Ischemia-induced activation of AMPK does not increase glucose uptake in glycogen-replete isolated working rat hearts

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Omar MA, Fraser H, Clanachan AS. Ischemia-induced activation of AMPK does not increase glucose uptake in glycogen-replete isolated working rat hearts. Am J Physiol Heart Circ Physiol 294: H1266–H1273, 2008. First published January 4, 2008; doi:10.1152/ajpheart.01087.2007. —Alterations in myocardial carbohydrate availability and metabolism are critical determinants of ischemic injury and postischemic left ventricular (LV) mechanical function. Thus myocardial carbohydrate availability and metabolism are critical determinants of ischemic injury and postischemic left ventricular (LV) mechanical function.

Glucose transport, the first step of myocardial glucose utilization, involves the facilitated diffusion of glucose across the sarcolemmal membrane. The rate of glucose transport is determined by the transmembrane concentration gradient of glucose as well as the abundance and affinity of glucose transporter proteins, GLUT1 and GLUT4 (27). Following transport, glucose is rapidly phosphorylated by hexokinase to glucose-6-phosphate that may be either used by glycolysis or stored as glycogen. Glycogen is an important store of endogenous glucose that undergoes simultaneous synthesis and degradation (19). Synthesis is accelerated by insulin and by increases in substrate availability, whereas degradation (glycolysis) is accelerated by ischemia or hypoxia (44). Thus the rate of glucose uptake is influenced not only by glucose influx (transport) but also by the pathways of glucose metabolism that may be affected by endogenous glycogen content, metabolic demand, availability of O2, and other energy substrates, as well as by insulin concentration (46).

Several studies have investigated ischemia-induced alterations in glucose uptake, but these have yielded conflicting conclusions. More than three decades ago, Neely and colleagues (35, 37) showed that ischemia reduces glucose uptake and glycolysis. However, those results contrast with positron emission tomography studies that show an increase in the uptake of 18F-deoxyglucose in relation to coronary flow in patients with coronary artery disease (10, 32). Similar observations in vivo (50) and ex vivo (40, 45) were attributed to a translocation of GLUT4 to the cell surface, due to ischemia-induced activation of adenosine 5'-monophosphate-activated protein kinase (AMPK) (39).

AMPK is a serine-threonine stress kinase and has been termed “the guardian of cardiac energy status” (23) since it is activated by energy-deficient states to stimulate energy production while inhibiting energy-consuming processes. AMPK stimulates GLUT4 translocation in skeletal muscle during hypoxia (34), and pharmacological activation of AMPK in isolated rat ventricular papillary muscle increases GLUT4 translocation as well as glucose uptake (38). The role of AMPK in mediating ischemia-induced increases in glucose uptake was further supported by studies using transgenic mice expressing a kinase dead form of AMPK, in which low-flow ischemia (LFI) fails to enhance glucose uptake or glycolysis (39). Indeed, it has become generally accepted that the activation of AMPK stimulates glucose uptake (13, 31, 41).

On the other hand, there is evidence that ischemia either reduces or has no effect on myocardial glucose uptake. Stanley et al. (43) showed in open-chest swine that acute myocardial ischemia increases glucose extraction but not glucose uptake. Similar results have been obtained in Langendorff-perfused (28) as well as working rat hearts (11, 18, 19). Indeed, AMPK activation fails to increase glucose uptake in skeletal muscle
(36) as well as in aerobically perfused isolated working rat hearts (25). The lack of consistency regarding the effects of ischemia, as well as AMPK, on glucose uptake may be attributed to a number of factors, the most important of which concern the methods and conditions used to measure glucose uptake.

Given the importance of glucose uptake and utilization in cardiac function, this study was designed to compare the temporal changes in glucose uptake and utilization during ischemia in hearts with either normal or low levels of glycogen. Also, since the roles of AMPK in cellular metabolism and ischemia-reperfusion injury are the subject of intense investigation, we sought to determine the relationship between alterations in glucose uptake and utilization and the concomitant ischemia-induced activation of AMPK. Studies were performed in isolated working rat hearts that were perfused with Krebs-Henseleit solution containing insulin as well as glucose and fatty acids as energy substrates. LV mechanical function, glucose uptake, glycogen turnover, glycolysis, and AMPK activity were assessed during aerobic perfusion as well as during graded periods of severe LFI.

