Additive Effects of Hyperinsulinemia and Ischemia on Myocardial GLUT1 and GLUT4 Translocation In Vivo

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Background—Myocardial ischemia increases glucose uptake through the translocation of GLUT1 and GLUT4 from an intracellular compartment to the sarcolemma. The present study was performed to determine whether hyperinsulinemia causes translocation of myocardial GLUT1 as well as GLUT4 in vivo and whether there are additive effects of insulin and ischemia on GLUT1 and GLUT4 translocation.

Methods and Results—Myocardial glucose uptake and transporter distribution were assessed by arteriovenous measurements, cell fractionation, and immunofluorescence. In fasted anesthetized dogs, hyperinsulinemia increased myocardial glucose extraction 3-fold ($P<0.01$) and the sarcolemmal content of GLUT4 by 90% and GLUT1 by 50% ($P<0.05$ for both) compared with saline infusion. In subsequent experiments, glucose uptake and transporter distribution were determined in ischemic and nonischemic regions of hearts from hyperinsulinemic animals during regional myocardial ischemia. Glucose uptake was 50% greater in the ischemic region ($P<0.05$). This was associated with a 20% increase in sarcolemmal GLUT1 and a 60% increase in sarcolemmal GLUT4 contents in the ischemic region ($P<0.05$ for both).

Conclusions—Insulin stimulates myocardial glucose utilization through translocation of GLUT1 as well as GLUT4. Insulin and ischemia have additive effects to increase in vivo glucose utilization and augment glucose transporter translocation. We conclude that recruitment of both GLUT1 and GLUT4 contributes to increased myocardial glucose uptake during moderate reductions in coronary blood flow under insulin-stimulated conditions. **(*Circulation*. 1998;98:2180-2186.)**

Key Words: glucose ■ insulin ■ ischemia ■ metabolism

The transport of glucose across the sarcolemma is the first step in myocardial glucose metabolism, and the rate of transport is determined by the number of the facilitative glucose transport proteins, GLUT1 and GLUT4, present in the sarcolemma. Glucose uptake and metabolism in the glycolytic pathway are further controlled by the transmembrane glucose gradient,[1] the rates of glucose phosphorylation[2] and glycogen turnover,[3] and flux control at each of the reactions of the glycolytic pathway.[2]

GLUT1 is expressed ubiquitously and is responsible for "basal" glucose transport. The GLUT1 present in heart localizes predominantly to cardiomyocytes and also translocates to the sarcolemma in response to ischemia.[4] Although one recent in vitro study has demonstrated an insulin-mediated increase in sarcolemmal GLUT1 in cultured cardiomyocytes,[5] the effect of hyperinsulinemia on myocardial GLUT1 distribution in vivo is unknown. GLUT4 translocates from an intracellular pool to the cell surface in response to insulin in skeletal[6] and heart[7] muscle. Furthermore, GLUT4 translocates in response to myocardial ischemia in vitro[7] and in vivo.[4] The combination of exercise and supraphysiological hyperinsulinemia increases the content of GLUT4 in the cardiac sarcolemma in rats.[8] However, the effect of physiological hyperinsulinemia on myocardial GLUT1 and GLUT4 translocation in vivo has not been determined.

Our previous studies demonstrating ischemia-mediated translocation of GLUT1 and GLUT4 were performed in fasted animals, in which baseline myocardial glucose uptake rates are low.[4] It remains unclear whether ischemia also increases myocardial transporter translocation and glucose utilization during hyperinsulinemia. Hypoxia and insulin stimulation have been shown to have additive effects on glucose uptake in skeletal muscle,[9] but these studies did not assess the subcellular distribution of GLUT4. Furthermore, no studies have investigated whether these two stimuli have additive effects on myocardial glucose transporter translocation in vivo.

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Thus, the present studies were performed to determine whether insulin-stimulated glucose uptake is associated with GLUT1 and GLUT4 translocation in vivo and to determine the effects of hyperinsulinemia and regional myocardial ischemia on GLUT1 and GLUT4 translocation and glucose uptake.

