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Inhibition of Glycogen Synthase Kinase-3 β Improves Tolerance to Ischemia in Hypertrophied Hearts

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Abstract

Background—Hypertrophied myocardium is more susceptible to ischemia/reperfusion injury, in part owing to impaired insulin-mediated glucose uptake. Glycogen synthase kinase-3 β (GSK-3 β) is a key regulatory enzyme in glucose metabolism that, when activated, phosphorylates/inactivates target enzymes of the insulin signaling pathway. Glycogen synthase kinase-3 β is regulated upstream by Akt-1. We sought to determine whether GSK-3 β is activated in ischemic hypertrophied myocardium owing to impaired Akt-1 function, and whether inhibition with lithium (Li) or indirubin-3'-monoxime,5-iodo- (IMI), a specific inhibitor, improves post-ischemic myocardial recovery by improving glucose metabolism.

Methods—Pressure-overload hypertrophy was achieved by aortic banding in neonatal rabbits. At 6 weeks, isolated hypertrophied hearts underwent 30 minutes of normothermic ischemia and reperfusion with or without GSK-3 β inhibitor (0.1 mM Li; 1 μ M IMI) as cardioplegic additives. Cardiac function was measured before and after ischemia. Expression, activity of Akt-1 and GSK-3 β , and lactate were determined at end-ischemia.

Results—Contractile function after ischemia was better preserved in hypertrophied hearts treated with GSK-3 β inhibitors. Activity of Akt-1 was significantly impaired in hypertrophied myocardium at end-ischemia. Glycogen synthase kinase-3 β enzymatic activity at end-ischemia was increased in hypertrophied hearts and was blocked by Li or IMI concomitant with significantly increased lactate production, indicating increased glycolysis.

Conclusions—Regulatory inhibition of GSK-3 β by Akt-1 in hypertrophied hearts is impaired, leading to activation during ischemia. Inhibition of GSK-3 β by Li or IMI improves tolerance to ischemia/reperfusion injury in hypertrophied myocardium. The likely protective mechanism is an increase in insulin-mediated glucose uptake, resulting in greater substrate availability for glycolysis during ischemia and early reperfusion.

Cardiac hypertrophy is an adaptive response to compensate for increased workload. It is accompanied by distinct qualitative and quantitative changes of the myocardium, such as multiplication of sarcomeres, a switch of contractile proteins to fetal isoforms, reintroduction of fetal isoforms for several enzymes including lactate dehydrogenase or creatine kinase, changes in intracellular Ca²⁺ handling, and metabolic alterations [1]. Early in the development of hypertrophy, abnormalities of fatty acid metabolism occur, accompanied by compensatory increase in glucose utilization for high-energy phosphate production [2]. These alterations should result in adaptation of the hypertrophied heart to

withstand ischemic injury, as anaerobic glycolysis is the major source of energy production. However, paradoxically, glucose uptake and metabolism is impaired in severe pressure-overload hypertrophy during ischemia and early reperfusion [1].

Hypertrophied hearts use not only exogenous carbohydrates, such as glucose and lactate, but also endogenous glucose from glycogen stores to produce energy [2, 3]. However, during myocardial ischemia, hypertrophied hearts exhibit accelerated loss of high-energy nucleotides, earlier onset of ischemic contracture, and accelerated calcium overload during early reperfusion [4]. A number of morphologic, metabolic, and physiologic changes in the hypertrophied heart contribute to increased susceptibility to ischemic injury [5]. As we and others have previously shown, in hypertrophied myocardium, insulin-stimulated glucose transport and uptake rate is reduced, and this impairment likely contributes to decreased tolerance to ischemia [1, 6, 7]. The decreased glucose uptake rate in hypertrophy is not due to a decreased expression of the insulin-sensitive glucose transporter Glut-4, but appears to be due to a defect in the insulin signaling pathway [6–9].

