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Transmural Distribution of Metabolic Abnormalities and Glycolytic Activity during Dobutamine Induced Demand Ischemia

Mohammad N Jameel, Xiaohong Wang, Marcel H.J. Eijgelshoven, Abdul Mansoor, and Jianyi Zhang

Cardiovascular Division, Departments of Medicine, University of Minnesota Medical School. Minneapolis, Minnesota 55455

Abstract

The heterogeneity across the LV wall is characterized by higher rates of oxygen consumption, systolic thickening fraction, myocardial perfusion and lower energetic state in the subendocardial layers (ENDO). During dobutamine stimulation induced demand ischemia, the transmural distribution of energy demand and metabolic markers of ischemia are not known. In this study, hemodynamics, transmural high energy phosphate (HEP) and 2-deoxyglucose-6-phosphate (2DGP) levels and myocardial blood flow (MBF) were determined under basal conditions (B), during dobutamine infusion (DOB: 20 $\mu\text{g/kg/min}$ iv.), and during coronary stenosis+DOB+2-deoxy-glucose (2DG) infusion. DOB increased rate pressure products (RPP) and MBF significantly without affecting subendocardial to subepicardial blood flow ratio (ENDO/EPI) or HEP levels. During coronary stenosis+DOB+2-deoxy-glucose (2DG) infusion RPP, ischemic zone (IZ) MBF and ENDO/EPI decreased significantly. IZ PCr/ATP decreased significantly (2.30 $\pm$ 0.14, 2.06 $\pm$ 0.13 and 2.04 $\pm$ 0.11 to 1.77 $\pm$ 0.12, 1.70 $\pm$ 0.11 and 1.72 $\pm$ 0.12; EPI, MID and ENDO, respectively) and 2DG6P accumulated in all layers as evidenced by the 2DG6P/PCr (0.55 $\pm$ 0.12, 0.52 $\pm$ 0.10 and 0.37 $\pm$ 0.08; EPI, MID and ENDO respectively; $p < 0.05$, EPI $>$ ENDO). In the IZ the wet weight/dry weight ratio was significantly increased as compared to the normal zone (5.9 $\pm$ 0.5 vs. 4.4 $\pm$ 0.4; $p < 0.05$). Thus, in stenotic perfused bed, during dobutamine induced high cardiac workstate, despite higher blood flow the subepicardial layers showed the greater metabolic changes that characterized by a shift toward higher carbohydrate metabolism suggesting a homeostatic responses to high cardiac workstate is characterized by more glucose utilization in energy metabolism.

Keywords

Ischemia; energetics; glycolytic metabolism; left ventricle

INTRODUCTION

It is well-established that myocardial ischemia caused by a decrease in coronary flow secondary to a coronary stenosis (*supply ischemia*) is most severe in the inner layers of the LV wall (21, 37), and metabolic changes could be disassociated from transmural blood perfusion pattern (10). In contrast, it was shown that in normal hearts (without coronary artery stenosis) during catecholamine induced maximal workstate myocardial high energy phosphate (HEP) levels decreased and inorganic phosphate levels increased (9,17,34,36). This phenomenon was named *demand ischemia*. During demand ischemia the metabolic ischemic markers were either transmurally uniform or more pronounced in the outer layers, despite higher blood flow in that

region (34). This indicates that in normal hearts, during catecholamine induced high workstates the subepicardial layer of the LV wall has the highest level of energy expenditure. Catecholamine stimulation in the presence of coronary stenosis (*demand induced ischemia in the setting of coronary stenosis*) also produced similar transmural patterns of metabolic markers with the subepicardial layer showing reduction in energetic states (3). These unexpected experimental observations lead to the postulation that transmural patterns of myocardial ischemia are significantly different between supply ischemia and demand ischemia.