Methods

Animal Preparation

Fasted adult male mongrel dogs were used in experiments approved by the Yale Animal Care and Use Committee. The dogs were initially anesthetized with intravenous sodium thiamyl (20 mg/kg), intubated, and mechanically ventilated with halothane (1%), nitrous oxide (69%), and oxygen (30%) to maintain adequate anesthesia.

Effects of Insulin on Myocardial Glucose Uptake and Transporter Distribution

Closed-chest dogs either underwent a hyperinsulinemic, euglycemic clamp (insulin group, n=5) or received intravenous saline (control group, n=6) for 4 hours. Pulmonary artery, coronary sinus, and arterial catheters and a left ventricular micromanometer catheter were inserted and kept patent with heparinized saline (total dose, 500 to 1000 U). Insulin was infused at a rate of 8 $mU \cdot kg^{-1} \cdot min^{-1}$ for 10 minutes, followed by 4 $mU \cdot kg^{-1} \cdot min^{-1}$ for the remainder of the experiment. Glucose was infused at a variable rate to maintain euglycemia. Cardiac output was determined by the thermodilution method. At the end of the experiment, the heart was rapidly excised, placed in ice-cold 0.9% NaCl, and processed as described below to isolate intracellular and sarcolemmal membranes.

Effects of Insulin and Ischemia on Myocardial Glucose Uptake and Transporter Distribution

The effect of ischemia on glucose transporter translocation during insulin stimulation was studied in an open-chest canine model of regional ischemia (n=13).[4,10] Epicardial Doppler thickening crystals (10 MHz) were used to measure transmural myocardial thickening in both the ischemic and nonischemic regions. Catheters were inserted into the distal left anterior descending coronary artery (LAD) to monitor pressure and into veins draining the LAD and left circumflex artery (LCx) regions to determine arteriovenous substrate differences. A pulmonary artery catheter was inserted to determine cardiac output, and micromanometer-tipped catheters were placed into the left ventricular cavity and the aorta for pressure measurement. Catheters were kept patent with heparinized saline. After baseline measurements were obtained, insulin was infused as described above. After 60 minutes, regional ischemia was induced during hyperinsulinemia by inflating a hydraulic occluder around the proximal LAD to reduce distal LAD pressure by 50% for 80 minutes. The heart was then removed and placed into ice-cold 0.9% NaCl, and the central ischemic and nonischemic regions were divided into epicardial and endocardial portions.[4] The subcellular membrane fractions from the ischemic LAD and nonischemic LCx regions were prepared as described below.

A subset of these animals (n=8) were injected with 2.5 mCi of the flow tracer ^{99m}Tc-sestamibi 10 minutes before euthanasia to determine the subendocardial, subepicardial, and transmural blood flow in the ischemic LAD region relative to the LCx region. An aliquot of heart homogenate was counted in a gamma well counter to determine the ratio of blood flow in the LAD and LCx regions. The relative transmural blood flow, combined with the arteriovenous glucose difference, was used to calculate the glucose uptake in the ischemic region relative to the nonischemic region.

Metabolic Determinations

Paired arterial and venous blood samples were drawn in quadruplicate during the final 15 minutes of each experimental period and used to determine plasma arteriovenous metabolite differences, which were then averaged to yield a mean value for each period. Plasma glucose, lactate, free fatty acid, and insulin concentrations were measured as previously described.[4]

Membrane Vesicle Preparation and Immunoblotting

Crude, intracellular (IC), and sarcolemmal (SL) membranes were prepared from heart samples as described previously.[4] Membrane fraction enrichment was based on the quantification of the β_1-subunit of the Na^+/K^+-ATPase (SL marker) from immunoblots and Ca^{2+} ATPase activity (IC marker).[4] The IC and SL fractions demonstrated high degrees of enrichment for their respective markers (Table 1). Immunoblots of IC and SL proteins were probed with specific polyclonal rabbit anti-GLUT1 or anti-GLUT4 antibodies and incubated with ^{125}I-labeled protein A.[4] Bands corresponding to either GLUT1 or GLUT4 were excised from the filters and counted in a gamma well counter (coefficient of variation, 8.7%). The SL and IC contents of GLUT4 and GLUT1 were quantified as previously described.[7]

Immunofluorescence Studies

The subcellular distribution of GLUT1 and GLUT4 was further defined by immunofluorescence confocal microscopy of myocardial tissue samples from saline-infused and insulinized animals as previously described.[4] Contrast and brightness settings were chosen so that all pixels were within the linear range. All images are the product of 8-fold line averaging.

