Impaired Glucose Transporter Activity in Pressure-Overload Hypertrophy Is an Early Indicator of Progression to Failure

Ingeborg Friehs, MD; Adrian M. Moran, MD; Christof Stamm, MD; Steven D. Colan, MD; Koh Takeuchi, MD; Hung Cao-Danh, PhD; Christine M. Rader, BA; Francis X. McGowan, MD; Pedro J. del Nido, MD

Background—Severe hypertrophy and heart failure are important risk factors in cardiac surgery. Early adaptive changes in hypertrophy include increased ventricular mass-to-cavity volume ratio (M/V ratio) and increased dependence on glucose for energy metabolism. However, glucose uptake is decreased in the late stages of hypertrophy when ventricular dilatation and failure are present. We hypothesized that impaired glucose uptake would be evident early in the progression of hypertrophy and associated with the onset of ventricular dilatation.

Methods and Results—Ten-day-old rabbits underwent banding of the descending aorta. Development of hypertrophy was followed by transthoracic echocardiography to measure left ventricular M/V ratio. Glucose uptake rate, as determined by $^{31}$P-nuclear magnetic resonance spectroscopy measuring 2-deoxyglucose conversion to 2-deoxyglucose-6-phosphate, was measured in isolated perfused hearts obtained from banded rabbits when M/V ratio had increased by 15% from baseline (compensated hypertrophy) and by 30% from baseline (early-decompensated hypertrophy). In age-matched control animals, the rate of glucose uptake was $0.61 \pm 0.08 \, \text{mmol} \cdot \text{g of wet weight}^{-1} \cdot \text{30 min}^{-1}$ (mean±SEM). With a 15% M/V ratio increase, glucose uptake rate remained at control levels (0.6 $\pm 0.05 \, \text{mmol} \cdot \text{g of wet weight}^{-1} \cdot \text{30 min}^{-1}$), compared with hearts with 30% increased M/V ratios, where glucose uptake was significantly lower (0.42 $\pm 0.05 \, \text{mmol} \cdot \text{g of wet weight}^{-1} \cdot \text{30 min}^{-1}$; $P<0.05$). Glucose transporter protein expression was the same in all groups.

Conclusions—Glucose uptake rate is maintained during compensated hypertrophy. However, coinciding with severe hypertrophy, preceding ventricular dilatation, and glucose transporter protein downregulation, glucose uptake is significantly decreased. Because of the increased dependence of the hypertrophied hearts on glucose use, we speculate that this impairment may be a contributing factor in the progression to failure. (Circulation. 1999;100[suppl II]:II-187–II-193.)

Key Words: hypertrophy ■ echocardiography ■ glucose ■ metabolism

One of the earliest events during adaptive remodeling in pressure-overload hypertrophy is an increase in ventricular muscle mass; this is considered an initial compensatory response to maintain normal wall stress. If pressure overload persists, progressive ventricular dilatation ensues, which then leads to increased wall stress, afterload mismatch, and decreased cardiac output. Several adaptive mechanisms occur during the development of hypertrophy; these include the multiplication of sarcomeres, a switch to immature isoforms of contractile proteins, and greater dependence on transsarcolemmal calcium influx for excitation-contraction coupling. In addition, an increase occurs in glucose use as a substrate for energy production. The transition from compensated to decompensated hypertrophy with ventricular dysfunction is poorly understood.

