In failing hearts, coronary flow is normal, but the coronary flow reserve (CFR) is reduced, so demand-induced ischemia (DII) may occur in response to greater demand for O₂. The objectives of this study were: (i) to verify that dobutamine stimulation produces DII in isolated rat hearts having, like failing hearts, increased left ventricular end-diastolic pressure (LVEDP) and hence reduced CFR and (ii) to study the effects of stimulation of glucose oxidation and of inhibition of fatty acid oxidation in this new model of DII. Isolated rat hearts perfused with 11 mM glucose and 0.6 mM palmitate (or no palmitate) were studied. Stepwise increments in the volume of a balloon placed in LV resulted in reciprocal impairment of CFR, supporting the role of the extravascular compressive forces in determining CFR. CFR was 1.82±0.1 and 1.32±0.1 (p<0.05) in the hearts with LVEDP set to 5 mmHg (controls) and 40 mmHg (expanded), respectively. In controls, dobutamine increased coronary flow, myocardial oxygen consumption (MVO₂), LVDP, mechanical efficiency, and the rates of palmitate and glucose oxidation, however, the effluent lactate concentration remained unchanged. In the expanded hearts vs. controls, dobutamine-induced increases in coronary flow and MVO₂ were reduced by ~50%, the increases in LVDP, efficiency, and rates of glucose and fatty acid oxidation were completely prevented, and lactate production greatly increased with dobutamine, indicating DII. Pyruvate dehydrogenase activator, dichloroacetate (DCA 1 mM) and a putative inhibitor of fatty acid β-oxidation, trimetazidine (5 µM), both increased the rate of glucose oxidation and attenuated myocardial lactate production during DII, however they did not improve myocardial function during DII. Likewise, palmitate-free perfusion had no beneficial effect during DII although it attenuated lactate production. In the hearts subjected to palmitate-free perfusion plus DCA, lactate overproduction during DII was completely abolished, however, the deterioration of LVDP and mechanical efficiency was only partially prevented. Thus, interventions shifting this balance toward glucose oxidation are not beneficial in the setting of DII in our model although they are known to effectively mitigate contractile dysfunction in the post-ischemic myocardium.

Key words: isolated rat heart, demand induced ischemia, trimetazidine, dichloroacetate, dobutamine, substrate metabolism, contractile function, mechanical efficiency
Thereby they lessen the degree of ischemia by better matching the delivery of \( O_2 \) to the amount of work the myocardium performs. The use of drugs that relieve myocardial ischemia by reducing cardiac output may be contraindicated in heart failure. An alternative approach is pharmaceutical modulation of cardiac fatty acid or carbohydrate metabolism aimed at optimization of cardiac energy metabolism and increasing the mechanical efficiency (measured as cardiac work per \( O_2 \) consumed) of the ischemic myocardium without increasing myocardial perfusion and/or suppressing contraction (12-14).

Current understanding of the anti-ischemic potential of the metabolic therapies is based almost exclusively on experiments aimed at studying the mechanism of impaired functional recovery of ischemic myocardium during reperfusion (a phenomenon referred to as stunning) rather than the mechanisms of the contractile dysfunction during DII itself. These "anti-stunning oriented" studies suggest the following scenario (12, 14). In the myocardium the oxidation of fatty acids acts to inhibit glucose and lactate oxidation under both well-perfused conditions and during myocardial ischemia. This effect is primarily mediated at the level of pyruvate dehydrogenase (PDH) in the mitochondrion, where products of fatty acid oxidation inhibit flux through this enzyme. The pyruvate flux produced from glycolysis cannot be fully metabolized by PDH and oxidized in mitochondria during ischemia and is reduced to lactate that can further uncouple glycolysis from pyruvate oxidation. In reperfused myocardium, lactate and \( H^+ \) accumulation and efflux have negative effects on the ability of cardiac muscle to maintain \( Ca^{2+} \) homeostasis and consequently to use the energy released from the breakdown of ATP to perform contractile work resulting in the impaired mechanical efficiency of cardiac contraction (ATP is used for maintaining \( Ca^{2+} \) homeostasis instead for supporting the contraction). In support of this concept, a direct PDH activator, dichloroacetate (DCA) (15, 16) and various partial inhibitors of fatty acid oxidation, acting presumably by reducing inhibition by fatty acid oxidation, reduced lactate accumulation, and improve contractility of stunned myocardium. Alternatively, decreasing fatty acid oxidation and increasing glucose oxidation may improve efficiency by decreasing the amount of \( O_2 \) required for ATP synthesis (12).