Statistics

Results are reported as mean±SEM. ANOVA was performed, followed by Tukey's test where appropriate. A value of $P<0.05$ was considered statistically significant.

TABLE 1. Myocardial Membrane Fraction Enrichments

	Na^+/K^+-ATPase β-subunit			Ca^{2+} ATPase		
	SL	IC	SL/IC	SL	IC	SL/IC
Saline vs insulin protocol						
Saline	61.6±15.3*	8.2±2.0	7.8±1.6	3.8±1.3*	12.7±2.5	0.3±0.1
Insulin	59.5±8.5*	7.0±1.7	9.6±2.1	3.8±0.5*	15.5±1.9	0.3±0.1
Insulin+LAD stenosis protocol						
Nonischemic	61.1±14.4*	8.7±3.2	11.5±4.1	3.1±1.4*	11.4±2.7	0.2±0.1
Ischemic	64.0±26.1*	6.4±1.4	10.4±2.9	3.2±1.2*	10.8±1.8	0.4±0.2

The intracellular (IC) and sarcolemmal (SL) Na^+/K^+-ATPase β-subunit protein and Ca^{2+} ATPase activity enrichments relative to the values for the crude membrane fraction.

*$P<0.05$ vs IC fraction.

TABLE 2. Metabolic Effects of Saline or Insulin Infusion

	Saline	Insulin
Arterial [glucose], mmol/L		
Control	5.8±0.3	6.6±0.2
Infusion	5.0±0.1	5.6±0.3
Myocardial glucose extraction, mmol/L		
Control	0.3±0.1	0.2±0.1
Infusion	0.2±0.1	0.6±0.1*
Arterial [free fatty acids], mmol/L		
Control	1.09±0.35	0.92±0.09
Infusion	1.18±0.32	0.38±0.01*
Plasma [insulin], pmol/L		
Control	39±11	30±6
Infusion	21±4	1242±102†

*$P<0.01$ or †$P<0.001$ vs control time point and the saline group at the end of the study.

Results

Effects of Hyperinsulinemia

Insulin infusion did not significantly affect heart rate (104±8 versus 120±4 bpm), mean aortic pressure (89±7 versus 93±8 mm Hg), or left ventricular systolic (105±6 versus 103±6 mm Hg) or diastolic (8±1 versus 8±1 mm Hg) pressure compared with baseline. However, hyperinsulinemia did increase cardiac output 20% (3.1±0.2 versus 3.7±0.2 L/min, $P<0.05$), reflecting a small decrease in afterload through insulin-induced arterial vasodilation.[11] Saline infusion caused no changes in heart rate, blood pressure, or cardiac output (data not shown).

To maintain euglycemia during hyperinsulinemia, glucose was infused at a rate of 10±1 mg · kg^{-1} · min^{-1}. Hyperinsulinemia increased the arteriovenous glucose difference 2.5-fold compared with baseline (Table 2). There was a trend toward a decrease in glucose extraction in the saline group that was not statistically significant. Glucose extraction was 3-fold higher during insulin infusion than during saline infusion at the end of the experiment. Hyperinsulinemia caused a 68% decrease in the plasma free fatty acid concentration.