Under normal conditions majority of the total cellular ATP is derived from the oxidation of fatty acids and pyruvate with only a small portion coming from glycolysis (11,13,18,28). Even during supply ischemia caused by a reduction of coronary blood flow by 30-60%, fatty acid oxidation continues to provide the majority (60-80%) of the ATP formed in the heart (11,13,18,28). However, in supply ischemia there is an increase in glucose extraction that maintains glucose uptake despite a decrease in myocardial blood flow (29). This is accompanied by an increase in net tissue glycogen breakdown and lactate accumulation suggesting an increase in non-oxidative glycolysis under ischemic conditions (29). It is important to note that the accumulation of lactate during demand ischemia, could be the limitation in glycolytic reducing equivalents transfer to the mitochondria. The reducing equivalents that would otherwise be shuttled into mitochondria for oxidative ATP synthesis, remain in the cytosol because the malate aspartate shuttle exchange becomes the limiting factor for glucose oxidation due the very high flux rates through the mitochondrial dehydrogenases (20). A similar increase in non-oxidative glycolysis is seen in demand induced ischemia in the presence of coronary stenosis (2). Under basal conditions, cardiomyocyte contractile shortening uses about 60-80% of the ATP produced in the heart and the rest is used to support ion pumps, especially the sarcoplasmic reticulum Ca^{2+} -ATPase pump and the Na^{+} - K^{+} exchanger (2,30). It has been shown that the glycolytically produced ATP is preferentially used to support cellular ion homeostasis (14, 27). However, the function of the glycolytic ATP production may be different in different forms of ischemia: maintaining cellular ion homeostasis during supply ischemia versus maintaining contractile performance during demand ischemia. It is not known whether the myocardium perfused by a stenotic coronary artery in the setting of suppressed glycolysis, can maintain its ion hemostasis after catecholamine stimulation. We hypothesized that the transmural pattern of demand ischemia is different from supply ischemia, and inhibition of glucose uptake can cause both metabolic changes and myocardial edema in the ischemic region during catecholamine stimulation. Spatially localized ^{31}P NMR spectroscopy was used to evaluate the transmural distributions of the high energy phosphate (HEP), inorganic phosphate (Pi) and 2-deoxyglucose-6-phosphate (2DG6P) levels, which permitted transmurally differentiated evaluation of distributions of energy demand under these experimental conditions.

METHODS

Surgical preparation

Ten adult mongrel dogs weighing 18 - 23 kg were anesthetized with sodium pentobarbital (30 mg/kg and 4 mg/kg/hr, IV.). The animals were intubated and ventilated with a respirator using room air supplemented with oxygen. A heparin filled polyvinyl chloride catheter (3.0 mm o.d.) was inserted into the left femoral artery and advanced into the ascending aorta. A left thoracotomy was performed through the fourth intercostal space. The pericardium was opened and the heart was suspended in a pericardial cradle. Heparin filled catheters were inserted into the left ventricle through the apical dimple and into the left atrium through the atrial appendage and secured with purse string sutures. A 1.5 - 2.0 cm segment of the proximal left anterior descending coronary artery (LAD) was dissected free, and a hydraulic occluder constructed of polyvinyl chloride tubing (2.7 mm o.d.) was placed around the artery, proximal to the first major arterial branch. A silicone elastomer catheter (0.75 mm i.d.) was placed into the LAD

distal to the occluder by the method of Gwartz (4). A Doppler flow probe was placed onto the LAD proximal to the occluder.

The region of the left ventricle which became cyanotic upon inflation of the arterial occluder was determined by visual inspection and a 28 mm diameter NMR surface coil was sutured onto the pericardium overlying the ischemic area. The pericardial cradle was then released and the heart was allowed to assume its normal position. The surface coil leads were connected to a balanced, tuning circuit and the animals were placed within the magnet.

All experimental procedures followed were in accordance with the animal use guidelines of the University of Minnesota and the experimental protocol was approved by the University of Minnesota Animal Resources Committee. The investigation conformed to the *Guide for the care and use of laboratory animals* published by the US National Institutes of Health (NIH publication No 85-23, revised 1985).

NMR methods

Measurements were performed in a 40 cm bore 4.7 T magnet interfaced with a SISCO (Spectroscopy Imaging Systems Corporation, Fremont, CA) console. The left ventricular pressure signal was used to gate NMR data acquisition to the cardiac cycle while respiratory gating was achieved by triggering the ventilator to the cardiac cycle between data acquisitions (6,12,25,35). ^{31}P and ^1H NMR frequencies were 81 and 200 MHz, respectively. Spectra were recorded in late diastole with a pulse repetition time of 6-7 seconds. This repetition time allowed full relaxation for ATP and inorganic phosphate (Pi) resonances, and approximately 90% relaxation for the Creatine phosphate (PCr) resonance. The correction factor for PCr was determined for each heart from two spectra recorded consecutively without transmural differentiation, one with 15 second repetition time to allow full relaxation and the other with the 6-7 second repetition time used in all the other measurements. PCr resonance intensities were then corrected for this minor saturation.