It is uncertain if and how these metabolic mechanisms underlie the mechanism of contractile dysfunction during DII. Indeed, pharmacological agents that partially inhibit fatty acid oxidation (trimetazidine, ranolazine, oxemazine, etomoxir) have been shown to reduce the symptoms of DII in patients with stable angina (14, 17-19). Likewise, DCA (20), trimetazidine (21, 22), and etomoxir (23) have been reported to improve myocardial contractility in heart failure patients. In the experimental models of DII, however, although partial inhibitors of fatty acid oxidation consistently stimulated myocardial glucose oxidation and reduced lactate production during DII they managed to improve cardiac contraction during DII only incidentally (24-27, 27). One reason for this discrepancy might be that the experimental models with a completely suppressed CFR were studied in this context (10, 27) that may not be clinically relevant. Therefore, we aimed at verifying that dobutamine stimulation produces DII in rat hearts having, like failing hearts, increased LVEDP and partially reduced CFR, but without contact with air as required for \( PO_2 \) and \( ^{14}CO_2 \) determinations, as described previously (32). Cardiac function was monitored via a fluid-filled balloon placed in the left ventricle (LV) and connected to a pressure transducer. Coronary flow was measured with Transonic Flowprobe (Transonic Systems, Inc.) placed in the perfusion line just above the aortic cannula and recorded with Hugo Sachs software. During the equilibration perfusion, LV end-diastolic pressure (LVEDP) was set to 5 mmHg by adjusting balloon volume and it was eventually changed thereafter according to experimental protocols. The hearts were enclosed in a water-jacketed chamber, and the temperature of the perfusate was controlled at 37°C. The hearts were not paced. At the end of each perfusion protocol, the ventricles were dried to obtain their dry weight.

### Perfusion solutions

The hearts were perfused either with a standard KHB containing (in mM): 118 NaCl; 23.8 NaHCO\(_3\); 4.7 KCl; 1.2 KH\(_2\)PO\(_4\); 2.5 CaCl\(_2\); 1.2 MgSO\(_4\); and 11 glucose or with a modified KHB containing in addition 0.6 mM sodium palmitate bound to 1.2% BSA (32). These solutions were gassed with 95% \( O_2 \) + 5% \( CO_2 \) gas mixture giving pH 7.4 and \( PO_2 \) 580-640 mmHg at 37°C. To bind palmitate to BSA, the palmitate (unlabeled plus an aliquot of [9,10 (n)-3H] palmitate) was first dissolved in 20 ml of a water: ethanol mixture (75:25 vol/vol) containing 0.5 g Na\(_2\)CO\(_3\)/g palmitate. The mixture was heated with constant stirring until the ethanol was removed. This mixture was added to...
the KHB containing BSA (without glucose) while stirring rapidly to ensure adequate mixing. This solution was dialyzed overnight at 4°C against 20 vol of the standard KHB solution (without glucose) using 12000 mol wt cut-off dialysis tubing (Sigma). Glucose (11 mM, unlabeled plus an aliquot of [U-14C]glucose) was added to the perfusate the next day just before use.

**Experimental protocols**

To probe for the effect of LV volume/pressure on CFR, LVEDP was gradually set from the basal 5 mmHg, to 10, 15, 20, 30, 40 and 60 mmHg by increasing LV balloon volume. After each change in LVEDP, 5 min was allowed to stabilize functional variables and CFR was measured. Next, LVEDP was set again to 5 mmHg, the heart was allowed to equilibrate for 15 min, the procedure was repeated, and the measurements from these two runs of LVEDP increments were averaged. To assess CFR, adenosine was given to determine maximal coronary flow. Adenosine was infused via a side arm of the aortic cannula as a constant 15 sec infusion 1/100 of coronary flow (final adenosine concentration ~1 μM) with a digital infusion pump (Kwapisz, Poland). Preliminary concentration-response studies established 1 μM adenosine to be supra-effective in producing maximum coronary vasodilation in our model. CFR was defined as the ratio between the maximal and the basal coronary flow.