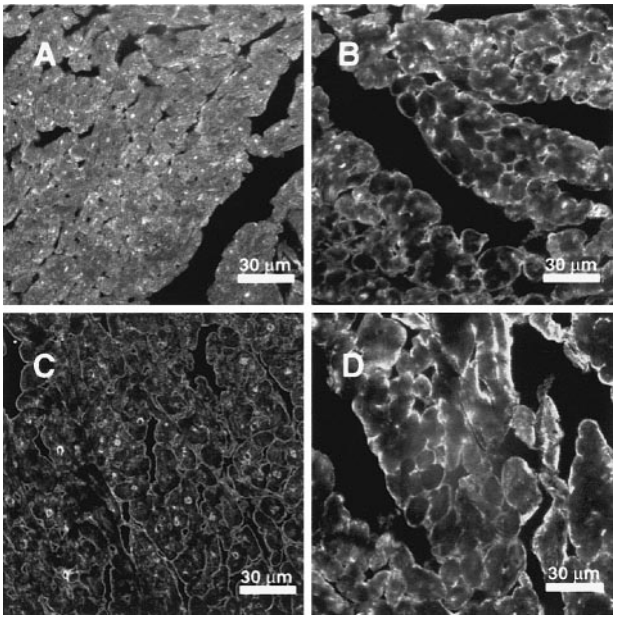

Figure 2. Immunofluorescence photomicrographs of hearts from saline-infused (A and C) or insulin-infused (B and D) animals. GLUT4 localization is shown in A and B; GLUT1 localization in C and D.

The percentage of GLUT4 in the SL fraction doubled in response to hyperinsulinemia (19±3% versus 36±7%; Figure 1), with a concomitant decrease in the percentage of GLUT4 in the IC fraction. Confocal microscopy confirmed the redistribution of myocardial GLUT4 to the sarcolemma with insulin stimulation. Specifically, heart GLUT4 immunolocalized to the intracellular compartment during saline infusion (Figure 2A). In contrast, there was predominant myocyte surface labeling for GLUT4 in insulinized hearts (Figure 2B). The proportion of GLUT1 present in the SL fraction was higher than that for GLUT4 during saline infusion and increased 1.5-fold with insulin stimulation (42±5% versus 63±8%; Figure 3). GLUT1 immunolocalized primarily to cardiomyocytes and had a more prominent surface labeling pattern than GLUT4 (Figure 2C), so that it was difficult to appreciate insulin-stimulated changes in the amount of GLUT1 present in the sarcolemma (Figure 2D). Nonetheless, the immunofluorescence results confirm the predominant

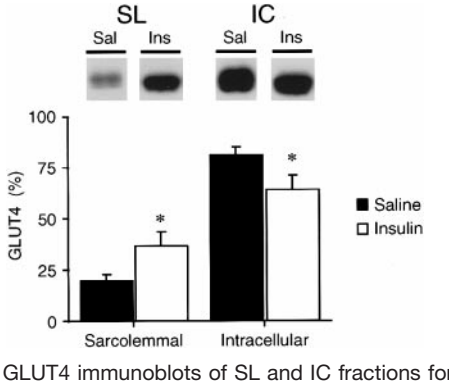

Figure 1. GLUT4 immunoblots of SL and IC fractions for hearts after either saline or insulin infusion (top) and percentage of myocardial GLUT4 present in SL and IC fractions (bottom) after saline or insulin infusion. *$P<0.05$ vs saline.

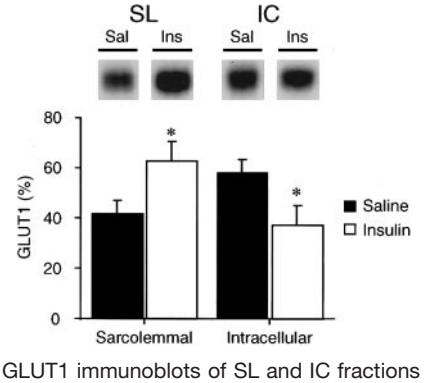

Figure 3. GLUT1 immunoblots of SL and IC fractions for hearts after either saline or insulin infusion (top) and percentage of myocardial GLUT1 present in SL and IC fractions (bottom) after saline or insulin infusion. *$P<0.05$ vs saline infusion.

TABLE 3. Hemodynamic Parameters During Insulin Infusion and Regional Ischemia

	Control	Insulin	Insulin+LAD Occlusion
Mean aortic pressure, mm Hg	84±3	82±3	86±4
Mean distal LAD pressure, mm Hg	77±3	74±2	37±3*
LCx thickening fraction, %	17.1±1.4	16.6±1.7	17.3±1.8
LAD thickening fraction, %	19.0±2.4	19.5±2.7	7.2±1.1*
Left ventricular systolic pressure, mm Hg	96±3	94±3	97±4
Left ventricular diastolic pressure, mm Hg	11±4	5±1	6±1
Heart rate, bpm	114±5	120±6	128±5
Cardiac output, L/min	2.6±0.2	2.8±0.2	2.6±0.2

*$P<0.002$ vs the other two periods.

sarcolemmal distribution of GLUT1 in saline-infused and hyperinsulinemic animals.

