GLUCOSE PHOSPHORYLATION AS A BARRIER TO MUSCLE GLUCOSE UPTAKE

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SUMMARY

1. Glucose phosphorylation is the first irreversible step of the muscle glucose uptake pathway and is catalysed by a hexokinase isozyme.
2. While glucose transport is the primary barrier to muscle glucose uptake during basal conditions, glucose phosphorylation becomes an important barrier to muscle glucose uptake during stimulated conditions such as hyperinsulinaemia or exercise.
3. High fat feeding markedly impairs insulin- and exercise-stimulated muscle glucose uptake. As hexokinase II over-expression corrects this dietary-induced deficit during exercise, glucose phosphorylation is a site of impairment following high fat feeding.
4. Exercise is an important tool for diagnosing deficits in glucose phosphorylation.

Key words: 2-deoxyglucose, distributed control, exercise, hexokinase, insulin clamp, mice.

INTRODUCTION

Intracellular phosphorylation of glucose to form glucose-6-phosphate (G6P) is the final step of the muscle glucose uptake (MGU) pathway and is catalysed by a hexokinase isozyme (ATP:D-hexose 6-phosphotransferase, EC 2.7.1.1; HK I and II) in skeletal muscle. Glucose phosphorylation is critical because, at this point, glucose carbons are committed to the myocyte. That is, as shown in Fig. 1, once G6P is formed in muscle, glucose can either be stored as glycogen or further metabolized as myocytes lack significant G6Pase activity. Interestingly, G6P can feed back on HK I or II and inhibit their activity. Thus, the metabolite G6P is an important hub of the muscle’s metabolome that links the supply of glucose carbons from extracellular and intracellular pools to their eventual intracellular metabolism.

Several elegant studies utilizing isotopic glucose analogues combined with modelling approaches in rats2–7 and humans8–12 have provided evidence that glucose phosphorylation can become a significant barrier to net MGU of exercise- and insulin-stimulated muscles. In addition, it has been demonstrated that glucose phosphorylation capacity can become impaired in insulin resistant states,13–15 and that an accumulation of long chain fatty acylcoenzyme A (acyl-CoA) inside the muscle inhibits HK II activity.16 With the ability to manipulate the mouse genome, an additional tool has been developed to study the regulation of metabolic pathways. In order to study the role that glucose delivery and transport make in controlling MGU, manipulations have been made to the endothelial nitric oxide synthase17 and GLUT-418–20 genes, respectively. Manipulations have also been made to increase or decrease muscle glucose phosphorylation capacity by altering HK II content21,22 or adding glucokinase23 to skeletal muscle. The remainder of the present review will focus primarily on mice with three levels of HK II activity in skeletal muscle (50, 100 and 300–700% of wild type levels). These studies, conducted in conscious, postabsorptive mice, are in direct agreement with data from other models and humans and suggest that glucose phosphorylation is not a barrier to MGU in the basal state, but becomes an important site of regulation in stimulated states such as during exercise and hyperinsulinaemia.

EXPERIMENTAL APPROACH TO STUDY METABOLIC REGULATION IN VIVO

The theoretical approach adopted for these experiments designed to study metabolic regulation is that: (i) if a step such as glucose phosphorylation is a barrier to MGU, then sufficient over-expression of the protein that catalyses that step will functionally reduce the resistance to that step and increase overall glucose influx; and (ii) selectively reducing the protein may create a site of resistance that could decrease overall muscle glucose influx. Therefore, in order to determine if glucose phosphorylation is a barrier to MGU, mice both overexpressing and underexpressing HK II have been used.

In order to take advantage of the experimental opportunities provided by the ability to manipulate the mouse genome, several
Hexokinase and muscle glucose uptake

The technological hurdles needed to be overcome to comprehensively study glucose metabolism in an organism of such small size. Significant advances have, in fact, been made in the experimental techniques utilized to study glucose metabolism in the mouse, including miniaturizing assays and advanced surgical procedures. In order to minimize the confounding influences of anaesthesia and stress from surgery or handling, catheters are surgically implanted into the left common carotid artery and right jugular vein. While technically challenging, arterial catheterizations in mice are imperative for obtaining well-mixed blood and to minimize unwanted stress responses. In addition, the arterial catheterization affords the opportunity to obtain blood during treadmill exercise. The venous catheterization provides access to the circulation with the purpose of infusing various substances to either stimulate (e.g. insulin) or trace (e.g. isotopic glucose analogues) glucose metabolism in the conscious, freely moving animal.