Radiofrequency transmission and signal detection were performed with a 28 mm diameter surface coil. The coil was cemented to a sheet of silicone rubber 0.7 mm in thickness and approximately 20% larger in diameter than the coil itself. A capillary containing 15 μl of 3 M/L phosphonoacetic acid was placed at the coil center to serve as a reference. The proton signal from water detected with the surface coil was used to homogenize the magnetic field and to adjust the position of the animal in the magnet so that the coil was at or near the magnetic isocenter. This was accomplished using a spin-echo experiment and a readout gradient. The information gathered in this step was also utilized to determine the spatial coordinates for spectroscopic localization (6,12). Chemical shifts were measured relative to PCr, which was assigned a chemical shift of -2.55 ppm relative to 85% phosphoric acid at 0 ppm.

Spatial localization across the left ventricular wall was performed with the RAPP-ISIS/FSW method (6,24,35). Detailed data with regard to voxel profiles, voxel volume and extensive documentation of the accuracy of the spatial localization obtained in phantom studies and *in vivo* have been published elsewhere (6,24,25). Briefly, signal origin was restricted using B_0 gradients and adiabatic inversion pulses to a column coaxial with the surface coil and perpendicular to the left ventricular wall. The column dimensions were 17 mm $\times$ 17 mm. Within this column, the signal was further localized using the B_1 gradient to 5 voxels centered about 45°, 60°, 90°, 120° and 135° spin rotation increments (6,24,25,35). FSW localization utilized a 9-term Fourier series expansion. The Fourier coefficients, number of free induction decays acquired for each term in the Fourier expansion and the multiplication factors employed to construct the voxels are reported previously (5,6,25,35). The position of the voxels relative to the coil was set using the B_1 magnitude at the coil center which was experimentally determined in each case by measuring the 90° pulse length for the phosphonoacetic acid reference located

in the coil center. Each set of spatially localized transmural spectra were acquired in 10 minutes. A total of 96 scans were accumulated within each 10 minute block.

Resonance intensities were quantified using integration routines provided by the SISCO software. ATP γ resonance was used for ATP determination. Since data were acquired with the transmitter frequency positioned between the ATP γ and PCR resonance, off resonance effects on these peaks were virtually non-existent. However, ATP β suffers from off-resonance phenomenon, and this effect varies with proximity to the surface coil due to the inhomogeneity of the magnetic field generated by the surface coil (6,12). Consequently, ATP β is not suitable for evaluating the relative ATP contents or the PCr/ATP ratio. The numerical values for PCR, ATP and 2DG6P in each voxel were expressed as ratios of PCr/ATP and 2DGP/PCr. Inorganic phosphate (P_i) levels were measured as changes from baseline values, using integrals obtained in the region covering the P_i resonance. The change of P_i level was expressed as ΔP_i /PCr ratio. Data are reported for the subepicardial (EPI), mid-myocardial (MID) and subendocardial (ENDO) voxels.

Hemodynamic measurements

Aortic, LV and mean distal LAD pressures were measured using Spectromed TNF-R pressure transducers positioned at mid-chest level. All data were recorded on an 8-channel Coulbourn R14-28 direct writing recorder.

Myocardial blood flow measurements

Transmural myocardial blood flow was measured using radionuclide labeled microspheres, 15 μ m in diameter, suspended in low molecular weight dextran. Microspheres labeled with 4 different radioisotopes (^{51}Cr , ^{85}Sr , ^{95}Nb and, ^{46}Sc), were agitated in an ultrasonic mixer for 10 minutes prior to injection. The microsphere suspension containing 2×10^6 microspheres was injected through the left atrial catheter and flushed with 10 ml of normal saline. A reference sample of arterial blood was drawn from the aortic catheter at a rate of 15 ml/min beginning 5 sec before microsphere injection and continuing for 120 sec. Radioactivity in the myocardial and blood reference specimens was determined using a gamma spectrometer with multi-channel analyzer (model 5912, Packard Instrument Company, Downers Grove, IL) at window settings chosen for the combination of radioisotopes used during the study. Activity in each energy window, background activity and sample weight were entered into a digital computer programmed to correct for overlap between isotopes and for background activity, and to compute the corrected counts/minute/gram of myocardium. Knowing the rate of withdrawal of the reference blood specimen (Q_r) and the radioactivity of the reference specimen (Cr), myocardial radioactivity (C_m) was used to compute myocardial blood flow (Q_m) as: $Q_m = Q_r (C_m/Cr)$. Blood flow was expressed as ml/g of myocardium/minute.