Combined Effects of Hyperinsulinemia and Ischemia

Based on the above findings, together with our previous demonstration of translocation of both GLUT1 and GLUT4 in response to ischemia,[4] we examined whether insulin and ischemia have additive effects on myocardial glucose transporter distribution. In these studies, insulin infusion had no significant effect on myocardial function or hemodynamics before partial occlusion of the LAD, which caused moderate hypokinesis in the LAD region (Table 3).

Insulin infusion increased the insulin concentration from a low fasting to a high physiological level (Table 4). Euglycemia was maintained with glucose infused at a rate of 8.2±0.5 $mg \cdot kg^{-1} \cdot min^{-1}$ before regional ischemia and 8.8±0.8 $mg \cdot kg^{-1} \cdot min^{-1}$ during regional ischemia. These values are ≈20% lower ($P<0.05$) than the rate of glucose infusion in the closed-chest dogs in the first set of experiments. The plasma free fatty acid concentration at baseline (Table 4) was lower than during the control period in the experiments described above (Table 2) but decreased during hyperinsulinemia to a level comparable to that of the earlier experiments. The arterial lactate concentration did not change throughout the experiment (Table 4).

Insulin increased myocardial glucose extraction 2-fold in both the LAD and LCx regions before the onset of ischemia (Figure 4). Acute ischemia led to a further doubling of the arteriovenous glucose difference in the ischemic LAD region, whereas glucose extraction in the nonischemic LCx region

TABLE 4. Arterial Substrate Concentrations During Insulin Infusion and Regional Ischemia

	Control	Insulin	Insulin+LAD Occlusion
Glucose, mmol/L	5.5±0.2	5.4±0.2	5.7±0.1
Lactate, mmol/L	1.2±0.1	1.3±0.1	1.3±0.1
Free fatty acids, mmol/L	0.55±0.05	0.34±0.03*	0.31±0.02*
Insulin, pmol/L	24±6	1932±126*	2052±138*

*$P<0.01$ vs control conditions.

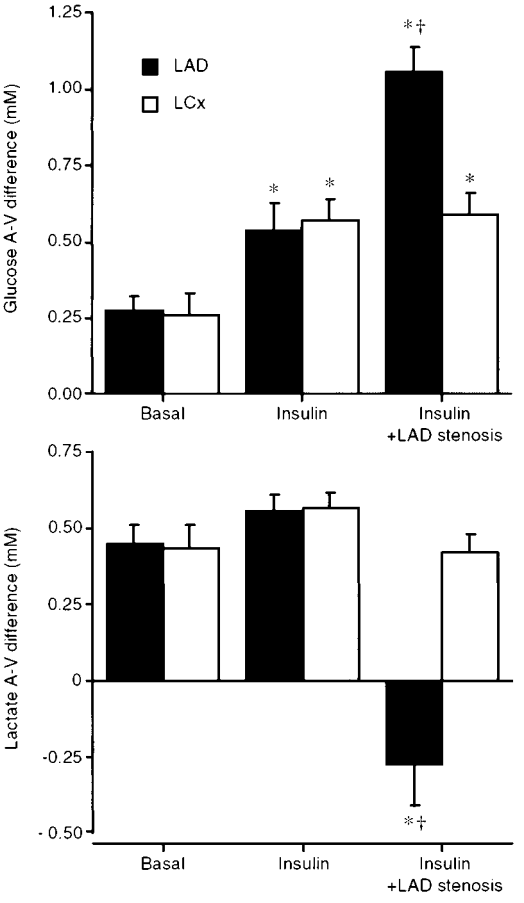

Figure 4. Myocardial arteriovenous differences for glucose (top) and lactate (bottom) during control conditions, insulin infusion, and insulin infusion with regional ischemia for LCx and ultimately ischemic LAD regions. *$P<0.05$ vs control, †$P<0.05$ vs LCx.

was not affected. Transmural blood flow in the LAD region was reduced by 21±5% relative to the nonischemic LCx territory, which is in keeping with values determined in a similar preparation using microspheres.[10] With this relative blood flow measurement used to correct the arteriovenous glucose difference, there was a 50% greater flow-normalized glucose extraction in the LAD than in the LCx region (13.1±1.6% versus 8.8±1.4%, respectively, $P<0.05$). There was also net lactate release in the LAD region during ischemia (Figure 4, bottom).