In mammalian cells, glucose is not freely permeable across the lipid bilayer; it enters the cells by facilitated diffusion. Specific membrane proteins that passively transport glucose down a concentration gradient achieve this process. In cardiac myocytes, 2 types of glucose transporters have been described (GLUT-1 and GLUT-4); they are responsible for most of the uptake of glucose under basal conditions and in response to insulin stimulation, respectively. We previously showed that the glucose uptake rate is impaired in hearts with decompensated pressure-overload hypertrophy when ventricular dilatation and contractile dysfunction have occurred in response to thoracic aortic banding.
paired glucose transport occurs early in the progression to ventricular dilatation. In a rabbit model of thoracic aortic banding, we monitored the progression of hypertrophy using transthoracic echocardiography. In previous work with this model, we observed that after aortic banding, an initial increase occurs in the left ventricular (LV) mass to LV volume ratio (M/V ratio), which reaches a plateau ≈30% above prebanding levels. At this point, the rate of LV cavity volume increases faster than the rate of LV mass production, resulting in a fall in M/V ratio. This progression mimics the progression seen clinically with pressure overload. To determine the relationship between glucose uptake and progression of hypertrophy, we measured the glucose uptake rate and glucose transporter expression in control and aortic-banded rabbits during the early rise and the plateau phase of LV M/V ratio after aortic banding.

**Methods**

Pressure-overload hypertrophy was achieved by placing a 2-0 silk suture around the descending aorta just distal to the ligamentum arteriosum in 10-day-old New Zealand White rabbits, as previously described. Implanting a fixed constriction in an immature animal and allowing it to grow induced pressure-overload hypertrophy by 2 to 3 weeks of age in this model. The progression of LV hypertrophy was determined by transthoracic echocardiography ( Hewlett-Packard Sonos 1500 Cardiac Imager) with a 7.5-MHz transducer. During these procedures, the animals remained unsedated to avoid the influences of anesthetics on the results.

**Echocardiography**

Two-dimensional cross-sectional images and M-modes of the left ventricle were obtained by echocardiography. Simultaneous measurements of ECG and LV short-axis dimensions by M-mode were recorded on hard copy at a paper speed of 100 mm/s. These examinations were started at 3 weeks of age and performed at 1-week intervals. LV epicardial and endocardial surfaces were traced by computer-aided offline analysis on a bit-mapped digitizing tablet. End-diastolic (maximum dimension) and end-systolic (minimum dimension) LV wall thicknesses were determined using previously described methods. The M/V ratio was calculated, and the serial evaluation of M/V ratio was expressed as percent change from baseline.

**2-Deoxyglucose Uptake**

Glucose uptake rate was determined using 31P-nuclear magnetic resonance (NMR) spectroscopy. Spectra were acquired on an 8.45 Tesla vertical Bruker spectrometer operating at a proton frequency of 145 MHz. The isolated heart in its perfusion chamber was positioned within a 20-mm solenoid radiofrequency coil. During the stabilization period of 30 minutes, the magnetic field was optimized by shimming on the free-induction decay of the proton signal from the heart and the surrounding perfusate. Spectra were obtained by signal-averaging 120 scans with a 2-s delay. Each spectrum took 4 minutes. Spectral peak areas were quantified by integration after baseline correction with software provided by Bruker. The determined areas were normalized to an external standard (using 500 μmol/L of dimethylene phosphonic acid) contained in a balloon adjacent to the heart.

The animals were euthanized by intravenous injection of an overdose of ketamine (50 to 100 mg/kg); heparin (500 U/kg) was also given intravenously. The hearts were rapidly excised and placed in cold Krebs-Henseleit (KH) solution. After aortic cannulation, the hearts were perfused in the nonworking, nonrecirculating Langendorff mode at a perfusion pressure of 80 mm Hg with oxygenated KH solution containing (in mmol/L): NaCl 117, KCl 4.7, MgSO4 1.2, CaCl2 1.8, NaHCO3 23.7, and glucose 10 (37°C, pH 7.4). After a 30-minute stabilization period, the perfusate was switched to a modified KH solution containing 10 I/U/L bovine insulin, a reduced glucose content (1 mmol/L), and a concentration of 3 mmol/L 2-deoxyglucose (2-DG; Sigma). 2-DG acts as a competitive inhibitor of glucose, and it is transported into the cell via the same mechanism (facilitative diffusion). Phosphorylation of 2-DG by hexokinase to 2-deoxyglucose-6-phosphate (2-DG-6-P) results in a distinct resonance peak. The rate of rise of this peak is proportional to glucose uptake in the cell and serves to quantify the rate of glucose uptake. The rate of degradation of 2-DG-6-P was determined in a separate group of hearts by perfusion with a KH buffer with 0.03 U of glucose-6-phosphate dehydrogenase (Sigma). Control assays were performed on a buffer solution in which glucose or homogenate was omitted. The rate of reduction of NADP+ with glucose-6-phosphate dehydrogenase was measured spectrophotometrically at a wavelength of 340 nm. One milliunit of hexokinase activity that forms in 1 nmol of glucose-6-phosphate in 1 minute.