The glucose tracer utilized in these studies to provide an index of MGU is 2-deoxy[$^3$H]glucose (2DG). The 2DG method for determining MGU has been described by Kraegan et al. and is an extension of the technique described by Sokoloff et al. for measuring cerebral glucose uptake. Because 2DG is irreversibly trapped inside the muscle, it is an ideal substance for measuring MGU in vivo. Upon steady state conditions, 2DG is infused into the venous circulation as a bolus. Clearance of the glucose tracer ($k_c$) is calculated as the accumulation of radioactivity from phosphorylated 2DG in muscle divided by the area under the curve for the disappearance of radioactivity from arterial 2DG. The glucose concentration-dependent index of MGU ($R_g$) is $k_c$ multiplied by the average arterial blood glucose concentration. While both indices are useful for comparing the effects of transgenic manipulation on MGU, $R_g$ is useful as it provides an index that reflects an actual mass of glucose entering the muscle.

The experimental details for studying MGU during various physiological conditions (i.e. basal, insulin-stimulated and exercise-stimulated) have been described in detail previously. For stimulated MGU, mice were either infused with 4 mU/kg/min of insulin to achieve insulin concentrations of 60–80 μU/mL or were exercised at ~80% of maximal oxygen consumption by running on a treadmill at 16.7 m/min with a 0% grade. Insulin- and exercise-stimulated MGU were compared to basal MGU from saline-infusion and sedentary control experiments, respectively.

**Fig. 1** Control of muscle glucose uptake. Intracellular phosphorylation of glucose to glucose-6-phosphate (G6P) is catalysed by hexokinase. This step of the muscle glucose uptake pathway, unlike glucose transport, is irreversible. Glucose 6-phosphate can inhibit hexokinase activity, be metabolized glycolytically, or be stored as glycogen. The control of glucose influx is dependent on the state of the muscle (i.e. basal versus insulin- or exercise-stimulated).

**Fig. 2** Relationship between hexokinase II (HK II) activity and muscle glucose uptake. The HK II activity is expressed relative to wild type (WT) activity (i.e. WT equals 1). Muscle glucose uptake ($R_g$) data are from gastrocnemius muscles during basal, insulin- and exercise-stimulated experiments and are expressed in μmol/100 g/min.

GLUCOSE PHOSPHORYLATION IS A BARRIER TO STIMULATED MUSCLE GLUCOSE UPTAKE

In conscious, postabsorptive mice, glucose phosphorylation is not a significant barrier to MGU in the non-stimulated state. It can be clearly seen in Fig. 2 that basal $R_g$ is not influenced by HK II content. GLUT-4 content at the plasma membrane, however, does influence basal MGU and thus glucose transport is the formidable barrier to basal MGU. Data supporting this concept have been generated using the techniques described herein, as well as by other methods. With GLUT-4 overexpression, the plasma membrane has an increased concentration of glucose transporters thereby minimizing the glucose transport barrier. The net result is that basal MGU is increased approximately twofold even in the face of a reduction in arterial blood glucose concentration resultant from the GLUT-4 transgene.
Insulin or contractions stimulate the translocation of GLUT-4 to the plasma membrane and thereby increase the net rate of glucose transport (Fig. 1). When glucose transport is stimulated, does glucose phosphorylation then become a barrier to MGU? The answer is yes. Glucose phosphorylation, indeed, becomes a barrier to MGU during stimulated conditions such as hyperinsulinaemia or exercise. Figure 2 demonstrates the dependence of stimulated MGU on HK II content and hence glucose phosphorylation capacity. In other words, relative to wild type levels, as HK II content is increased so too is R₉. Similarly, as HK II content is decreased, stimulated R₉ is also decreased. A correlation such as this cannot be made with regards to GLUT-4 content. For example, overexpression of GLUT-4 has no effect on exercise-stimulated kₙ or R₉ in conscious mice. These findings in conscious mice are consistent with previous work using a conscious rat model.⁵–⁷