A key regulatory enzyme in insulin stimulated glucose metabolism, which targets many of the signaling intermediaries, is glycogen synthase kinase-3 (GSK-3). Glycogen synthase kinase-3 was first characterized for its ability to phosphorylate and inhibit glycogen synthase, a key regulatory step in the synthesis of glycogen for glucose storage [10–13]. There are two homologous isoforms of GSK-3, α and β . Glycogen synthase kinase-3 α is mostly required for amyloid production [14], and GSK-3 β is a critical central figure in many cellular signaling pathways as it phosphorylates a diverse group of substrates, such as metabolic and signaling proteins, structural proteins, and transcriptional factors [10, 11, 15, 16]. Inhibition of GSK-3 β by the mood-stabilizing drug lithium (Li) has been shown to augment insulin action, increasing glucose uptake and synthesis of glycogen from glucose by activating glycogen synthase [11, 14, 17, 18]. Several other compounds have also been shown to inhibit GSK-3 β , including indirubin-3'-monoxime, 5-iodo-IMI, a specific inhibitor of the enzyme [19].

The mechanism regulating GSK-3 β is not fully understood but phosphorylation by Akt-1 plays an important role [20]. The serine/threonine kinase Akt-1 regulates cellular growth, survival, and metabolism. The coupling of Akt-1 and GSK-3 β has been suggested to lead to inverse changes in their activities, when Akt-1 activity is increased, it maintains serine-phosphorylation of GSK-3 β , whereas decreased Akt-1 activity leads to dephosphorylation and activation of GSK-3 β [21]. Because glucose uptake is reduced in hypertrophied hearts, we hypothesized that Akt-1 activity is decreased and consequently GSK-3 β activity is increased in these hearts, particularly during ischemia. We sought to determine whether direct inhibition of GSK-3 β with Li or IMI stimulates anaerobic glycolysis and improves tolerance to ischemia in a rabbit model of pressure-overload hypertrophy subjected to ischemia and reperfusion.

Material and Methods

Model of Left Ventricular Hypertrophy from Pressure Overload

A model of left ventricular (LV) pressure-overload hypertrophy, achieved by banding the descending aorta in 10-day-old New Zealand White rabbits was used for this study. Details of this model have been published previously [6, 7, 22]. This model results in severe concentric hypertrophy by 4 to 5 weeks of age, and ventricular dilatation and dysfunction by 7 weeks. For this study, we used hearts from rabbits at early decompensated hypertrophy (6 weeks). We have found that at this stage of hypertrophy, tolerance to ischemia/reperfusion is significantly decreased, with depressed postischemic recovery of myocardial function [7].

Isolated Perfused Heart Model and Contractile Function Measurements

The animals were administered heparin (500 U/kg intravenously) and euthanized with an overdose of ketamine (150 mg/kg intravenously) and xylazine (2.5 mg/kg intravenously). The hearts were excised and, after aortic cannulation, were perfused in a nonworking, nonrecirculating Langendorff method, as described previously in more detail [7]. After 30 minutes of perfusion, hypertrophied hearts (n = 6 per group), and age-matched control hearts (n = 6) were arrested with a 2-minute infusion of normothermic cardioplegic solution (50 mL) of modified Krebs-Henseleit buffer containing 10 U/L insulin and a final concentration of 22.5 mmol/L KCl. One group of hypertrophied hearts received KH + potassium cardioplegia alone; in a second group of hypertrophied hearts, 0.1 mmol/L Li (Hyp + Li) was added to the cardioplegia solution; and in the third group, 1 μ mol/L IMI (Hyp + IMI) was added. The hearts were subjected to 30 minutes of global ischemia at 37°C followed by 30 minutes of reperfusion.

Left ventricular contractile function was measured with an intracavitary fluid-filled latex balloon connected to a catheter-tipped micromanometer (Millar Instruments, Houston, Texas) inserted through the left atrial appendage. Left ventricular developed pressure (systolic pressure minus diastolic pressure) was calculated with an end-diastolic pressure preset between 5 and 8 mm Hg at the end of the 30-minute stabilization period for baseline measurements. The same balloon volume was used to measure LV pressures after 30 minutes of reperfusion. The balloon remained deflated during the entire ischemic period and the reperfusion period.