Glucose transporter distribution was measured in the endocardium of both the LAD and LCx regions. These portions of the myocardium were used because the reduction in flow (relative to the nonischemic region) was greater ($P<0.05$) in the ischemic subendocardium (27.3±6.1%) than in the ischemic epicardium (12.8±5.1%). There was a significant increase in the sarcolemmal GLUT4 content in the ischemic region compared with the nonischemic region (40±6% versus 25±4%; Figure 5). Although the percentage of GLUT4 present in the sarcolemma of the insulin-stimulated LCx region tended to be lower than in myocardium from closed-chest, insulinized animals in the first set of experiments, the difference was not statistically significant. In addition, the arteriovenous glucose difference for the nonischemic, insulin-

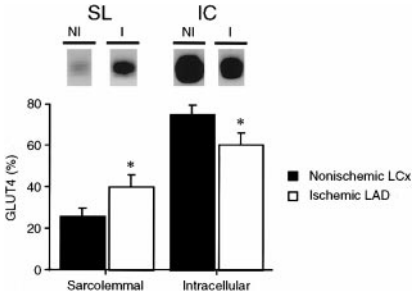

Figure 5. GLUT4 immunoblots of SL and IC fractions from nonischemic LCx and ischemic LAD regions from insulinized animals (top) and effects of either insulin infusion alone (LCx) or insulin infusion together with ischemia (LAD) on percentage of GLUT4 protein present in SL and IC fractions (bottom). *$P<0.05$ vs LCx.

stimulated LCx region (0.6±0.1 mmol/L, Figure 4) was similar to the arteriovenous glucose difference for the insulinized, closed-chest animals (0.6±0.1 mmol/L, Table 2). The sarcolemmal GLUT1 content also increased in response to ischemia in insulinized animals (67±6% versus 53±4%; Figure 6). The increases in sarcolemmal GLUT1 and GLUT4 were mirrored by decreases in the intracellular content of GLUT1 and GLUT4 in the ischemic region, indicating translocation from the IC pool to the cell surface (Figures 5 and 6). As with GLUT4, there were no significant differences in the sarcolemmal GLUT1 content in the nonischemic, insulinized LCx region compared with the myocardium from insulinized animals in the first set of experiments.

Discussion

The present study demonstrates several important aspects of the regulation of myocardial glucose transporter distribution in vivo. Specifically, physiological hyperinsulinemia causes translocation of both GLUT4 and GLUT1 from an intracellular pool to the sarcolemma. Furthermore, in hyperinsulinemic animals, ischemia causes additional translocation of both GLUT1 and GLUT4. This additive effect on glucose transporter translocation is associated with increased glucose uptake by the ischemic myocardium. These studies are the first to demonstrate in vivo translocation of myocardial GLUT1 and GLUT4 in response to insulin as well as the first to demonstrate additive effects of ischemia and hyperinsulinemia on GLUT1 and GLUT4 translocation and glucose uptake.

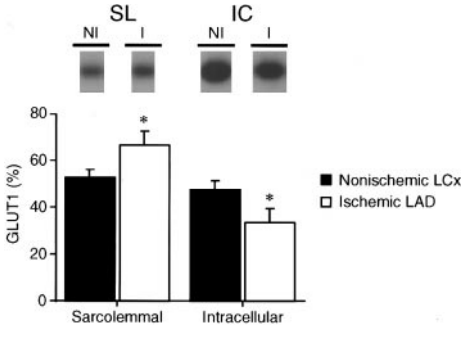

Figure 6. GLUT1 immunoblots of SL and IC fractions from nonischemic LCx and ischemic LAD regions from insulinized animals (top) and effects of either insulin infusion alone (LCx) or insulin infusion together with ischemia (LAD) on percentage of GLUT1 protein present in SL and IC fractions (bottom). *$P<0.05$ vs LCx.