MATERIALS AND METHODS

Heart perfusions. The investigation conforms to the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996), and the guidelines of the Canadian Council of Animal Care and has been approved by the Animal Care and Use Committee of the University of Alberta. Male Sprague-Dawley rats (300–350 g) were anesthetized using pentobarbital sodium (60 mg/kg ip), and hearts were rapidly excised and placed in ice-cold Krebs-Henseleit solution followed immediately by aortic cannulation and perfusion in nonworking Langendorff mode (16). Hearts were then switched to working mode and perfused at 37°C at constant workload (11.5 mmHg preload, and 80 mmHg afterload) and rate (paced at 300 beats/min). The perfusate (recirculating, 100 ml) consisted of a modified Krebs-Henseleit solution (consisting of (in mM) 118.0 NaCl, 4.7 KCl, 1.2 KH2PO4, 1.2 MgSO4, 25 NaHCO3, and 2.5 Ca2+2) containing insulin (100 μU/ml) and both palmitate (1.2 mM, prebound to 3% BSA) and glucose (11 mM) as energy substrates. Gassing with carbogen (95% CO2-5% O2) and both palmitate (1.2 mM, prebound to 3% BSA) and glucose (11 mM) was used to maintain perfusate pH and O2 saturation. Aortic systolic and diastolic pressures (in mmHg), cardiac output (in ml/min), aortic flow (ml/min), and LV work (mmHg·1·min−1) were measured as described previously (15, 16, 18, 19). LFI was initiated by switching hearts to Langendorff mode and delivering oxygenated perfusate at a constant flow (0.5 ml/min) into the aorta via the aortic cannula (18, 19).

Perfusion protocol. Two series of hearts with different preischemic glycogen contents were studied. In the first series, glycogen-replete (G replete), glycogen stores were replenished before the onset of LFI to near the normal levels reported for the rat heart in vivo (120–150 μmol/g dry wt) (49). These hearts were perfused initially for 10 min in Langendorff mode (unpaced), followed by 60 min of aerobic perfusion in the working mode. In the second series, glycogen-depleted (G depleted), glycogen stores were depleted nonischemically before the onset of LFI during an initial 35-min Langendorff perfusion with substrate-free solution (no glucose or palmitate) while pacing at 420 beats/min, as described previously (15). This was followed by 20 min of aerobic perfusion in working mode with modified Krebs-Henseleit solution containing both glucose and palmitate to reestablish stable LV mechanical function. Hearts from both groups were then either rapidly frozen or were subjected to LFI (0.5 ml/min) for 10, 15, or 60 min and then frozen. Frozen tissues were pulverized at the temperature of liquid N2, and the resulting powders were stored at −80°C.

Measurement of the rate of glycolysis. Glycolysis rates were measured during aerobic baseline at 10-min intervals as described previously (15, 16, 18, 19) by the quantitative determination of 3H2O liberated from [5-3H]glucose (at the enolase step of glycolysis). Rates (expressed as μmol glucose metabolized·min−1·g dry wt−1) were calculated for each time interval and were averaged for the period of aerobic perfusion. During LFI, glycolysis rates were calculated for the initial 10-, 10–15-, and 15–60-min periods. Total rates of glycolysis were calculated by the addition of the rate of liberation of 3H2O from labeled glucose (exogenous glucose as well as glucose liberated by glycogenolysis from labeled glycogen) and the rate of change of the unlabeled glycogen pool at the different time points.

Measurement of glycogen content and rates of glycogen turnover. Glycogen contents (in μmol glucosyl units/g dry wt) in frozen tissues were determined as described previously (19). In addition, the amount of radiolabeled glucose in glycogen extracts was also determined to evaluate the degree of incorporation of radiolabeled glucose into glycogen. The rates of glycogen synthesis (Gsyn) and degradation (Gdeg) were calculated as described previously (19). This method accounts for changes in both the unlabeled and labeled components of the glycogen pool during each phase of the perfusion protocol. This allows the assessment of rates that are independent of the extent of labeling of the glycogen pool, such as might arise due to the “first-on, first-off” concept of glycogen turnover (19).