**Hexokinase Activity**

Hypertrophied and control hearts from separate groups of animals were rapidly excised and afterward perfused via KH containing (in mmol/L): TrisHCl 20, KCl 900, MgCl2 10, EDTA 2, and glucose 10 and 0.5% Triton (Sigma). The assay was performed on the homogenate in a buffer containing (in mmol/L): TrisHCl 40, KCl 100, MgCl2 20, EDTA 4, ATP 2, NADP+ 0.25, and glucose 10 and 0.03 U of glucose-6-phosphate dehydrogenase (Sigma). Control assays were performed on a buffer solution in which glucose or homogenate was omitted. The rate of reduction of NADP+ with glucose-6-phosphate dehydrogenase was measured spectrophotometrically at a wavelength of 340 nm. One milliunit of hexokinase represents the amount of enzyme activity that forms in 1 nmol of glucose-6-phosphate in 1 minute.

**Western Immunoblotting**

Ventricular tissue from a separate set of hypertrophied and age-matched littermates (control animals) was homogenized in ice-cold buffer containing (in mmol/L): TrisHCl 20, EDTA 2, EGTA 0.5, and PMSF 1, and 25 μg/L leupeptin and 0.03 mol/L sucrose (pH 7.4). It was then centrifuged at 1000g for 20 minutes. The crude supernatant fraction was stored at −80°C for later analysis. Gel electrophoresis with 10% SDS-PAGE gels was performed on samples of 25 μg of protein from total homogenates in accordance with the method of Laemml. Proteins were then electrophoretically transferred to nitrocellulose membranes. After transfer, the membranes were incubated in 5% nonfat dry milk in 10 mmol/L TrisHCl (pH 7.5), 100 mmol/L NaCl, and 0.1% Tween 20 for 1 hour at room temperature to block unspecific binding sites; they were then incubated with antibodies. GLUT-1 and GLUT-4 (Genzyme Diagnostics) were used as primary antibodies at a dilution of 1:1000; samples were then incubated with horseradish peroxidase-conjugated secondary antibody (Jackson Immuno Research Labs) at a dilution of 1:10000. The bound antibody was detected by the enhanced chemiluminescence method according to the manufacturer’s instructions (Amersham Life Science). This method depends on the production of light after
the oxidation of luminol by horseradish peroxidase in the presence of 
H₂O₂. After exposure on films, quantitative protein analysis was 
undertaken by laser densitometry.

**Statistical Analysis**

Data are expressed as mean±SEM and were analyzed using Sig- 
maStat software (Jandel Scientific). Comparisons between groups 
were made with 1-way ANOVA. Probability values were corrected 
by Bonferroni’s post hoc correction. If normality and equal variance 
testing were passed, a standard $t$ test was used. For all these tests,
$P≤0.05$ 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 prepared by the National Academy of Sciences and pub-
lished by the National Institutes of Health (NIH Publication No. 86 
to 23, revised 1985). The protocol was reviewed and approved by 
the animal care committee at Children’s Hospital in Boston.

**Results**

Weekly measurements of LV M/V ratios demonstrated that 
after aortic banding, an initial rapid increase of LV wall 
thickness occurred, resulting in a rise in LV M/V ratio. This 
reached a plateau $≈30\%$ above prebanding levels by 3 to 4 
weeks of age. We termed the phase leading up to this plateau 
“compensated hypertrophy.” By 4 to 5 weeks of age, a 
gradual decline in M/V ratio occurred due to a more rapid 
increase in LV cavity volume relative to LV mass, indicative 
of LV dilatation (decompensated phase). Figure 1 shows 
representative M-mode echocardiographic images of a heart 
with compensated hypertrophy and an age-matched control 
heart.