Oxygen consumption measurement

Blood specimens were withdrawn anaerobically into iced syringes from the aortic and coronary sinus catheters (34). pO_2 and pCO_2 were measured with a blood gas analyzer (model 1304, Instrumentation Laboratory, Lexington, MA) calibrated with known gas mixtures. Hemoglobin content was determined by the cyanmethemoglobin method. Blood oxygen content was calculated as $\text{hemoglobin} \times 1.34 \times O_2 \text{ saturation} + (0.0031 \times pO_2)$, using the oxygen dissociation curve for dog blood. Myocardial oxygen consumption was then computed as the arterial-venous oxygen content difference multiplied by the myocardial blood flow.

Myocardial Specimen Weight Measurements

Tissue pieces were de-blotted and weighed when obtained (before they dehydrate at all). This was termed the wet weight. 5 grams of myocardial tissue was placed overnight in the oven at

100 degrees C, that caused it to fully dehydrate. Dry weight was measured immediately after removal from the oven in order to prevent rehydration.

Study protocol

Ventilation rate, volume and inspired oxygen content were adjusted (on the basis of arterial blood gas and pH measurements) as required to maintain physiologic values. Aortic, LV and mean LAD (distal to occluder) pressures were monitored continuously throughout the study. During each intervention myocardial contractile function, blood flow and hemodynamic measurements were collected simultaneously with the acquisition of transmural ^{31}P NMR spectra.

Following control observations, animals received Dobutamine 20 $\mu\text{g/kg/min}$ iv. After waiting for about 5 minutes to allow systemic hemodynamics to stabilize all measurements were repeated. The occluder was then slowly inflated with a micrometer driven syringe to decrease the mean coronary flow, as indicated by the Doppler signal, to the basal level and maintained constant thereafter. The distal coronary perfusion pressure of this mean flow was recorded. The 2DG infusion was then started at a rate of 15 $\mu\text{mol/kg}$ body weight/minute iv. All measurements, except myocardial blood flow measurements, were repeated every 15 min in the presence of the coronary stenosis with Dobutamine and 2DG infusion continued. Microspheres were injected only at 45 min after the start of 2DG infusion.

Data analysis

HEP and Pi measurements were obtained as described above. Hemodynamic data were determined from the strip chart recordings. Transmural blood flow distribution was determined from the microsphere measurements. Data was analyzed with one-way analysis of variance for repeated measurements. A value of $p < 0.05$ was considered significant. When significant results were found, individual comparisons were made using the Bonferroni Correction (26). For comparing regional myocardial blood flow data or NMR data of the PCr/ATP ratios, there are two factors being compared with experimental conditions and myocardial region of interest between subepicardial, mid-wall, and subendocardial layers. Therefore, a 2 way ANOVA analysis is applied (26)

RESULTS

Hemodynamic measurements

Hemodynamic data are shown in Table 1. Heart rate and left ventricular systolic pressure increased significantly during dobutamine infusion. RPP reached 30,000 mm Hg/min, a level which has been shown not to cause demand ischemia (34). Following application of the stenosis distal coronary pressure decreased to 48 mm Hg. RPP also decreased significantly, which was mainly caused by a decrease in LV systolic pressure since heart rate did not change during this intervention. LVEDP did not change significantly during any of the experimental conditions.

Myocardial blood flow

Myocardial blood flow data are summarized in Table 2. In response to dobutamine stimulation blood flow increased significantly parallel across the LV wall compared to baseline ($P < 0.05$, Table 2). Coronary stenosis (with continued dobutamine infusion) induced a significantly decrease in myocardial blood flow based on the Doppler flow probe (data not shown). During Db20 + coronary stenosis+2DG infusion, myocardial flow in the posterior wall remained significantly increased compare to baseline (Table 2). The anterior wall myocardial blood flow was successfully restricted to the baseline level (Table 2) by observing the Doppler flow and adjusting the hydraulic occluder.