Glucose uptake and percent extraction. Glucose uptake (in μmol glucose·min−1·g dry wt−1) was calculated, as described previously (25), as the sum of the rates of glycolysis and the rate of incorporation of radiolabeled glucose into glycogen during the different phases of the perfusion protocol. This method of assessment of glucose uptake accounts for each of the two main fates of glucose (metabolism by the glycolytic pathway or incorporation into glycogen stores). Glucose extraction (in %) was calculated from the rate of glucose uptake as a percentage of glucose delivery (perfusate glucose concentration × coronary flow).

Measurement of ATP, AMP, creatine, and phosphocreatine. Frozen heart tissue (100 mg) was homogenized and extracted with 6% perchloric acid. The tissue-perchloric acid mixture was centrifuged heart tissue (100 mg) was homogenized and extracted with 6% perchloric acid. The tissue-perchloric acid mixture was centrifuged and the supernatant neutralized with 5 M K2CO3, and then analyzed by high-performance liquid chromatography for ATP, AMP, creatine (Cr), and phosphocreatine (PCr) (2).

Measurement of AMPK activity. AMPK activity (in nmol·min−1·mg protein−1) was determined in 6% polyethylene glycol fractions extracted from 200 mg of frozen LV tissue. Activity of AMPK was measured by following the incorporation of [γ-32P]-ATP into a synthetic SAMS [HMRSAMSGHIVKVPR peptide as described previously (8, 30).

Plasma membrane GLUT4 content. Membrane fractions of frozen extracts of G-replete and G-depleted hearts were prepared as described previously (21). To validate that the procedure could detect changes in plasma membrane GLUT4 content, fractions were also prepared from additional working hearts perfused aerobically in the absence or presence of insulin (100 μU/ml). Powdered tissue was incubated for 30 min in a high-salt solution containing 2 M NaCl, 20 mM HEPES (pH 7.4), and 5 mM NaN3 at 4°C. Thereafter, tissue was recovered by centrifugation for 5 min at 1,000 g; resuspended in homogenization solution containing (in mM) 20 HEPES (pH 7.4), 2 EDTA, 1 MgCl2, and 250 sucrose; and recentrifuged. The pellet was then homogenized (10% wt/vol) using a handheld glass homogenizer (DUALL, Kontes). Homogenates were sequentially centrifuged at 100 g for 10 min and then 5,000 g for 10 min to prepare a plasma membrane fraction (7), confirmed by enrichment of the plasma membrane marker Na+−K+ ATPase α1-subunit (data not shown). Plasma membrane fractions were subjected to SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes as previously described (26), and then immunoblotted using rabbit anti-Na+−K+ ATPase α1-subunit or rabbit anti-GLUT4 (1:1,000 dilution; Cell Signaling Technology). After being extensively washed, membranes

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were incubated with a peroxidase-conjugated goat anti-rabbit secondary antibody (1:2,000 dilution; Cell Signaling Technology). After being further washed, antibodies were visualized using the Pharmacia enhanced chemiluminescence Western blot analysis and detection system (ECL Plus). Densitometric analyses of immunoblots (n = 4 per experimental group) were performed using ImageJ software. Densitometric values for GLUT4 protein were normalized to the corresponding value for Na\(^+\)-K\(^+\) ATPase α\(_1\)-subunit.

Statistical analyses. Results are expressed as means ± SE of n observations. The significance of the differences for multiple comparisons was estimated by one-way analysis of variance (ANOVA). If significant, selected data sets were compared with Bonferroni’s multiple comparison test. Differences were considered statistically significant when P < 0.05.

RESULTS

LV mechanical function. LV work was similar in G-replete and G-depleted groups (7.07 ± 0.40 and 6.42 ± 0.17 mmHg·l·min\(^{-1}\), respectively). Cardiac output, aortic flow, and coronary flow also remained stable throughout aerobic working mode perfusion in both groups (data not shown). During LFI, LV work was not detectable in either group.