On the basis of the echocardiographic findings, animals 
with a $≈15\%$ increase in M/V ratio from baseline (4 weeks of 
age, compensated hypertrophy) and with a $≈30\%$ rise in M/V 
ratio (5 weeks of age, early-decompensated phase) were 
studied and compared with age-matched control animals. 
Paralleling these echocardiographic findings, the banded 
animals showed a significant degree of hypertrophy, as 
estimated by LV weight to body weight ratios, which were 
3.4±0.4 (compensated phase) and 3.3±0.4 (early-decompensated phase) in the hypertrophied groups compared 
with 2.3±0.2 in controls. Body weights were not different 
between the groups, and LV weight was 3.08±0.26 g in 
hearts with compensated hypertrophy, 2.85±0.06 g in hearts 
with early-decompensated hypertrophy, and 2.16±0.29 g in 
control hearts ($P≤0.05$).

$^{31}$P-NMR spectra before and after a 30-minute infusion of 
2-DG are shown in Figures 2A and 2B. Figure 2C depicts a 
representative curve of the 2-DG-6-P peak during 2-DG 
infusion and wash-out, indicating that 2-DG-6-P is not further 
metabolized during the observation period, even after subse-
quent wash-out with glucose-containing buffer.

Figure 3A shows the rate of 2-DG-6-P accumulation over 
a period of 30 minutes in the aortic-banded groups and 
control animals. 2-DG accumulation was significantly lower 
in hearts with early-decompensated hypertrophy ($≈30\%$ in-
crease of M/V ratio) than in control hearts ($P≤0.05$), with a 
slower rate of rise and lower total accumulation after 30 
minutes. Figure 3B shows the rate of glucose uptake mea-
sured by cumulative tritiated water production from 
[2-3H]glucose over a period of 30 minutes. In aortic-banded 
animals at a $≈30\%$ rise of M/V ratio, the glucose-uptake rate 
was significantly lower compared with age-matched control 
animals ($P≤0.05$).

In a separate group of hearts ($n=4$ per group), myocardial 
protein was obtained from the left ventricle of rabbits with 
compensated, early-decompensated, and late-decompensated 
hypertrophy (6 weeks of age, M/V ratio beginning to fall). 
The GLUT-1 and GLUT-4 protein content from the myocar-
dium of aortic-banded groups and age-matched littermates, as 
determined by immunoblotting, was not significantly differ-
ent between any of the groups. A representative immunoblot 
of GLUT-4 is depicted in Figure 4A, and a summary of the 
densitometry results is shown in Figure 4B.

In a separate group of hearts perfused with KH solution 
containing insulin and glucose, total hexokinase activity was 
the same in hypertrophied and control hearts at a M/V ratio 
increase of $≈30\%$ (38.2±0.47 mU/mg protein compared with 
42.8±1.62 mU/mg protein, respectively).
Discussion

In this study, echocardiography was used to monitor the progression of LV hypertrophy and to determine the timing for the study of glucose uptake and glucose transporter protein content. The observations derived from this investigation reveal that a decrease in glucose uptake rate is evident early in the transition from compensated to decompensated pressure-overload hypertrophy, as determined by an index of LV M/V ratio. The defect in insulin-stimulated glucose uptake precedes the downregulation of sarcoplasmic reticulum Ca-ATPase (SERCA-2) and glucose transporter expression (GLUT-4 and GLUT-1).15,26