While glucose transport is a significant barrier to MGU in the basal state, it is not one when the muscle is sufficiently stimulated. Conversely, glucose phosphorylation is not a barrier in the basal state but becomes one during exercise and hyperinsulinaemia. Therefore, the notion that MGU is controlled by transport as a single rate-limiting step is inaccurate in vivo. The shift from glucose transport being a barrier in the basal state to glucose phosphorylation becoming a barrier in stimulated states is characteristic of the distributed control of metabolic pathways.

**GLUCOSE PHOSPHORYLATION IS FUNCTIONALLY IMPAIRED BY HIGH FAT FEEDING**

Chronic high fat feeding creates insulin resistance, which is exemplified by a reduction in rates of exogenous glucose infusion required to maintain euglycaemia during insulin infusion compared to the normal glucose infusion rate. While ample data exists regarding high fat feeding-induced deficits in glucose transport, it is less is known regarding high fat feeding-induced deficits to steps both proximal and distal to glucose transport. Functional impairments to steps distal to glucose transport (e.g. glucose phosphorylation) can occur as a result of high fat feeding or accumulation of intracellular lipids. Work in high fat fed conscious rats using a modelling approach has demonstrated that dietary-induced insulin resistance can be manifested by impairments to both delivery of glucose to the muscle and intracellular phosphorylation to G6P.

As in other species, high fat feeding markedly impairs net MGU in stimulated states of mice when the glucose fluxes are increased, whereas it is difficult to detect any impairment in the basal state as a result of the low glucose fluxes. Hexokinase II overexpression corrected this dietary-induced deficit to rates observed in wild type mice during exercise but not during stimulation with insulin. These results suggest that: (i) glucose delivery is impaired by high fat feeding; (ii) GLUT-4 translocation is impaired during insulin stimulation but not exercise stimulation; or (iii) some combination of these two hypotheses exists. Whichever hypothesis prevails, it remains certain that the best means to correct dietary-induced insulin resistance will target multiple steps of the MGU pathway.

It is interesting that a functional phosphorylation deficit was not detected during insulin stimulation. It is assumed that, in high fat fed relative to chow fed mice, exercise is still able to increase glucose delivery and exercise-stimulated GLUT-4 translocation is not dysregulated. Thus exercise is able to unmask impairments in glucose phosphorylation resultant from high fat feeding. Therefore, exercise is a useful physiological tool for diagnosing deficits in glucose phosphorylation.

**CONCLUSION**

Work in conscious mice has highlighted the importance of each step in regulating MGU. Only when glucose influx is studied in vivo in conscious animals can all facets of this process be appreciated. For example, when animals are studied in the anaesthetized state, profound insulin resistance is created. Likewise, when a muscle is excised and studied in vitro, insulin resistance occurs and, in addition, the component of glucose delivery is abolished.

The range of glucose fluxes that muscles achieve is quite remarkable and thus it is not surprising that diverse mechanisms are required to control this process in order to meet energy demands. Future studies will be needed to further characterize and quantify the specific role that glucose appearance and delivery plays in regulating MGU and how it may become impaired by high fat feeding. In addition, the steps beyond glucose phosphorylation (i.e. glycolysis and glycogen metabolism) will need to be considered in order to appreciate more completely the regulation of muscle glucose metabolism.

In summary, glucose transport is a barrier to glucose influx in muscles during basal conditions. However, when the muscle is stimulated by either insulin or exercise, glucose phosphorylation becomes a critical barrier to MGU in vivo. High fat feeding-induced impairments in MGU are, in part, a result of deficits in glucose phosphorylation capacity. Therefore, HK activity and inhibition of its activity are crucial for understanding the regulation of MGU.

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**REFERENCES**