Glycogen content of G-replete and G-depleted hearts. The stress associated with heart extraction markedly reduces glycogen content, and, when perfused with Krebs-Henseleit solution containing glucose as the sole energy substrate glycogen content remains low (19) relative to in vivo values (44, 49). Thus, to generate G-replete hearts, a 60-min period of aerobic perfusion was required to replenish glycogen content to 114 ± 6 μmol/g dry wt (n = 6). In G-depleted hearts, the initial substrate-free Langendorff perfusion and the shorter period of aerobic working mode perfusion resulted in significantly lower myocardial glycogen content before LFI (71 ± 6 μmol/g dry wt).

Effect of LFI on glycogen content. LFI rapidly reduced glycogen content during the initial 10 min of LFI in both G-replete and G-depleted groups, reaching 66 ± 9 μmol/g dry wt and 23 ± 2 μmol/g dry wt, respectively. Glycogen content continued to decrease in the G-replete group during more prolonged periods of LFI, whereas in G-depleted hearts, no further reductions were observed (Fig. 1A).

Effect of LFI on rates of glycolysis. Glycolysis during aerobic perfusion was lower in G-depleted hearts by 44% compared with the G-replete group. In G-replete hearts, total glycolysis was initially increased at 10 min LFI (unpaired t-test, P = 0.0008) and then returned close to aerobic values during the remainder of LFI. In G-depleted hearts, total glycolysis increased significantly during LFI to reach its peak at 15 min, and it remained elevated during the remainder of LFI compared with preischemic values (Fig. 1B).

Effect of LFI on glucose uptake and glucose extraction. In G-replete hearts, glucose uptake was reduced by 59% during the first 10 min of LFI, which was followed by gradual recovery to near aerobic values after 60 min of LFI. Glucose uptake was also reduced in G-depleted hearts during the initial 10 min of LFI by 90%, but this was followed by a significant increase in glucose uptake at 15 min and a return to preischemic values at the end of LFI (Fig. 2A). Percent glucose extraction was very low during aerobic perfusion in G-replete and G-depleted hearts (0.64 ± 0.09% and 0.58 ± 0.07%, respectively). It increased gradually in G-replete hearts during LFI to 21 ± 5% after 60 min of LFI (Fig. 2B). In G-depleted hearts, it increased more rapidly to reach its maximum at 15 min (40 ± 2%).

Effect of LFI on glycogen turnover. During baseline aerobic perfusion, G\(_{\text{out}}\) was similar in the two groups, whereas G\(_{\text{in}}\) was significantly higher in the G-depleted group (Fig. 3A). LFI for 10 min caused a marked increase in G\(_{\text{out}}\). In G-replete hearts, G\(_{\text{out}}\) recovered only partially during LFI but still remained higher than the preischemic rate. In contrast, in G-depleted hearts, the acceleration of G\(_{\text{out}}\) was not maintained (Fig. 3A). LFI did not alter G\(_{\text{in}}\) in G-replete hearts but significantly reduced the higher preischemic rate of G\(_{\text{in}}\) in G-depleted hearts (Fig. 3A). There was a significant inverse correlation between rates of glucose uptake and glycogen degradation (Fig. 4). However, there were no significant correlations between glucose uptake and rates of glycolysis, glycogen synthesis, glycogen content, or AMPK activity (data not shown).

Effect of LFI on AMPK activity. AMPK activity before LFI was similar to values reported previously for the aerobically perfused working rat heart (3, 5, 18). LFI elicited similar increases in AMPK activity in both G-replete and G-depleted groups. However, AMPK activity peaked at 15 min in G-replete hearts and remained elevated throughout the rest of LFI, whereas in G-depleted hearts, it peaked earlier (at 10 min) and then recovered to preischemic values at the end of 60 min of LFI (Fig. 3B).

Effect of LFI on high-energy phosphates and their metabolites. Values for high-energy phosphates are presented in Table 1. The AMP-to-ATP and Cr-to-PCr ratios were similar in both G-replete and G-depleted groups during aerobic conditions. LFI resulted in a gradual increase in AMP-to-ATP ratio in both groups, which reached its maximum at 60 min of LFI (Fig. 5A).
However, LFI resulted in more rapid increases in Cr-to-PCr ratios in both G-replete and G-depleted hearts (Fig. 5).