Insulin-Mediated Translocation of GLUT1 and GLUT4

Previous studies have demonstrated insulin-mediated GLUT4 translocation from an intracellular pool to the sarcolemma of cardiomyocytes[12] and perfused rat hearts.[5,7] However, these previous studies were performed in vitro at supraphysiological insulin concentrations. The present study expands on these previous reports by examining the effects of physiological hyperinsulinemia when the heart is exposed to other substrates and performing physiological work in vivo. Our findings are also in keeping with studies using immunogold labeling that demonstrate redistribution of GLUT4 from an intracellular pool to the sarcolemma of cardiomyocytes in response to the combination of supraphysiological insulin stimulation and exercise.[8]

The present study is the first to demonstrate in vivo effects of insulin on GLUT1 translocation and is consistent with previous in vitro reports that insulin stimulates GLUT1 translocation in isolated cardiomyocytes[12] and the perfused obese Zucker rat heart.[5] There is also indirect evidence, based on immunoblot analysis of intracellular membrane proteins, that insulin causes GLUT1 translocation in vitro in the perfused normal rat heart.[13] Although insulin-mediated translocation of GLUT4 requires activation of phosphatidylinositol 3-kinase,[14] the mechanism responsible for insulin-mediated GLUT1 translocation remains unknown.

The present findings indicate that like ischemia,[4] insulin stimulation causes GLUT1 translocation. These results suggest that increased myocardial glucose demand, because of insulin stimulation or ischemia, causes translocation of both GLUT1 and GLUT4 from intracellular sites to the sarcolemma. These results also suggest that the role of GLUT1 in glucose metabolism may differ between heart and skeletal muscle. The metabolic demands and the GLUT1 content of resting skeletal muscle are much lower than those of the heart.[15] Furthermore, skeletal muscle GLUT1 is found predominantly on neurofilaments rather than on myocytes,[6] in contrast to our findings of GLUT1 in the sarcolemma of cardiomyocytes. This may explain, in part, why insulin-stimulated GLUT1 translocation is not normally seen in skeletal muscle cells but occurs in heart, in which GLUT1 contributes to insulin-stimulated glucose uptake.

Additive Effects of Ischemia and Insulin Stimulation on Glucose Transporter Translocation

In the present study, ischemia increased the sarcolemmal GLUT1 and GLUT4 contents and in vivo myocardial glucose uptake in insulinized animals. Specifically, using a moderate reduction in coronary blood flow plus physiological hyperinsulinemia, we were able to demonstrate directly the additive effects of hyperinsulinemia and ischemia on GLUT1 and GLUT4 translocation. These findings are consistent with recent in vitro studies that demonstrated additive effects of global ischemia and insulin stimulation on glucose uptake in the isolated working rat heart,[16] although glucose transporters

were not studied. In one previous in vitro study of isolated, retrogradely perfused rat hearts, total, global ischemia did not increase the percentage of GLUT4 present in the sarcolemma of maximally insulin-stimulated hearts, but the combined effects of insulin and ischemia on GLUT1 distribution were not examined.[7] In another previous study, glucose uptake increased, whereas the IC contents of GLUT1 and GLUT4 tended to decrease, in anoxic rat hearts perfused with insulin.[13] The sarcolemmal GLUT1 and GLUT4 contents were not measured, however; therefore, these findings only indirectly suggest that the combined effects of anoxia and insulin stimulation may increase the sarcolemma GLUT1 and GLUT4.

The present demonstration of the additive effects of insulin and ischemia on glucose transporter translocation is of interest in view of previous studies that indicate that insulin and hypoxia signal translocation through different mechanisms. In isolated skeletal muscle cells, chemically induced hypoxia causes GLUT4 translocation via an undefined mechanism distinct from the phosphatidylinositol 3-kinase–dependent pathway of insulin-mediated GLUT4 translocation.[14] The mechanism by which ischemia causes translocation of myocardial GLUT1 and GLUT4 remains to be elucidated.