Glycogen Synthase Kinase-3 β Expression and Activity

At end-reperfusion, hearts were snap-frozen and samples stored at -80°C for later analysis of GSK expression and activity. Glycogen synthase kinase-3 β protein content in LV myocardium was determined by immunoblotting of myocardial tissue collected from all groups. Left ventricular tissue was homogenized on ice in buffer, as described previously, and the crude supernatant fraction was stored at -80°C for later analysis [6]. Gel electrophoresis with 10% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) gels was performed on samples of 50 μ g of protein from total homogenates. Electrophoretically separated proteins were transferred to nitrocellulose membranes by electroblotting. Blots were blocked against nonspecific protein binding by incubation in TBST (tris-buffered saline tween-20) with 5% nonfat milk for 30 minutes at room temperature, followed by incubation with the GSK-3 β antibody overnight (1:1000 dilution; Cell Signaling Technology, Danvers, Massachusetts). Primary antibody binding was detected with horseradish peroxidase-conjugated secondary antibody (1:10,000 dilution; Amersham Life Science, Arlington Heights, Illinois). The bound antibody was detected by the enhanced chemoluminescence method according to the manufacturer's instructions (Amersham Life Science). Band intensity was quantified by laser densitometry.

The GSK-3 β activity was measured with the method described by Ryves and colleagues [23], in which GSK-3 activity is determined by ^{32}P transfer from [γ - ^{32}P] adenosine triphosphate (ATP) to a GSK-3 substrate peptide (GSM). Assay components were incubated at room temperature for 10 minutes and the reaction terminated by spotting 25 μ L onto P81 ion-exchange paper (Whatman, Florham Park, New Jersey). The paper was then washed in phosphoric acid three times, and bound radioactivity was quantified by liquid scintillation counting. To determine inhibition of GSK-3 myocardial tissue activity by Li and IMI, GSK-3 activity was determined in vitro incubating tissue obtained from untreated, nonischemic hypertrophied hearts with the experimental concentrations of both inhibitors (0.1 mM Li, and 1 μ M IMI). This was done to determine if the concentrations used in the isolated heart ischemia/reperfusion experiments were sufficient to completely inhibit kinase

activity. In addition, activity was measured in tissue collected from untreated hypertrophied hearts and compared with the treated groups, in the presence of 200 mM Li, which was added in vitro to every group to measure background of enzyme activity. For final experiments, ventricular tissue from a separate set of hypertrophied hearts (n = 6 per group), untreated, or treated with Li or IMI, was collected at end-ischemia, snap-frozen in liquid nitrogen, and proteins isolated as described above. In this LV myocardial tissue extract, GSK-3 β activity was determined.

Metabolic Measurements

In a separate group of hypertrophied hearts, coronary effluent was collected for the first 2 minutes of reperfusion to determine lactate production during the ischemic period. Lactate release was measured with a commercially available blood gas analyzer (pHOx plus L stat profile; Nova Biomedical, Waltham, Massachusetts). Values are expressed as milligrams per milliliter per minute of coronary flow per gram of wet tissue.

Akt-1 Expression and Activity

Expression levels of Akt-1 were determined in LV myocardium obtained from hypertrophied and age-matched control hearts. Immunoblotting using a mouse monoclonal antibody directed against Akt-1 (Cell Signaling, Danvers, Massachusetts) determined the protein levels of Akt-1. The Akt-1 activity was measured by immunoprecipitating LV myocardial tissue samples which were obtained at end-ischemia from hypertrophied hearts and controls, and activating Akt-1 in vitro by incubation with ATP. A GSK-3 fusion protein was used as substrate and activity was determined by measuring phosphorylation of this fusion protein by immunoblotting. All assay components were obtained from Cell Signaling.

Statistical Analysis

Data were analyzed using GraphPad InStat Software (GraphPad Software, San Diego, CA) and are reported as mean $\pm$ SEM. Analysis of variance was used for comparison among and between groups, followed by the Bonferroni's correction. A value of *p* less than or equal to 0.05 was considered statistically significant.

Animal Care

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication 85-23, revised 1985). The protocol was reviewed and approved by the Animal Care Committee at Children's Hospital in Boston.

Results

Left Ventricular Weight to Body Weight Ratio

Left ventricular weight (g) to body weight (kg) ratio at the time of study was 1.94 ± 0.3 g/kg in nonhypertrophied, age-matched controls, and 3.65 ± 0.5 g/kg in aortic banded animals (*p* < 0.01). None of the aortic banded animals had clinical evidence of heart failure.