Myocardial high energy phosphate levels

Figure 1 shows an example of transmural set of ^{31}P NMR spectra from one heart. Panel A displays the baseline spectra for five layers spanning the LV wall from subepicardium to subendocardium, while Panels B and C illustrate the responses during subsequent interventions. The accuracy of this type of spectral localization was previously tested rigorously in phantoms using voxel profiles and multichambered arrangements, and directly in the heart with surgically implanted markers on the inner and outer surfaces of the left ventricular wall (5,6,12,24,25). In the RAPP-ISIS experiment, it is possible to shift the voxels in the phase encode direction (i.e. perpendicular to the LV wall) to obtain voxels centered about any phase angle and consequently any “depth”, including the LV chamber. However, only five voxels centered around the phase angles specified were displayed in the current study. In these five voxel transmural sets of spectra, every other voxel is virtually non-overlapping; however, adjacent voxels contain some overlap (5,24). The spectrum at the bottom of the transmural stack (voxel 1) is nearest to the surface coil and accordingly is labeled “EPI”. Spectra from the midwall and the subendocardium are labeled as “MID” and “ENDO”, respectively. Under the basal conditions, myocardial PCr and ATP resonances are well observed whereas the Pi signal is too low to be detected across the wall (Panel A). In response to catecholamine stimulation, this heart increased RPP from 18750 mmHg $\times$ beats/min to 44000 mmHg $\times$ beats/min. Under this workstate there is a clear increase of myocardial Pi level with a tendency towards more pronounced changes in the outer layers of the LV wall (Panel B). When coronary stenosis was applied to reduce to mean coronary flow (measured by Doppler flow probe) to the basal level, combined with continued infusion of dobutamine (same rate as before) and additional infusion of 2DG (15 $\mu\text{mol/kg}$ body weight per minute, iv), the Pi level further increased and 2DG6P accumulation was clearly seen (Panel C; spectra acquired between 30-40 minutes after 2DG infusion started). Again these changes are most prominent in subepicardial layers of the LV wall (Panel C).

The summarized PCr/ATP, $\Delta\text{Pi}/\text{PCr}$ and 2DG6P/PCr ratios are shown in Table 3. PCr/ATP did not change significantly in response to catecholamine stimulation. Two of the 8 animals studied showed increase of Pi in the outer layers during dobutamine stimulation, but the mean value of the group did not reach a significant change (Table 3). During stenosis plus dobutamine stimulation together with intracoronary infusion of 2DG, myocardial PCr/ATP decreased significantly (Table 3). $\Delta\text{Pi}/\text{PCr}$ increased significantly across the wall uniform. Because during this experimental condition, the levels of PCr were not significantly different across the transmural different layers (Table 3), the levels of accumulation of 2DG6P across the LV wall were expressed as 2DG6P/PCr ratio. This ratio is not subject to variability in PCr across the LV wall. 2DG6P accumulated markedly and the 2DG6P/PCr ratio was significantly higher in the Epi region than in Endo (Table 3).

Myocardial oxygen consumption and lactate metabolism

Myocardial oxygen consumption (MVO_2) of the LAD-perfused area increased significantly in response to dobutamine infusion (Table 4). When stenosis was applied with continuous infusion of dobutamine and 2DG, the MVO_2 in the ischemic region returned to baseline values, despite the fact that RPP was still significantly higher compared to the control situation. Net lactate uptake also increased with dobutamine and showed similar decrease with combined dobutamine and 2DG infusion in the presence of stenosis (Table 4).

Myocardial specimen data

In the ischemic zone the myocardial wet weight/dry weight ratio was significantly increased as compared to the normal zone (5.9 $\pm$ 0.5 vs. 4.4 $\pm$ 0.4; $p < 0.05$).

DISCUSSION

The results of the present study have further elucidated the metabolic abnormalities occurring in demand-induced ischemia caused by dobutamine infusion in the setting of coronary flow restriction. Firstly, the transmural energetic abnormalities in demand induced ischemia are discordant to the transmural changes in myocardial blood flow, such that the sub-epicardial layer has the most pronounced energetic changes despite having higher blood flow in this experimental setting. Secondly, it has shown that ^{31}P NMR detectable 2DG6P accumulates in the in vivo canine hearts in the setting of demand-induced ischemia. Moreover, there is a transmural gradient to the accumulation of 2DG6P such that the concentration of 2DG6P is highest in the subepicardium. Thirdly, glycolytic inhibition as a result of 2DG6P accumulation in hearts undergoing demand induced ischemia leads to pronounced metabolic abnormalities and consequently myocardial edema.