In the main study, all the hearts were subjected to 60-min perfusion, and all had the initial 20-min equilibration perfusion with the standard KHB, and with LVEDP set at 5 mmHg. Next, the hearts were allocated to one of the following protocols:

I. Further 40-min perfusion at LVEDP 5 mmHg;
II. Further 40-min perfusion at LVEDP 5 mmHg and in the presence of 1 mM DCA or 5 μM trimetazidine;
III. Further 20 min perfusion plus 20 min perfusion with 100 nM dobutamine, all at LVDP 5 mmHg;
IV. Further 40-min perfusion at LVEDP 40 mmHg;
V. Further 40-min perfusion at LVEDP 40 mmHg and in the presence of 1 mM DCA or 5 μM trimetazidine;
VI. Further 20 min perfusion plus 20 min perfusion with 100 nM dobutamine, all at LVDP 40 mmHg;
VII. Further 20 min perfusion plus 20 min perfusion with 100 nM dobutamine, all at LVDP 40 mmHg, and in the presence of 1 mM DCA lub 5 μM trimetazidine;

The hearts within these protocols were perfused either with the standard KHB (glucose 11 mM) throughout the study or perfusion with the modified KHB (11 mM glucose + 0.6 mM palmitate) was started immediately after 20-min equilibration perfusion.

Starting from the 20 min of the protocol, a 5-minutes sample of the perfusate exiting the heart via cannulated pulmonary artery was collected every 5 minutes until the end of the experiment. They were used for lactate determination, and for 14CO2 and 3H2O counting, to measure myocardial glucose and palmitate oxidation, respectively.

**Glucose and palmitate oxidation**

Oxidation of radiolabeled glucose and palmitate was measured simultaneously in one set of hearts. All determinations of substrate oxidation at each time point were made in duplicate. The values obtained were normalized for the dry weight of the ventricles.

Glucose oxidation was determined by trapping and measuring 14CO2 released by the metabolism of [U-14C]glucose (specific activity ~500 MBq/mmol), as previously described (32-34). In brief, 14CO2 present in the venous perfusate (mostly in the form of bicarbonate anion) was released by injecting 1 ml of the perfusate sample into 5 ml of 80% lactic acid inside a sealed metabolic flask. The flask were gently shaken for at least 3 hrs, and 14CO2 released from the solution was subsequently trapped in a center well containing 800 μ of 1.0 M hyamine hydroxide. The hyamine samples containing 14CO2 were counted using scintillation cocktail.

The rate of glucose oxidation (μmol min⁻¹ g dry wt⁻¹) was calculated as glucose oxidation = coronary flow x [14CO2]/[glucose specific radioactivity where coronary flow was in ml/min/g dry wt, [14CO2] was the activity of 14C labeled CO2 in the coronary venous effluent (dpm/ml), and the glucose specific radioactivity (dpm/µmol) was the concentration of 14C-glucose in the modified KHB solution inflowing the heart (dpm/ml) divided by the total glucose concentration (µmol/ml), measured using an enzymatic-spectrophotometric assay, Sigma Diagnostics) in this solution. Palmitate oxidation was determined by measuring 3H2O released by the metabolism of [9,10(α)-3H] palmitate (specific activity ~3.5 GBq/mol). The 3H2O concentration was measured by distillation of an aliquot of the venous effluent (to separate 3H2O and [9,10(α)-3H] palmitate counted in the effluent) and counting tritiated water in the distillate.

The rate of palmitate oxidation (μmol min⁻¹ g dry wt⁻¹) was calculated as palmitate oxidation = coronary flow x [3H2O]/[palmitate specific activity where [3H2O] was the activity of tritiated water in the venous effluent (dpm/ml), and the palmitate specific activity (dpm/µmol) was the concentration of 3H-palmitate in the perfusion buffer (dpm/ml) divided by the total palmitate concentration (µmol/ml, measured using enzymatic spectrophotometric kit, Wako Chemicals, USA, Richmond, VA) in the perfusion buffer.

**Lactate outflow**

Lactate in the venous effluent was determined using an enzymatic-spectrophotometric kit (Sigma-Diagnostics). Cardiac lactate production was calculated as a product of the coronary flow and the lactate effluent concentration.

**Myocardial oxygen consumption and efficiency index**

Arterial perfusate and venous effluent samples were analyzed for the pO2 using a Ciba-Corning 248 pH & Gas-Analyzer (Ciba-Corning, Kalstead, Essex, UK). MVO2 was calculated as a product of the coronary flow and the arterial-venous oxygen concentration difference. The heart rate-pressure product (RPP=HR x LVDP, in mmHg/min) was calculated and used as an index of external cardiac work. The mechanical external efficiency of the heart was calculated from RPP divided by the MVO2 (35) that was referred to as the "efficiency index".