Cardiac Function

There was no difference in preischemic values for heart rate, end-diastolic pressure, and developed pressure between the control and study groups (data not shown). Heart rate remained unchanged in all groups after ischemia. As indicated in Figure 1, hypertrophied hearts treated with Li or IMI (H + Li: 16 ± 0.7 and H + IMI: 16 ± 0.2 mm Hg) had lower diastolic pressure at end-reperfusion when compared with untreated hypertrophied hearts

(27 ± 0.4 mm Hg; $p < 0.001$). After 30 minutes of normothermic ischemia and 30 minutes of reperfusion, developed pressure in the treated hypertrophied hearts (H + Li: 91 ± 0.4 ; and H + IMI: 89 ± 1.2 mm Hg) and control hearts (91 ± 0.6 mm Hg) was greater than that in untreated hypertrophied hearts (72 ± 4.2 mm Hg; $p < 0.001$; see Fig 2).

Glycogen Synthase Kinase-3 Expression and Activity

There was no difference in GSK-3 β protein content between nonhypertrophied, age-matched control hearts and aortic banded hypertrophied hearts (Fig 3). As indicated in Figure 4, GSK-3 activity at end-ischemia, as determined in vitro using the experimental concentrations of both inhibitors (0.1 mM Li and 1 μ M IMI) showed significantly higher activity in untreated hypertrophied myocardial tissue compared with the Li and IMI treated tissue ($63,256 \pm 2,743$ counts per minute [CPM] versus $11,467 \pm 1,754$ and $7,029 \pm 1,545$ CPM, $p < 0.001$). Enzymatic activity measured directly in tissue samples collected at end-ischemia with or without Li or IMI as cardioplegic additives is shown in Figure 5. The Li and IMI treated hearts had significantly lower GSK-3 β activity compared with untreated hypertrophied hearts (H + Li $19,993 \pm 2,704$ CPM and H + IMI $33,081 \pm 3,497$ CPM versus hypertrophy $68,149 \pm 1,850$ CPM; $p < 0.001$).

Lactate Production During Ischemia

Lactate production/release was measured during the first 2 minutes of reperfusion. Lactate was found to be significantly higher in the hypertrophied hearts treated with Li and IMI in the cardioplegia solution compared with untreated hypertrophied hearts (9.5 ± 0.5 versus H + Li 18.8 ± 0.7 and H + IMI 21.9 ± 1.5 mg $\cdot$ mL $^{-1} \cdot$ min $^{-1}$ CF/g wet weight; $p < 0.001$; Fig 6).

Akt-1 Expression and Activity

There was no difference in the protein levels of Akt-1 in hypertrophied versus age-matched control hearts. A representative immunoblot is depicted in Figure 7A. The Akt-1 activity at end-ischemia, however, was significantly higher in nonhypertrophied control hearts compared with hypertrophied hearts. The band on the representative immunoblot indicates the substrate phosphorylated by activated Akt-1 (Fig 7B), and a summary of the densitometry data is shown in Figure 7C.

Comment

In this study, we found that GSK-3 β is activated during ischemia in pressure-overload hypertrophied myocardium compared with nonhypertrophied age-matched controls. In addition, we determined that activity of Akt-1, the enzyme phosphorylating/inactivating GSK-3 β , was decreased in hypertrophied myocardium during the ischemic period. Inhibition of GSK-3 β by lithium or the specific inhibitor IMI as cardioplegic additives results in significantly improved recovery of systolic and diastolic function after reperfusion concomitant with increased glycolysis during ischemia and early reperfusion, as indicated by increased lactate production.

Alterations in energy metabolism have been implicated as contributing to worse recovery of contractile function in hypertrophied myocardium after ischemia [5]. During ischemia, anaerobic glycolysis is the primary source for ATP production as lack of oxygen and accumulation of nicotinamide adenine dinucleotide phosphate in the mitochondria rapidly inhibit oxidative phosphorylation. Although the mechanisms responsible for the greater impairment of postischemic mechanical function in hypertrophied hearts remain unclear, we speculate that regulation of glucose transport plays an important role. The slower glucose uptake rate demonstrated in hypertrophied myocardium may in part explain the accelerated

