to immediately phosphorylate all glucose that enters the cell through the maximum contraction-induced permeability increase of the muscle plasma membrane. This “sufficiency” capacity of the hexokinase is further supported by the lack of accumulation of any significant free intracellular glucose during exercise (8, 13), which would be expected if phosphorylation of glucose was rate limiting.

As noted above, glucose uptake in skeletal muscle is a saturable process. Transport of glucose through the muscle plasma membrane follows Michaelis-Menten kinetics. Estimated with 3-O-methylglucose zero-trans influx measurements, fiber type-dependent \( K_m \) values of 5–17 mM and \( V_{\text{max}} \) values in excess of 14-fold, compared with basal unstimulated muscle, have been found immediately after contractions in perfused rat muscle (11). These values reflect transport of glucose through GLUT4 proteins located in the muscle plasma membrane and compare very favorably with \( K_m \) values of 5–13 mM for GLUT4 expressed in heterologous systems like Xenopus oocytes and yeast cells (2, 17). After transport through the membrane, glucose is irreversibly phosphorylated by the hexokinase. Mammalian skeletal muscles contain two hexokinase isozymes. In rat skeletal muscle, the predominant type is hexokinase II, whereas in human skeletal muscle hexokinase I accounts for around three-fourths of total hexokinase activity (Ref. 14 and references therein). \( K_m \) values of 0.03 mM for type I and 0.3 mM for type II hexokinases are found in vitro (18), which are at least 15- to 150-fold lower than \( K_m \) for endogeneous muscle GLUT4. Let us suppose for the sake of argument that we remove the entire muscle plasma membrane. In that case, as soon as glucose leaves the capillaries, it will encounter the hexokinases without any intervening barriers and...