This study confirms our previous finding that in myocardium perfused by a stenotic coronary artery dobutamine stimulation resulted in a distinct transmural ischemic pattern, with the most prominent changes appearing in the outer layers of the LV wall (3). In contrast, it is well established that during basal workstate in response to a partial or total occlusion of a coronary artery, the myocardial metabolic ischemic markers occur predominantly in the inner myocardial layers (21,37). In the present study, we used a model of demand ischemia, induced by imposing a high workload in a condition of fixed coronary stenotic flow, in which the decrease of PCr/ATP, increase of $\Delta\text{Pi}/\text{PCr}$ and 2DG6P/ATP were either uniform across the wall or most prominent in the outer layers (Table 3). These data are thereby different from results usually obtained during supply ischemia with the changes of metabolic ischemic markers being most pronounced in the inner layers of the LV wall. This suggests that the two types of ischemia have distinct metabolic and functional characteristics. In supply ischemia induced by a decrease of blood flow delivery, the metabolic ischemic markers were concordant with the pattern of myocardial hypoperfusion, with the largest changes occurring in the subendocardium (21,37). In contrast, when coronary stenosis is combined with dobutamine stimulation, the metabolic markers of ischemia were transmurally uniform or greater in the subepicardium and did not correspond to the pattern of myocardial hypoperfusion. In this regard, it is important to note that one important mechanism for the accumulation of lactate during demand ischemia, is a limitation in glycolytic reducing equivalents transfer into the mitochondria (10,20). The reducing equivalents, that would otherwise be shuttled into mitochondria for oxidative ATP synthesis, remain in the cytosol because of the malate aspartate shuttle exchange becomes the limiting factor due to the high flux rates through the mitochondrial dehydrogenases. This mechanism could also contribute to observation that the metabolic changes are disassociated from transmural blood perfusion pattern. This pattern has also been observed in hearts without partial stenosis where demand ischemia occurred after cardiac work load had been increased to a very high level (34). During a medium level dobutamine infusion RPP increased significantly to about 30,000 mmHg/min. At this workstate myocardial ATP synthetic rate can keep up with energy demand and consequently the PCr/ATP and $\Delta\text{Pi}/\text{PCr}$ ratios did not change (Table 3). This is consistent with the previous studies (34). However, when stenosis was applied to reduce the mean flow to approximately basal level, in combination with continued infusion of dobutamine and infusion of 2DG, PCr/ATP decreased, $\Delta\text{Pi}/\text{PCr}$ increased, and 2DG6P accumulated significantly. These changes were either uniform across the LV wall or more pronounced in the subepicardium (Table 3). Taken together with the significantly higher blood flow in this region (Table 2), these data suggest that under this experimental condition energy utilization is significantly higher in the outer layers of the LV wall, assuming that mitochondrial density and ATP synthetic capacity are similar in the inner and outer layers of the myocardium. The higher blood flow in the outer layer and therefore higher 2DG delivery could also contribute to the higher 2DG6P accumulation. However, the myocardial blood flow was not ceased in the inner layers during

the 2DG and dobutamine infusion. In fact the inner layer perfusion is similar to the basal level (Table 2), suggesting the higher 2DG6P/PCr ratio in the outer layer more likely reflect a higher glycolytic activity and a limitation in glycolytic reducing equivalents transfer into the mitochondria in the outer layers under this experimental condition. It is however not clear why the workload is distributed more to the outer layers. It is likely that when inner layers experienced underperfusion as consequence of stenosis the workload is redistributed to the outer layers where flow is higher. If dobutamine receptors are not completely saturated under this condition and their distribution is uniform across the wall, then higher blood flow in the outer layer means that more dobutamine is delivered to this region which may result in more energy utilization. During partial coronary occlusion combined with dobutamine and 2DG infusion, RPP was significantly lower as compared with dobutamine stimulation alone (Table 1). Whether this was caused by partial glycolytic inhibition by 2DG6P is not certain. However, Yoshiyama M *et al* (33) have shown that 2DG infusion during baseline conditions as well as during hyperperfusion did not affect hemodynamics. Furthermore, since both LVEDP (Table 1) and the duration of the diastole (data not shown) did not differ during dobutamine infusion alone or during stenosis plus dobutamine and 2DG infusion, it is unlikely that decreased glycolytic ATP production due to inhibition by 2DG6P is the cause of the decreased RPP. The fact that RPP is still elevated during stenosis together with dobutamine and glycolytic inhibition by 2DG6P is probably caused by the continued increased pressure development of the posterior part of the LV wall. Posterior region blood flow, and thereby oxygen and substrate supply, increased even more after the LAD had been partially occluded (Table 2). Work and blood flow of the posterior region increased in parallel, thereby maintaining the balance between demand and supply in this region of the heart (Table 2).