Metabolic and Clinical Implications

Our studies characterize one of the mechanisms responsible for the acute adaptation of cardiomyocytes to the increased demand for glycolytically produced energy in the setting of ischemia. Glucose transport is determined by the number of glucose transporters present in the sarcolemma and the transsarcolemmal glucose concentration gradient. GLUT1 and GLUT4 translocation increases glucose extraction during ischemia[4] despite decreased glucose delivery and lower interstitial glucose concentrations.[1] Our findings indicate that the combination of hyperinsulinemia and ischemia causes greater recruitment of glucose transporters to the sarcolemma, thereby increasing the ability of ischemic cardiomyocytes to utilize extracellular glucose.

The present studies demonstrate an increase in myocardial glucose extraction during hyperinsulinemia in association with the translocation of GLUT1 and GLUT4 (direct insulin effect). However, glucose utilization is also controlled by substrate competition, and hyperinsulinemia decreases plasma free fatty acid concentrations and myocardial fatty acid oxidation, which increase glucose utilization through the Randle cycle (an indirect insulin effect). In vivo studies have demonstrated that decreasing free fatty acid concentrations through inhibition of lipolysis by niacin or nicotinic acid derivatives increases myocardial [^{18}F]fluorodeoxyglucose uptake without increasing the plasma insulin concentration.[17,18] Although these previous studies highlight the indirect effects of insulin, it might be noted that a high free fatty acid concentration decreases insulin-mediated glucose uptake by only 17% to 26%,[19,20] indicating the important roles of both direct and indirect insulin effects in myocardial glucose uptake.

There are several potentially beneficial metabolic effects of insulin-mediated augmentation of glucose uptake in the ischemic myocardium. These effects include greater glycolytic ATP production,[21] preservation of glycogen stores,[21] and increased contribution of exogenous glucose to citric acid cycle flux, which decreases the detrimental effects of ischemic and postischemic fatty acid oxidation.[22] These benefits have been exploited clinically with the use of glucose-insulin-potassium infusions in the setting of both myocardial infarction[23] and cardiac surgery[24] to maintain myocardial viability and enhance postischemic contractile recovery.

Patients with coronary artery disease are hyperinsulinemic and insulin resistant.[25] In the present studies, the glucose infusion rate required to maintain euglycemia in open-chest animals was 20% lower than in the closed-chest dogs, despite somewhat higher insulin concentrations, suggesting that thoracotomy caused mild insulin resistance. However, insulin still stimulated myocardial glucose uptake in these animals, suggesting that even in the presence of mild insulin resistance, insulin stimulation and ischemia have additive effects on myocardial glucose uptake.

Study Limitations

In the present study, changes in glucose transporter distribution were determined only at one level of ischemia. Because myocardial ischemia represents a spectrum of reductions in flow, GLUT1 or GLUT4 translocation may be affected by the degree of flow reduction. Further studies are needed to determine how the severity of ischemia affects the subcellular distribution of GLUT1 and GLUT4. In addition, glucose extraction was determined transmurally and was therefore affected by rates of glucose extraction from tissue subjected to heterogeneous degrees of ischemia. In contrast, the subcellular distributions of GLUT1 and GLUT4 were determined in the more ischemic subendocardium. It is therefore difficult to directly compare glucose uptake rates with the degree of transporter translocation. Studies with labeled 2-deoxyglucose might help to elucidate the relationship between regional glucose uptake and glucose transporter distribution.

Because of the differing affinities of the antibodies used in the immunoblotting studies, it is difficult to determine the relative contributions of GLUT1 and GLUT4 to myocardial glucose transport. However, the GLUT1 protein content of the heart is estimated to be 25% that of GLUT4.[15] On the basis of the relatively greater degree of GLUT4 translocation and content, GLUT4 is most likely primarily responsible for increased glucose transport under conditions of hyperinsulinemia and myocardial ischemia.

In summary, the present study demonstrates that insulin stimulation causes translocation of both GLUT1 and GLUT4, resulting in an increase in in vivo myocardial glucose uptake. Furthermore, we have also shown that ischemia and hyperinsulinemia have additive effects on myocardial GLUT1 and GLUT4 translocation and glucose uptake. These findings help to characterize the adaptation of the heart to an increased glucose demand and may be of importance in the design of strategies to support the heart metabolically during ischemia.

Acknowledgments

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