loss of high-energy phosphates during ischemia and worse recovery of contractile function during reperfusion [6, 24]. As suggested by several investigators, rapid recovery of aerobic glycolysis is crucial during early reperfusion for recovery of contractile function after ischemia [25, 26]. Improved glycolysis is associated not only with delayed onset of contracture during ischemia but also preserves diastolic function during reperfusion as evidenced by lower diastolic pressures for a given balloon volume. This beneficial effect of glycolysis has been attributed to a preferential ability of glycolytically derived ATP to restore calcium homeostasis during early reperfusion [25]. In this study, one of the main effects of inhibiting GSK-3 β was the preservation of diastolic function after ischemia. This beneficial effect was likely due to improved anaerobic glycolysis during ischemia since treated hearts produced significantly more lactate during ischemia. In normal hearts, fatty acid oxidation is the predominant source for energy production in general and also during reperfusion [27]. In hypertrophied myocardium, fatty acid oxidation is impaired [2] and glucose metabolism takes over a more important role in energy metabolism. Glucose utilization in the myocardium is controlled by many factors including cardiac work, ischemia, hypoxia, and insulin [28]. It has been reported that hypertrophied myocardium suffers from relative insensitivity to insulin, which results in an impaired ability to increase glucose uptake in response to either insulin or ischemia [6, 7, 9, 29–31].

The activity of GSK-3 β can be modulated by several mechanisms, including mood-stabilizing drugs such as lithium [32]. Lithium has been shown to compete with magnesium for binding to GSK-3 β and thereby blocks enzyme activity, then, inorganic phosphate attaches to the enzyme, further inactivating it [11, 14, 15, 17–19, 32–34]. Chemical agents of the indirubin family can also inhibit GSK-3 β , such as indirubin-3'-monoxime, 5-iodo-(IMI), a specific inhibitor of the enzyme, which inhibits GSK-3 β by competing with ATP for binding to the catalytic site [22]. In preliminary studies perfusing hearts with different concentrations of Li (0.1 mmol/L to 3 mmol/L) or IMI (0.5 μ mol/L to 50 μ mol/L), we have found that the concentrations chosen in this study (0.1 mmol/L Li and 1 μ mol/L IMI) had no effect on contractile function when administered to nonischemic control and hypertrophied hearts (data not shown). In addition, dose-response experiments on ischemic hearts determined the final concentrations for Li and IMI using recovery of contractile function after ischemia at the lowest concentration as the endpoint. Several reports indicate that inhibition of GSK-3 β increases insulin action, resulting in augmented glucose uptake [35, 36]. If that is the case, then inhibiting GSK-3 β during ischemia should result in increased anaerobic glycolysis (presumably from exogenous glucose) and result in greater lactate production.

In this study, we found that there was significantly more lactate released in the coronary effluent immediately after the ischemic period when GSK inhibitors were given during ischemia. Thus, our findings are consistent with the hypothesis that GSK inhibition during ischemia promotes anaerobic glycolysis. Improved anaerobic glycolysis during ischemia resulted in preservation of diastolic function after ischemia. This beneficial effect suggests a potential regulatory role for GSK-3 β in the insulin-signaling pathway. Glycogen synthase kinase-3 β has been shown to phosphorylate insulin-receptor substrate-1 and thereby regulates insulin-mediated glucose uptake [36, 37]. Another potential mechanism for the improved postischemic function may be increased glycogen storage, as GSK-3 β inhibition also preserves glycogen synthase activity, which results in an increase in glycogen production and greater endogenous substrate (glucose) availability during reperfusion. The exact role of glucose derived from glycogen, however, is controversial since it has been proposed that exogenous glucose exerts a beneficial effect on the postischemic myocardium by providing high energy substrates for intracellular cation homeostasis, while ATP derived from glycogen stored in the myofibrils attenuates ischemic contracture [38].

Another regulatory mechanism of GSK-3 β activity involves phosphorylation of its specific serine residue (Ser9) by Akt-1 [19, 36, 39]. The Akt-1 can be activated in response to various growth factors and hormones, including insulin, insulin-like growth factor-1, vascular endothelial growth factor, and β -adrenergic stimulation. In the case of insulin, the insulin receptor tyrosine kinase phosphorylates and activates insulin-receptor substrate-1, which then activates phosphatidyl-inositol-3 kinase. Phosphatidyl-inositol-3 kinase activation generates phosphoinositide-3,4,5 triphosphate, which mediates the phosphorylation and activation of Akt-1. Once activated, Akt-1 phosphorylates various intracellular signaling intermediates which include GSK-3 β [40]. In this study, we found that Akt-1 activation is significantly decreased in hypertrophied ischemic myocardium. The beneficial effect of Akt-1 activation during ischemia has also been shown in other models. In studies of regional cardiac ischemia, activation of Akt-1 by overexpression reduced infarct size, prevented apoptotic cardiomyocyte death, and preserved cardiac function [41, 42]. In addition, intracellular acidosis inhibits the insulin-mediated signaling pathway, and an intracellular pH of 6.75 seems to be the threshold [43]. As we have previously reported, hypertrophied hearts develop pH levels below the threshold compared with nonhypertrophied control hearts [9].