**Statistics**

All data are expressed as mean ±S.E.M. Significant intergroup differences were calculated using either one-way or two-way analysis of variance followed by Dunnett's post hoc test. Differences between groups were considered significant if the P value was<0.05.

**RESULTS**

**LVEDP and coronary flow reserve in isolated rat heart**

Compatible with the Frank-Starling mechanism, stepwise increments in LVEDP from 5 mmHg to 60 mmHg, initially caused a gradual increase and than fall in LVDP (Fig. 1a). The changes in basal coronary flow had a similar biphasic pattern,
and a progressive fall in CFR was associated with the increments in LVEDP (Fig. 1b).

In further experiments, the hearts with LVEDP set to 5 mmHg (control hearts) and 40 mmHg (expanded hearts) were studied. The expanded hearts had 27% smaller CFR compared to controls (1.52±0.01 vs. 1.82±0.1, p<0.05) (Fig. 1b). This corresponded to a slightly smaller basal coronary flow, LVDP, heart rate, and MVO₂, and a greater lactate outflow in the expanded vs. control hearts. These differences, however, did not reach statistical significance.

Baseline cardiac function was not significantly different between the hearts perfused with and without palmitate. The functional stability of the control and expanded hearts was confirmed by the lack of significant differences between cardiac functions measured at baseline and at the conclusion of the sham perfusion protocols (Table 1).

**Demand-induced ischemia (DII) in the expanded hearts**

Dobutamine caused a comparable ~35% increase in heart rate in control and expanded hearts. However, its other effects greatly differed between the groups (Table 1, Fig. 2).

In control palmitate-perfused hearts, dobutamine increased coronary flow, LVDP, and MVO₂ by approximately 15-20%, cardiac work by 60% (RPP 27,347±1,300 vs. 42,918±2,100 mmHg/min, p<0.05), mechanical efficiency by 20% (efficiency index 772±50 vs. 921±58 mmHg × g/mmol, p<0.05), and lactate outflow by 30%.

In expanded hearts, dobutamine-induced augmentation of coronary flow and MVO₂ was 7-11% only, LVDP actually decreased by 12%, cardiac work increased only by 20% (RPP 24,830±1,180 vs. 29,500±1,390 mmHg/min, p<0.05), dobutamine-induced increase in efficiency was prevented (baseline efficiency index 709±37 vs. 756±62 mmHg × g/mmol with dobutamine, p>0.05), and lactate outflow increased by 85%. This greatly augmented lactate outflow confronted with
reduced vasodilatory response implicated enhanced myocardial lactate formation (rather than enhanced washout) and hence DII in the dobutamine-treated expanded hearts. These data confirm that functional reserve with dobutamine existed in expanded hearts but to a lesser degree, and at an expense of lesser mechanical effectiveness, than in control hearts.

These dobutamine-induced effects were qualitatively similar in the hearts perfused with and without palmitate (Table 1).

Effects of DCA, trimetazidine, and palmitate-free perfusion on DII

DCA (1 mM) and trimetazidine (5 µM) affected functional characteristics neither in control nor in expanded hearts perfused with or without palmitate (Table 1). To study the effect of metabolic interventions on DII, the expanded hearts perfused with DCA, trimetazidine, palmitate-free KHB or with palmitate-free KHB plus DCA were treated with dobutamine.

Although DCA, trimetazidine and palmitate-free perfusion reduced DII-induced myocardial lactate production by 40%, 82%, and 96%, respectively (Fig. 3a), they failed to modify cardiac functional characteristics during DII (Table 1). In particular, none of these interventions appeared to prevent DII-induced deterioration in LVDP (Fig. 4), rate-pressure product and/or mechanical efficiency (Fig. 3b,c).

Only the combined treatment with palmitate-free perfusion and DCA appeared protective in the setting of DII. Indeed, this combination, which significantly reduced pre-ischemic lactate production and completely prevented DII-induced lactate overproduction (Table 1), managed to partially prevent DII-induced deterioration in LVDP (p<0.05, Fig. 4b), cardiac work, and mechanical efficiency (Fig. 3b,c).