In stunned myocardium, it has been shown that 2DG uptake is most pronounced in the inner layers of the myocardium (33). Yoshiyama M *et al.* (33) also showed that this gradient was flow independent. It has further been shown that a concentration gradient of glycolytic enzymes and glycogen exists with the highest levels being present in the subendocardial layers (7,15). In the present study, during demand ischemia 2DG uptake is higher in the outer layers of the heart suggesting that the two types of ischemia have different metabolic characteristics. The greater uptake of 2DG in the outer myocardial layers in the current study may be attribute to the increased glycolytic ATP production in that layer to support the cardiac performance.

Using a swine model of demand-induced ischemia in the setting of coronary stenosis, Chandler et al recently reported that an increase in net glycogen breakdown, glucose uptake and lactate production (2), suggesting an increase of non-oxidative glycolysis. 2DG is a glucose analog that is transported into the cell by the sarcolemmal glucose transporter and is subsequently phosphorylated by hexokinase (8). Clearance of phosphorylated 2DG from the heart is very slow because it cannot undergo further glycolytic metabolism, and it is very slowly dephosphorylated (8,23). In the present study, the partial inhibition of glycolysis by 2DG6P accumulation caused a myocardial edema as evidenced by the significant increase of wet weight to dry weight ratio. As a result of the accumulation of 2DG6P, glucose uptake is significantly inhibited (23). Although under normal circumstances glycolysis provides only a small fraction of total myocardial ATP production, there is increasing evidence that glycolytically produced ATP may be preferentially used to support cellular ion homeostasis. Indeed, glycolysis has been proposed to provide ATP for preferential use by the sarcolemmal Ca^{+} pump (19,22), and the Na^{+} - K^{+} pump (1,16). The reason for the apparent dependence of cellular ion homeostasis on glycolytically produced ATP is not clear, but may be related to the functional coupling of glycolytic enzymes with membrane channels and ion pumps. The function of glycolysis is in the same compartments as the ion pumps (31,32). Due to the functional compartmentation of metabolic processes possible damage to the sarcolemma during ischemia or hypoxia is delayed or even prevented by glycolytic ATP production. In the present study the wet to dry weight ratio in the ischemic (stenotic) 2DG perfused area of the

LV is significantly higher than in the control area, which is not observed in ischemic (stenotic) hearts without 2DG perfusion (21). This suggests that the edema present in the ischemic area is caused by the 2DG6P accumulation associated glycolytic inhibition and consequently failed to maintain the ion homeostasis.

In summary, the present study demonstrates that the subepicardial layer shows greater metabolic abnormalities and accumulation of 2-deoxyglucose despite higher blood flow during dobutamine induced demand ischemia in the setting of a stenosed coronary artery. These data suggests that energy expenditure is greater in the subepicardial-than in the endocardial-layers during dobutamine stimulation induced demand ischemia.

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Figure 1.

Typical spectra set obtained from one heart showing transmural distribution of high energy phosphates and 2-deoxyglucose 6 phosphate concentrations detected by ^{31}P nuclear magnetic resonance spectroscopy under basal conditions (A), during dobutamine infusion (B), and during combined dobutamine infusion, coronary stenosis and 2-deoxyglucose infusion (C). The decrease of P_i and accumulation of 2DG6P are more pronounced in EPI than in ENDO layers.

Table 1

Hemodynamic Data

Intervention	N	Heart Rate, beats/min	MAP, mmHg	LVSP, mmHg	LVEDP, mmHg	Coronary pressure, mmHg	RPP, 10 ³ mmHg × beats/min
Baseline	10	151 ± 8	95 ± 5	117 ± 7	7.1 ± 0.7	95 ± 5	17.2 ± 1.2
Db20	10	203 ± 11 [*]	109 ± 7	194 ± 15 [*]	7.0 ± 0.8	96 ± 5	29.8 ± 2.2 [*]
Db20+coronary stenosis+2DG	10	203 ± 11	88 ± 7 [†]	167 ± 16 [*]	7.5 ± 1.5	48 ± 2 ^{*,†}	24.6 ± 2.6 ^{*,†}

Values are means ± SE; N, no. of dogs. MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; RPP, rate pressure product; Db20, Dobutamine at 20 μg.kg⁻¹.min⁻¹; 2DG, 2-deoxyglucose at 15 μmol.kg⁻¹.min⁻¹.

^{*} P<0.05 compared with baseline.

[†] P<0.05 compared with Db20.