In conclusion, we have found that Li and IMI, as cardioplegic additives in low concentrations, had protective effects on hypertrophied myocardium subjected to global ischemia and reperfusion. We speculate that the improved recovery of function after ischemia is a result of an increase in insulin-mediated glucose uptake, by directly affecting the intracellular signaling pathway (insulin-receptor substrate-1) and potentially also activation of glycogen synthase, preventing glycogen breakdown during ischemia. Even though one of the limitations of our study is the use of a crystalloid perfused, isolated heart model, it is feasible to speculate on clinical applications of this treatment. Inhibition of GSK-3 β by low-dose Li provides a safe and effective as well as practical strategy to improve protection for the hypertrophied heart during open heart surgery.

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Fig 1.

End-diastolic pressure measured before ischemia and 30 minutes after reperfusion (expressed in mm Hg) with and without inhibitors (lithium [Li] or indirubin-3'-monoxime, 5-iodo- [IMI]) as cardioplegic additives. * $p < 0.001$ versus preischemia; # $p < 0.001$ versus hypertrophy (Hyp). (H + Li = hypertrophy plus Li; H + IMI = hypertrophy plus IMI.)

Fig 2.

Developed pressure before (black bars) and after (white bars) ischemia in untreated hypertrophied hearts and treated hypertrophied hearts. * $p < 0.001$ versus hypertrophy before ischemia; # $p < 0.001$ versus hypertrophy after ischemia. (H + Li = hypertrophy plus Li; H + IMI = hypertrophy plus IMI; IMI = indirubin-3'-monoxime,5-iodo-; Li = lithium.)

Fig 3.

A representative Western immunoblot of glycogen synthase kinase-3 β (GSK-3 β) expression in normal control hearts and hypertrophied hearts. There was no significant difference in protein levels in hypertrophied versus normal age-matched control hearts. (Hyp = hypertrophy.)

Fig 4.

In vitro measurement of glycogen synthase kinase-3 (GSK-3) activity. The GSK-3 activity (expressed as counts per minute [CPM] of protein content) was determined using the experimental concentrations of both inhibitors (0.1 mM lithium [Li], and 1 μ M indirubin-3'-monoxime,5-iodo-[IMI]) in untreated hypertrophied perfused hearts. * $p < 0.001$ versus untreated hypertrophy. (Hyp + Li = untreated hypertrophy plus Li; Hyp + IMI = untreated hypertrophy plus IMI.)

Fig 5.

In vivo measurements of glycogen synthase kinase-3 (GSK-3) activity. The GSK-3 activity (expressed as counts per minute [CPM] of protein content) was measured in tissue collected at end-ischemia in untreated and treated hypertrophied hearts. * $p < 0.001$ versus hypertrophy. (H + Li = hypertrophy plus Li; H + IMI = hypertrophy plus IMI; IMI = indirubin-3'-monoxime,5-iodo-; Li = lithium.)

Fig 6. Lactate production (coronary effluent during first 2 minutes of reperfusion) during ischemia in hypertrophied hearts receiving cardioplegic solution with and without lithium (Li) or indirubin-3'-monoxime, 5-iodo- (IMI). * $p < 0.001$ versus hypertrophy. (H + Li = hypertrophy plus Li; H + IMI = hypertrophy plus IMI.)

Fig 7.

(A) A representative immunoblot indicates that there is no difference in Akt-1 expression levels between hypertrophied hearts and age-matched control hearts. (B) The Akt-1 activity indicated by detection of degree of phosphorylation of a substrate. Glycogen synthase kinase-3 (GSK-3) fusion protein was used as substrate. The phosphorylated product is detected by immunoblotting at a molecular weight of 32 kDa. (C) The summary of densitometry data indicates that Akt-1 activity is decreased in hypertrophied hearts during ischemia. * $p < 0.05$ versus hypertrophy. (Black bar = control; white bar = hypertrophy.)