**Effect of LFI on sarcolemmal abundance of GLUT4.** GLUT4 content in plasma membrane fractions was threefold higher when hearts were exposed to insulin (Fig. 6A). LFI did not affect plasma membrane GLUT4 content in G-replete hearts but caused a significant elevation in G-depleted hearts (Fig. 6B).

**DISCUSSION**

This study investigated the effects of LFI on glucose uptake as well as glycogen turnover and glycolysis in working rat hearts perfused under conditions of normal (G replete) or low (G depleted) glycogen content. Relative to normal aerobic values, glucose uptake and GLUT4 translocation were not stimulated in G-replete hearts either by short-term (10 min) or by longer-term (60 min) LFI. Indeed, glucose uptake was inhibited during short-term LFI, and rates of glycolysis were maintained close to aerobic values by a marked acceleration of glycogenolysis. On the other hand, in G-depleted hearts, glucose uptake initially decreased during LFI in association with accelerated glycogenolysis as well as inhibition of glycogen synthesis. After 15 min of LFI, glucose uptake and GLUT4 translocation increased, effects that coincided with a decline in glycogenolysis as glycogen content became exhausted. As expected, LFI increased AMPK activity in both G-replete and G-depleted hearts, but this was not associated with an acceleration of glucose uptake. These results suggest that LFI increases glucose uptake only when myocardial glycogen stores are partially depleted. Glycogen stores, if replenished to normal values before LFI, provide sufficient endogenous substrate to maintain glycolysis during prolonged LFI. Activation of AMPK by LFI is not sufficient to stimulate glucose uptake but may alter glucose utilization by affecting glycogen turnover (inhibition of synthesis and acceleration of glycogenolysis).

The isolated working rat heart perfused with both glucose and palmitate provided an experimental system in which energy supply and demand, and hence rates of glucose uptake and utilization, were close to physiological values. The presence of adequate sources of endogenous and exogenous energy substrates is fundamental to any study on energy substrate metabolism but is of critical importance in studies on glucose uptake and utilization since glycogen provides a readily available source of glucose-6-phosphate for glycolysis. Heart extraction...
Table 1. Values for ATP, AMP, PCr, and Cr contents of left ventricular tissue

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<tr>
<th>Glycogen-Replete Hearts</th>
<th>Glycogen-Depleted Hearts</th>
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<tbody>
<tr>
<td></td>
<td>Aerobic</td>
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<tr>
<td>ATP</td>
<td>32.0±1.5</td>
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<tr>
<td>AMP</td>
<td>5.7±0.6</td>
</tr>
<tr>
<td>PCr</td>
<td>55.6±7.5</td>
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<tr>
<td>Cr</td>
<td>200.9±7.2</td>
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Values are means ± SE; n = 6–9 hearts. ATP, AMP, phosphocreatine (PCr), and creatine (Cr) contents (in μmol/g dry wt) were measured in extracts of both glycogen-replete and -depleted hearts frozen after baseline aerobic perfusion as well as after 10, 15, or 60 min of low-flow ischemia (LFI). *P < 0.05 compared with aerobic baseline value.

reduces glycogen, and, as shown in this and other studies (17, 19, 25), aerobic perfusion for 45 to 60 min with Kreb-Henseleit solution containing glucose, insulin, and palmitate is required to replenish glycogen content. Thus, in the G-replete group, preischemic glycogen content was close to normal values (120–150 μmol/g dry wt) (49).