Glucose and palmitate oxidation

The basal rates of glucose and palmitate oxidation were similar to those reported by others (36), and did not differ between control and expanded hearts (Fig. 5). Dobutamine increased glucose and palmitate oxidation by approximately 50% and 10%, respectively, (p<0.05), in control hearts. The effects reached a maximum within 5-10 min of dobutamine application and remained stable thereafter. Consequently, the 

\[ ^{14}C \text{CO}_{2} \] and \[ ^{3}H_{2}O \] measurements obtained at 10, 15, and 20 min of the dobutamine application (at 50, 55 and 60 min of the protocol) were averaged and treated as the intervention measurement.
Table 1. Effect of various treatments on functional characteristics, lactate outflow, and efficiency in perfused isolated rat heart.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Coronary flow (ml/min/g dry wt)</th>
<th>LVDP (mmHg)</th>
<th>Heart rate (beats/min)</th>
<th>Oxygen consumption (mmol/min/g dry wt)</th>
<th>Lactate outflow (mmol/min/g dry wt)</th>
<th>Efficiency index (Efficacy index)</th>
</tr>
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<td>5 mmHg (G)</td>
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<tr>
<td>Untreated</td>
<td>5</td>
<td>51 ± 3.4</td>
<td>71 ± 3.7</td>
<td>117 ± 3</td>
<td>114 ± 3</td>
<td>38 ± 6.0</td>
<td>7.8 ± 1.1</td>
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<tr>
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<td>5</td>
<td>72 ± 2.4</td>
<td>72 ± 3.2</td>
<td>120 ± 9</td>
<td>121 ± 8</td>
<td>38 ± 1.5</td>
<td>37 ± 3.3</td>
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<tr>
<td>Dobutamine, 100 nM</td>
<td>5</td>
<td>71 ± 5.2</td>
<td>81 ± 4.6</td>
<td>118 ± 7</td>
<td>145 ± 9*</td>
<td>37 ± 6.2</td>
<td>44 ± 7.1*</td>
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<tr>
<td>5 mmHg (G + FFA)</td>
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<td></td>
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<tr>
<td>Untreated</td>
<td>7</td>
<td>68 ± 4.4</td>
<td>67 ± 3.9</td>
<td>119 ± 6</td>
<td>117 ± 5</td>
<td>37 ± 3.6</td>
<td>35 ± 8.7</td>
</tr>
<tr>
<td>DCA, 1 mM</td>
<td>6</td>
<td>70 ± 4.4</td>
<td>71 ± 5.2</td>
<td>120 ± 5</td>
<td>120 ± 6</td>
<td>37 ± 3.3</td>
<td>36 ± 4.0</td>
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<td>6</td>
<td>69 ± 5.1</td>
<td>71 ± 4.8</td>
<td>116 ± 8</td>
<td>113 ± 8</td>
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<td>138 ± 9*</td>
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<td>6</td>
<td>68 ± 7.7</td>
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<td>40 mmHg (G + FFA)</td>
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<td>65 ± 2.5</td>
<td>70 ± 2.9</td>
<td>113 ± 8</td>
<td>100 ± 9*</td>
<td>21 ± 3.5</td>
<td>29 ± 3.5*</td>
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<td>69 ± 3.9</td>
<td>120 ± 7</td>
<td>99 ± 6*</td>
<td>22 ± 3.6</td>
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<tr>
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<td>68 ± 4.6</td>
<td>110 ± 9</td>
<td>102 ± 11</td>
<td>33 ± 2.4</td>
<td>38 ± 5.3*</td>
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</table>

Values are means±SEM N, number of hearts.

The hearts had LVDP set at either 5 mmHg or 40 mmHg and were perfused with 11 mM glucose (G) or 11 mM glucose + 0.6 mM palmitate bound to 1.2% albumin (G + FFA). In dichloroacetate (DCA) and trimetazidine (TMZ) groups, the drug was present in the perfusate for the whole perfusion protocol. In dobutamine groups, it was applied immediately after the baseline measurements were taken at 40 min and the measurements were repeated at 60 min of the protocol.

*p<0.05 vs. respective 40 min; **p<0.05 vs. respective untreated group.