Under pathophysiological conditions such as hypertrophy or during ischemia and early reperfusion, a high rate of cardiac glucose metabolism may be crucial.27–29 Glucose transport is thought to be rate-limiting for glucose use. In the heart, 2 distinctive glucose transporters are responsible for glucose uptake across the plasma membrane. The GLUT-1 transporter, which is present in low levels in most tissues, is non-insulin–stimulated and is responsible for basal glucose
GLUT-4 activity is regulated by insulin and is expressed in tissues in which glucose transport needs to be rapidly and markedly enhanced (adipocytes, skeletal and cardiac muscle). In the basal state, GLUT-1 is evenly distributed between the plasma membrane and low-density microsomal pools, whereas GLUT-4 is almost entirely stored in an intracellular pool. Insulin interacts with its receptor on the plasma membrane and stimulates the redistribution of GLUT-4 to the sarcolemma. When insulin levels fall, GLUT-4 is resequestered in intracellular vesicles. A decrease in GLUT-4 mRNA and protein is thought to be a mechanism for insulin resistance in various models of diabetes, and it has been associated with lower myocardial glucose uptake.

In the present study, we did not find altered GLUT-4 protein expression, as determined by immunoblotting of LV myocardial tissue, which indicates that insulin signal transduction and/or glucose transporter activity is altered in hypertrophied hearts.

In the present study, we used the distinct \(^{31}\)P-NMR peak of 2-DG-6-P to investigate the glucose transport capacity of hypertrophied hearts. 2-Deoxy-D-glucose, a competitive inhibitor of D-glucose, is transported into the cell via the same mechanism as glucose, is phosphorylated by hexokinase, and accumulates as 2-DG-6-P, which is metabolized very slowly. The rate of 2-DG-6-P accumulation, therefore, reflects both transport across the plasma membrane and phosphorylation of glucose, and it is a useful tracer of glucose transport and phosphorylation in the isolated perfused heart. To confirm our findings with 2-DG uptake in hypertrophied hearts, we used a radioactive tracer technique; the same results were obtained.

In some models, 2-DG-6-P is further metabolized by incorporation to glycogen at low rates. The rate of 2-DG-6-P degradation is slow with respect to the rate of uptake; degradation rates ranging from 70 to 110 minutes have been previously reported. In the present study, 2-DG uptake
reached a plateau after 30 minutes of perfusion, and little, if any, 2-DG-6-P was further metabolized during a wash-out period. Similar findings have been reported when hypertrophied and normal hearts were compared in a spontaneously hypertensive rat heart model.43 Therefore, we think that the use of 2-DG is a reliable method of glucose uptake determination in our model.

In the current study, in hearts with compensated hypertrophy (≤15% rise in M/V ratio), glucose uptake in response to insulin was still maintained at near-normal levels; impaired glucose uptake was detected before progression from compensated to decompensated hypertrophy at the peak M/V ratio (≤30% above baseline). It is conceivable that the impaired response of GLUT-4 to insulin stimulation (eg, defects in insulin receptor signaling or alterations in GLUT-4-containing vesicle trafficking) could contribute to the development of decompensated cardiac hypertrophy. Similar defects in the insulin-stimulation of glucose use and transport in the heart have been demonstrated in animal models of diabetes and insulin resistance.37,44–46 In the absence of insulin, glucose transport is rate-limiting for glycolysis, as reported for isolated rat cardiomyocytes.47 Therefore, an important relationship may exist between the progression of hypertrophy to failure and decreased glucose uptake because a diminished rate of cardiac glucose use is thought to be detrimental to heart function.48 We previously showed that severe hypertrophy with impaired glucose transport has functional significance with regard to impaired recovery after experimental myocardial ischemia. Normalizing glucose transport with vanadate significantly improved postischemic recovery in the hypertrophied heart.15

Our data indicate that a decline of glucose uptake rate occurs in the hypertrophied heart and, concomitantly with the lack of glucose available for the myocardium, hypertrophy progresses to myocardial failure. Insulin insensitivity of the GLUT-4 transport system, a potential underlying mechanism, may play a significant role. Because GLUT-4 protein levels remain unchanged at this stage, we speculate that impaired insulin signaling is responsible for this defect. Strategies to overcome this defect by either direct bypass of insulin-dependent GLUT-4 activation or augmenting glucose transport to the myocytes by other means may improve myocardial function and prevent the progression of hypertrophied hearts to failure.

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