Glycogen content is also an important consideration in studies designed to examine ischemia-induced signaling alterations. For example, there is an inverse correlation between glycogen content and AMPK activity in both skeletal (47, 48) and cardiac muscle (24). Indeed, abundant stores of glycogen depress activation of AMPK by 5-aminoimidazole-4-carboxamide riboside (AICAR) in skeletal muscles, as well as the associated acceleration of glucose uptake (47). An examination of the role of glycogen availability on the relationship between myocardial ischemia, glucose uptake, and AMPK activation was achieved using an additional group of hearts (G depleted) in which glycogen levels were reduced to values reported for hearts that are either perfused without insulin, perfused with glucose as the sole energy substrate, or where the preischemic aerobic baseline period may be too short to permit adequate resynthesis of glycogen. Importantly, in contrast to zero-flow ischemia models, the use of LFI in these experiments enabled the measurement of time-dependent changes in glucose uptake, glycogen turnover, and glucose utilization during the actual ischemic period in hearts with either normal or low levels of glycogen (18, 19).

Another important consideration in the present study is the approach used to assess glucose uptake. Glucose uptake is commonly measured using the nonmetabolizable glucose analogs, 2-deoxyglucose, or 18F-deoxyglucose, but their utility has been questioned by the observation that the constant (termed “lumped constant”) used to account for the difference in their kinetic properties from glucose (9) depends on the metabolic status of the heart as well as on the presence of insulin (12). Glucose uptake has also been assessed by the liberation of 3H2O from [2-3H]glucose, but studies often neglect to account...
for incorporation of [2-3H]glucose into glycogen, a process affected by numerous factors including insulin and ischemia. Glucose uptake may also be influenced by glycogen availability and turnover because glycogen synthesis is a route of glucose utilization and might enhance glucose uptake, whereas glycogenolysis produces endogenous glucose phosphate that may inhibit glucose uptake. Glucose uptake in this study was measured as the sum of the rates of glycolysis and glycogen synthesis and so accounts for both of the main fates of glucose following its uptake. Our data indicate that glycogen synthesis accounts for ~20% of glucose taken up during the initial aerobic phase in G-replete hearts, but this increases dramatically in G-depleted hearts to 66%. It should also be noted that although glucose uptake under these conditions is high (5 to 6 μmol/g dry wt), it is not maximal, since significant increases are demonstrable in response to the removal of palmitate from the perfusate (42). In the use of these experimental approaches, glucose uptake is significantly inhibited during LFI in G-replete hearts, a result that is in marked contrast with several reports that demonstrate ischemia-mediated increases in glucose uptake (39, 40, 45, 50). Ischemia-induced changes in glucose uptake are clearly dependent on glycogen content, and an increase is only observed in G-depleted hearts, and then only after glycogen stores are exhausted.

Inhibition of glucose uptake is not due to a lack of availability of exogenous glucose. Although glucose extraction is significantly increased during LFI, glucose availability did not become rate limiting for uptake since extraction never exceeded 40%. Glucose uptake is likely inhibited because LFI elicits marked alterations in glycogen turnover. The rapid increase in glycogenolysis in G-replete hearts provides more than 85% of glucose consumed through glycolysis during the initial 10 min of LFI, and the elevation of glucose-6-phosphate availability suppresses hexokinase activity (22) and thereby slows the rate of glucose uptake. Similar observations were reported for skeletal muscles during moderate exercise (20). Because of the finite supply of glycogen, glycogenolysis slows during more prolonged periods of LFI, and, consequently, glucose uptake recovers to maintain substrate availability for glycolysis. In G-depleted hearts, the greater inhibition of glucose uptake during the first 10 min of LFI is due to an increase in glycogenolysis as well as a marked inhibition of the accelerated rates of glycogen synthesis in this group. In the G-depleted hearts, the lower preischemic glycogen is almost exhausted after the initial 10 min of LFI and this leads to a stimulation of GLUT4 translocation and glucose uptake. Similarly, exercise-induced stimulation of glucose uptake in skeletal muscles is greater when glycogen content is low and unable to supply endogenous substrate for glycolysis (6, 14).

The significant inverse correlation between the rate of glycogen degradation and the rate of glucose uptake throughout the different phases of perfusion suggests that these two processes interact to provide sufficient substrate for glycolysis. Also, during ischemia, it is energetically more favorable for the heart to use endogenous, rather than exogenous, glucose. The degradation of glycogen provides a phosphorylated substrate for glycolysis, whereas the uptake of exogenous glucose requires the consumption of one mole of ATP per mole of glucose to convert glucose into glucose-6-phosphate. Therefore, the catabolism of each mole of glucose derived from glycogen yields one extra mole of ATP compared with the catabolism of exogenous glucose. Thus, during ischemia when the heart is energetically compromised, glycogen degradation becomes the preferred source of glucose-6-phosphate for energy production by glycolysis as long as glycogen stores are sufficient. However, when glycogen stores are depleted, glucose uptake is increased to maintain the supply of glucose-6-phosphate.