Fig. 5. Effect of 20-min dobutamine challenge on the rate of glucose (a) and palmitate oxidation (b) in control (●, 5 mmHg) and expanded ( ○, 40 mmHg) rat hearts perfused with 11 mM glucose and 0.6 mM palmitate. Arrows indicate the moment of dobutamine application. Values are mean±SEM; n=6-7; *p<0.05 vs. respective baseline at 40 min. **p<0.05 vs. control hearts.

The dobutamine-induced rise in glucose oxidation was completely prevented in the expanded hearts (i.e., during DII), and palmitate oxidation during DII was actually depressed.

Fig. 6. The rate of glucose (a) and palmitate oxidation (b) in the isolated rat hearts as affected by dobutamine, dichloroacetate (DCA), and trimetazidine (TMZ). The control (LVDP 5 mmHg) and expanded hearts (LVDP 40 mmHg) were perfused either without (open columns) or with dobutamine (crosshatched columns). Some expanded hearts were also perfused with 1 mM DCA or 5 µM trimetazidine given alone or with dobutamine. In each experimental group, the measurements obtained at 50, 55 and 60 min of the protocol (see Fig. 5) were averaged and treated as the control or intervention measurement, respectively. Values are mean±SEM; n=6-7; *p<0.05 vs. respective untreated; **p<0.05 vs. control dobutamine.
compared to that during the preischemia, although this was not significant (Fig. 5 and 6).

In the expanded hearts, DCA and trimetazidine increased glucose oxidation by approximately 80% and 35% (p<0.05), respectively, and had no significant effect on palmitate oxidation. These DCA- and trimetazidine-induced changes were not modified further by dobutamine treatment (Fig. 6).

DISCUSSION

This study demonstrates that in isolated rat heart: (i) gradual LV volume expansion caused proportional impairment of CFR, confirming the major role of the extravascular compressive forces in determining of CFR; (ii) DII was produced with dobutamine treatment in the expanded hearts; thus despite limited increase in coronary flow and MVO₂ with dobutamine, the hearts had increased lactate production, abolished contractile reserve, reduced mechanical effectiveness, and no typical increases in glucose and palmitate oxidation; (iii) although DCA, trimetazidine, and palmitate-free perfusion reduced lactate production, they failed to improve cardiac contraction and efficiency during DII, suggesting that lactate accumulation secondary to an imbalance between glycolysis and glucose oxidation is not a primary factor mediating contractile dysfunction during DII in our model.

Experimental model

CFR was found to amount 2.71±3.67 in healthy human subjects (2, 6, 37, 38) and it may be reduced to 1.96-2.22 (2, 37) and 1.45 (2) in the subjects with NYHA I/II, and NYHA III functional class of heart failure, respectively. Likewise, CHD patients have decreased CFR distal to coronary artery stenosis (8) and CFR <2 implies a functional stenosis, and CFR<1.5 - critical stenosis (39).

Crystallloid-perfused hearts, such as those in this study, have coronary flow approximately ten times greater, and CFR proportionately smaller than blood-perfused hearts. In addition, crystallloid-perfused hearts produce, rather than consume, small amounts of lactate. Nevertheless, convincing evidence accumulated to show adequacy of oxygenation of isolated perfused rat heart preparation (40). For instance, in our system, control rat hearts had CFR of 1.82±0.1 and they were releasing lactate. Despite that, when subjected to a moderately increased workload (dobutamine stimulation producing ~80% of maximal heart rate acceleration) their coronary flow, MVO₂, rate-pressure product and oxidation of glucose and palmitate, all increased while effluent lactate concentration remained unchanged. These imply that in the absence of dobutamine the hearts were not consuming all the oxygen they could, and that during dobutamine stimulation their oxygenation was still adequate, as evidenced by the increased cardiac work and no concomitant increase in lactate formation.

Dobutamine caused, however, DII in our model of the volume-expanded heart. These hearts had LVEDP increased to 40 mmHg (vs. 5 mmHg in controls) and CFR reduced to 1.32±0.1 (vs. 1.82±0.1 in controls), the values close to those observed in patients with advanced heart failure (2) or with subcritically stenosed coronary artery (39). Importantly, baseline coronary flow, MVO₂, LVEDP, and lactate outflow did not differ between the expanded and control hearts, further suggesting adequate baseline oxidation of the expanded hearts. Furthermore, the expanded hearts had preserved small reserve to increase coronary flow and MVO₂ with dobutamine that is in contrast to the models of DII available in the literature (10, 27). In the most widely studied open chest swine model of DII, the resting left anterior descending coronary artery flow was decreased by 20% and kept constant and demand was increased with dobutamine infusion (10, 24-26). This implies that ensuing ischemia was a mixture of resting low-flow ischemia (related to 20% restriction of the basal flow) and DII itself, and therefore, was probably more severe than DIs occurring in clinical settings. In the other model of DII, coronary flow was kept constant with a pump in isolated guinea-pig hearts and DII was created by increasing the rate of heart stimulation (27). Altogether we believe that our model of DII in expanded hearts is relevant to what may happen in response to increased demand for O₂ in the hearts with pathologies compromising CFR only partially, like hypertrophy and heart failure.