Table 2

Myocardial Blood Flow Data

N	Anterior Wall (Ischemic)				Posterior Wall (Reference)			
	Epi	Mid	Endo	Mean	Endo/Epi	Epi	Mid	Endo
Baseline	8	0.61±0.04	0.63±0.05	0.63±0.05	1.00±0.05	0.64±0.05	0.72±0.06	0.77±0.08 [‡]
Db20	8	1.52±0.13 [*]	1.35±0.14 [*]	1.40±0.13 [*]	0.99±0.03	1.75±0.14 [*]	1.58±0.15 [*]	1.56±0.20 [*]
Db20 + coronary stenosis+ 2DG	7	0.95±0.11 ^{‡a}	0.68±0.11 ^{‡a}	0.75±0.11 ^{‡a}	0.87±0.09	2.41±0.43 [*]	2.09±0.39 [*]	2.26±0.62 [*]
								2.25±0.48 [*]
								1.07±0.06
								0.97±0.04
								0.99±0.11

Values (in ml min⁻¹.g wet wt⁻¹) are means ± SE; N, no. of dogs. Epi, subepicardium; Mid, midmyocardium; Endo, subendocardium; Db20, Dobutamine at 20 µg.kg⁻¹.min⁻¹; 2DG, 2-deoxyglucose at 15 µmol.kg⁻¹.min⁻¹.

^{*} P<0.05 compared with baseline.
[‡] P<0.05 compared with Db20;
[‡] P<0.05 compared with Epi;
^a P<0.05 vs posterior wall.

Table 3

Transmural Myocardial Energetics Data

		PCr/ATP	2DG6P/PCr	ΔPi/PCr	%PCr	%ATP
Baseline	EPI	2.30±0.14	0	0	1	1
	MID	2.06±0.13	0	0	1	1
	ENDO	2.04±0.11	0	0	1	1
Db20	EPI	2.40±0.27	0	0.05±0.03	0.98±0.01	0.95±0.03
	MID	2.16±0.25	0	0.05±0.03	0.93±0.04	0.91±0.03
	ENDO	2.09±0.12	0	0	0.98±0.05	0.98±0.05
Db20+ coronary stenosis+2DG	EPI	1.77±0.12 [*] †	0.55±0.12 [*] †	0.54±0.08 [*] †	0.51±0.07 [*] †	0.62±0.05 [*] †
	MID	1.70±0.11 [*] †	0.52±0.10 [*] †	0.50±0.06 [*] †	0.47±0.05 [*] †	0.56±0.06 [*] †
	ENDO	1.72±0.12 [*] †	0.37±0.08 [*] †	0.48±0.07 [*] †	0.57±0.07 [*] †	0.69±0.07 [*] †

Values are means ± SE; PCr, phosphocreatine; ATP, adenosine triphosphate; ΔPi, change in inorganic phosphate from baseline; 2DG6P, 2-deoxyglucose-6-phosphate; Epi, subepicardium; Mid, midmyocardium; Endo, subendocardium; Db20, Dobutamine at 20 μg.kg⁻¹.min⁻¹; 2DG, 2-deoxyglucose at 15 μmol.kg⁻¹.min⁻¹.

* *P*<0.05 compared with baseline.

† *P*<0.05 compared with Db20;

P<0.05 compared with EPI.

Table 4
Myocardial Oxygen Consumption and lactate metabolism data

	Oxygen Consumption		Lactate Metabolism	
	a-v, ml O ₂ /100ml	MVO ₂ , ml.min ⁻¹ .100g ⁻¹	a-v, μmol/l	Net lactate uptake, μmol.min ⁻¹ .g ⁻¹
Baseline	8.16 ± 0.99	0.05 ± 0.01	0.25 ± 0.08	0.15 ± 0.05
Db20	8.26 ± 1.23	0.11 ± 0.01 [*]	0.37 ± 0.11	0.49 ± 0.13 [*]
Db20+coronary stenosis+2DG	8.04 ± 1.28	0.06 ± 0.01 [†]	0.10 ± 0.10	0.11 ± 0.08 [†]

Values are means ± SE; a-v, Arteriovenous; MVO₂, myocardial O₂ consumption; Db20, Dobutamine at 20 μg.kg⁻¹.min⁻¹; 2DG, 2-deoxyglucose at 15 μmol.kg⁻¹.min⁻¹.

^{*} *P*<0.05 compared with baseline.

[†] *P*<0.05 compared with Db20.