AMPK is a key kinase involved in the regulation of many aspects of cellular metabolism, including glucose metabolism (20, 29, 30, 51). AMPK activation by AICAR in rat skeletal muscles is accompanied by an activation of glycogen phosphorylase that increases glycogenolysis (51), but no such relationship occurs in rat ventricular papillary muscle (38). However, our previous data have shown that the adenosine-induced activation of AMPK in the stressed working heart does not affect glucose uptake but is accompanied by an alteration in glycogen turnover manifest as an inhibition of glycogen synthesis (25). The current study extends those observations and indicates that ischemia-induced activation of AMPK is not sufficient to increase glucose uptake in G-replete hearts but is accompanied by alterations in glycogen turnover that comprise an acceleration of glycogenolysis and an inhibition of glycogen synthesis. Although the activation of myocardial AMPK has been shown to increase glycolysis via activation of phosphofructokinase-2 (33), glycolysis was only accelerated in G-depleted hearts. The absence of such an effect in G-replete hearts may be attributed to the higher preischemic glycolysis rate and/or to a greater accumulation of protons or glycolytic by-products. The failure of AMPK activation to increase glucose uptake in G-replete hearts is not due to differences in the degree of AMPK activation between the G-replete and G-depleted groups since peak AMPK activity is similar. Also, the rapid, ischemia-induced increase in the Cr-to-PCr ratio and the more gradual increase the AMP-to-ATP ratio, as has been reported previously (4), were similar in each group. Interestingly, the time-dependent increase in AMPK activity had a profile more related to the increase in Cr-to-PCr ratio, an early and sensitive indicator of energy deficiency, than to increase in the AMP-to-ATP ratio.

In addition to myocardial glycogen content, other factors may account for the marked difference between this study and others that support the view that AMPK activity regulates glucose uptake. Stimulation of glucose uptake by the AMPK activator, AICAR, is dependent on both nutritional state (greater in fasted animals) and muscle fiber type (less in oxidative muscle) (1), suggesting that glucose uptake in G-replete cardiac muscle may be less responsive to AMPK activation. However, other studies using isolated LV papillary muscle (38) have shown that AICAR-induced activation of AMPK increases [2-3H]deoxyglucose uptake and that ischemia accelerates glucose uptake in wild-type mouse hearts but not in hearts with a kinase dead mutation of AMPK (39). In both those studies (38, 39), the effect of AMPK activation on glucose uptake was measured in the presence of either no or low (fasting) concentrations of insulin (that may 1) have limited glycogen resynthesis during preischemic conditions and/or 2) caused low aerobic (baseline) rates of glucose uptake. Our study indicates that LFI and subsequent AMPK activation can be accompanied by an increase in glucose uptake under conditions of glycogen depletion which resemble the conditions under which many of the studies showing ischemia-induced
increase in glucose uptake were performed. Lack of activation of glucose uptake in hearts with a kinase dead mutation of AMPK (39) may also be due to a lack of activation of glycolysis or inhibition of glycogen synthesis, effects that indirectly reduce the requirement for enhanced glucose uptake.

In conclusion, the present study provides clear evidence that in isolated working hearts, ischemia-induced alterations in glucose uptake and utilization are dependent on preischemic glycogen content. In G-replete hearts, the marked and sustained activation of glycogenolysis by LFI supplies sufficient substrate for glycolysis and so glucose uptake is inhibited. The ability of LFI to markedly stimulate AMPK activity in both G-replete and G-depleted hearts indicates that AMPK activation is not sufficient to accelerate glucose uptake. Instead, ischemia and AMPK may exert a greater influence on the regulation of glycogen turnover and glycolysis, effects that are dependent on glycogen content.

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