Contractile and metabolic responses during DII

Chronotropic responses to dobutamine were similar in our control and expanded hearts confirming unchanged sensitivity to dobutamine stimulation in the latter group. Despite the fact that expanded hearts increased their coronary flow and MVO₂ by 7-11% (vs. 15-20% in controls) with dobutamine they developed ischemia, as evidenced by their greatly increased lactate production. The consequences of DII were three-fold. (i) Dobutamine-induced increase in LVDP was prevented or even LVEDP slightly decreased relative to its predobutamine level. (ii) The fall in LVEDP and in rate-pressure product, occurring despite small accompanying increase in MVO₂, reflected impaired mechanical effectiveness of myocardial contraction (relative to that in the dobutamine-treated controls). In fact, DII only prevented dobutamine-induced augmentation in cardiac mechanical effectiveness, implying that this effect accounted for the impairment of cardiac contraction during DII. (iii) Dobutamine-induced augmentation in glucose and palmitate oxidation was completely prevented. Despite the differences between our model and the discussed swine model of DII (10), their responses to DII appeared to differ mostly quantitatively, confirming the complementariness of the models. Generally, in the pig model dobutamine clearly impaired basal cardiac contractility (by ~30%) and fatty acid oxidation (by ~70%). While the typical contractile and metabolic responses to dobutamine were only prevented in our model. Plausible explanation for these differences was that DII was milder in our model.

Effects of metabolic interventions

The metabolic therapies effectively mitigate contractile dysfunction in stunned myocardium. One frequently proposed mechanism of this beneficial action is the oxidative removal of lactate resulting from an increase in PDH activity. Alternative explanation is that shifting myocardial substrate metabolism from fatty acid oxidation to glucose oxidation may improve myocardial mechanical efficiency by decreasing the amount of O₂ required for ATP synthesis. There is an ~12% decrease in O₂ required for ATP synthesis in shifting from 100% palmitate oxidation to 100% glucose oxidation (12), although such extreme metabolic shifts are unlikely to occur under physiological conditions.

Recent experimental studies are consistent in showing that the partial inhibition of fatty acid oxidation results in stimulation of myocardial glucose oxidation and reduction in lactate production during DII. However no consistent improvement in the mechanical function was associated with these seemingly beneficial metabolic changes (24-27), suggesting that different mechanisms underlie contractile dysfunction in the setting of stunning and DII. To address this issue further, we have compared the effects of sole direct activation of carbohydrate...
oxidation, using DCA, with the effect of interventions known to shift cardiac substrate metabolism from fatty acid oxidation to glucose oxidation in our model of DII.

Neither DCA and trimetazidine nor palmitate-free perfusion affected basal cardiac functional characteristics in our model, implying that the effects of these interventions were attributable to their metabolic rather than hemodynamic actions.

The results of this study demonstrate that DCA applied before DII, strongly increased the rate of glucose oxidation at pre-ischemia and during DII and moderately reduced lactate production during DII with no improvement in mechanical function compared with untreated group. Earlier studies have demonstrated that the stimulation of glycolysis and glucose oxidation stimulation during pre-ischemia and during DII with hyperglycemia resulted in no mechanical improvement in the swine model of DII (25). Likewise, DCA, applied during a mild low-flow ischemia (30% reduction in LAD flow in pig), did not improve contractile function despite that it increased myocardial glucose utilization and depressed lactate production during ischemia (41). Altogether, these DCA experiments (and those with trimetazidine discussed later) add support to the notion (25) that sole increasing myocardial glucose oxidation (DCA had no effect on palmitate oxidation in our model) and/or improving the balance between glycolysis and glucose oxidation before and during DII is not beneficial in the setting of DII itself.

Trimetazidine was used in this study in 5 μM. This and smaller concentrations have been shown to shift cardiac energy metabolism from fatty acid oxidation to glucose oxidation and to mitigate stunning in the isolated rat hearts perfused with exogenous palmitate (28, 30, 31). In our model trimetazidine failed to improve contractile dysfunction during DII, although it stimulated glucose oxidation before and during DII and clearly reduced myocardial lactate production during DII. The mechanism of these metabolic effects is uncertain, as, in contrast to some (28), but not all studies (31), trimetazidine had no effect on palmitate oxidation in our system. Nevertheless, the metabolic effects of trimetazidine are compatible with improved coupling between glycolysis and glucose oxidation by this agent and further support the notion that this effect is not beneficial in the setting of DII, although it is beneficial in the setting of the post-ischemic stunning (12).

The removal of fatty acids from the perfusate is one of the best ways to depress fatty acid oxidation and simultaneously to stimulate PDH and glucose oxidation in isolated rat hearts (27, 29). In our expanded hearts subjected to palmitate-free perfusion there was even more dramatic reduction in lactate production during DII, compared to DCA and trimetazidine groups perfused with palmitate nevertheless there was still no improvement in the mechanical function during DII.

DCA has been shown to stimulate PDH activity in isolated rat hearts perfused without fatty acids (42) suggesting that PDH stimulation by DCA adds to the stimulation resulting from fatty acid-free perfusion. In the expanded hearts subjected to palmitate-free perfusion plus DCA, DII-induced lactate overproduction and the deterioration of mechanical efficiency were prevented. The only marker of DII that remained was the deterioration of LVDP that was, however, less expressed compared to other DII groups. Thus DCA-mediated protection is clearly dependent on the absence of the exogenous fatty acids. Hypothetical mechanism of this effect would be that DCA effectively overcomes PDH inhibition by fatty acid only when the inhibition is as weak as that exerted by endogenous fatty acids.

Although we did not directly measure glucose oxidation in the palmitate-free perfused hearts, our results suggest that: (i) the mechanism underlying lactate overproduction during DII involves fatty acid-mediated inhibition of glucose oxidation resulting in the imbalance between glycolysis and glucose oxidation; (ii) lactate accumulating in the myocardium, as a result of this mechanism, is not a primary factor adversely affecting cardiac mechanical function during DII; (iii) shifting cardiac energy metabolism from fatty acid oxidation to glucose oxidation alone is not beneficial either and (iv) only combined drastic inhibition of fatty acid oxidation and stimulation of glucose oxidation appeared beneficial in the setting of DII, however, the resulting extreme metabolic shifts are unlikely to be easily achieved under clinical conditions, particularly in states like CHD and heart failure in which fatty acid blood levels are frequently elevated (43).

Clinical implications

The results of this and previous studies (24-27) are consistent in showing that the metabolic interventions, including inhibition of fatty acid oxidation, can stimulate glucose oxidation hence reducing myocardial lactate production during DII. Such metabolic changes have been generally proved to be beneficial in the setting of the myocardial stunning (12, 14). They, however, appeared not to improve function in the setting of DII, both in this and some previous studies (25, 26). It is unclear how these seemingly negative experimental results can be reconciled with the fact that the metabolic therapies can improve the symptoms of patients with exercise-induced angina (12-14, 17, 18) and can improve myocardial contractility in patients with chronic heart failure (20-23). As hypothesized already before (25, 26), it is possible that the metabolic modulators by reducing lactate and H+ production during DII improve such consequences of DII like ST segment elevation and chest pain even without improving mechanical function. On the other hand, it is possible that the changes induced by the metabolic modulators during DII may result in less stunning during the rep erfusion following episodes of DII. In the presence of severely reduced CFR, repeated episodes of DII may occur in daily life that are followed by stunning that is cumulative. If chronic stunning contributes to chronic LV dysfunction (1, 11, 44), heart failure might be ameliorated by intervening on stunning, but not necessary on DII itself. Indeed, the inhibitors of fatty acid oxidation have been generally proved protective in experimental models of stunning, no matter if they were administered before (28, 30, 45) or after low-flow or zero-flow ischemia (29, 45). Likewise, increasing glycolytic substrates to the myocardium (e.g., with DCA) during low-flow ischemia (46-48) or during reperfusion only (15, 16), but not before and/or during zero-flow ischemia (15, 49, 50) can reduce stunning in isolated hearts.

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