

Trimetazidine Normalizes Postischemic Function of Hypertrophied Rat Hearts

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ABSTRACT

The fraction of glucose passing through glycolysis that is oxidized is low in hypertrophied hearts, a pattern of glucose use associated with poor postischemic contractile function. We tested the hypothesis that trimetazidine, a partial 3-ketoacyl coenzyme A thiolase inhibitor, would stimulate glucose oxidation and, thereby, improve fractional glucose oxidation and postischemic function of hypertrophied hearts. Function, glycolysis, and oxidation of glucose, lactate, and palmitate were measured before and after global no-flow ischemia in isolated working hearts from sham-operated (control) and aortic-constricted (hypertrophied) male Sprague-Dawley rats in the presence or absence of 1 μ M trimetazidine. Heart function was significantly improved by trimetazidine after ischemia, but only

in hypertrophied hearts, with function improving to values in untreated control hearts. This effect occurred in association with relatively minor changes in oxidative metabolism. However, trimetazidine reduced glycolysis by ~30% but did so only in hypertrophied hearts, an unexpected novel action of this agent that resulted in a larger fractional oxidation of glucose, effectively normalizing it in hypertrophied hearts. Thus, trimetazidine normalizes postischemic function and fractional glucose oxidation in hypertrophied hearts, mainly by reducing glycolysis. These data extend the potential usefulness of trimetazidine and provide support for its use as a means to improve postischemic function of pressure overload hypertrophied hearts.

Left ventricular dysfunction after ischemia is greater in hearts hypertrophied in response to pressure overload than in nonhypertrophied hearts (Anderson et al., 1990; Wambolt et al., 2000). During the hypertrophic response to pressure overload, changes occur in the myocytes, vasculature, and interstitium of the heart (Frohlich et al., 1992; Swyngedauw, 1999), many of which may be responsible for the detrimental effects of hypertrophy on the outcome after ischemia. Alteration in myocardial energy metabolism, particularly glucose metabolism, is one of the changes in myocytes thought to contribute to the increased susceptibility of hypertrophied hearts to dysfunction following ischemia and reperfusion (Anderson et al., 1990; Wambolt et al., 2000; Sambandam et al., 2002).

Glucose utilization is enhanced in hearts exposed to prolonged pressure overload (Allard et al., 1994; Wambolt et al., 2000; Hajri et al., 2001; Sambandam et al., 2002). However,

flux through the different pathways responsible for the catabolism of glucose is not uniformly enhanced in the hypertrophied heart. Glycolysis is accelerated in hypertrophied hearts before, during, and after ischemia (Allard et al., 1994; Wambolt et al., 1999, 2000; Sambandam et al., 2002; Vincent et al., 2003), possibly in response to a reduced energy reserve (Nascimben et al., 2004) and/or low rates of fatty acid oxidation (Hajri et al., 2001; Tian, 2003). Despite the acceleration of glycolysis and low fatty acid oxidation, glucose oxidation is not correspondingly increased in hypertrophied hearts (Allard et al., 1994; Sambandam et al., 2002) and may, in fact, be lower than in nonhypertrophied hearts (Wambolt et al., 2000). As a result, the fraction of glucose passing through glycolysis that is oxidized (i.e., fractional oxidation of glucose) is lower in hypertrophied hearts than in nonhypertrophied hearts (Wambolt et al., 2000).

In nonhypertrophied hearts, low rates of glucose oxidation and reduced fractional oxidation of glucose are considered by some to be detrimental during reperfusion after ischemia (Lopaschuk et al., 1993; Stanley et al., 1997). Stimulation of glucose oxidation, which improves fractional oxidation of glu-

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ABBREVIATIONS: TMZ, trimetazidine.

cose, has been shown to be beneficial to the nonhypertrophied heart during reperfusion after ischemia (Lopaschuk et al., 1993; Stanley et al., 1997). In a similar way, pharmacologically increasing fractional glucose oxidation (by stimulating glucose oxidation and, in some cases, by reducing glycolysis) improves function of hypertrophied hearts, especially after ischemia (Wambolt et al., 2000). It has recently been demonstrated that fractional oxidation of glucose is enhanced in rat hearts hypertrophied by exercise training, a metabolic alteration accompanied by an improved functional outcome after ischemia (Burelle et al., 2004). Taken together, these data suggest that low rates of glucose oxidation, high glycolytic rates, and the resultant low fractional oxidation of glucose contribute to the increased postischemic dysfunction of hypertrophied hearts compared with nonhypertrophied hearts.

On the basis of these data, we speculate that any agent capable of stimulating glucose oxidation will have a beneficial effect on postischemic function of the hypertrophied heart. Trimetazidine [TMZ; 1-((2,3,4-trimethoxyphenyl) methyl) piperazine], a drug with documented antianginal activity (Stanley and Marzilli, 2003), is one such agent. Trimetazidine is a pharmacological agent that partially but selectively inhibits the fatty acid β -oxidation enzyme 3-keto-acyl-CoA thiolase in a reversible competitive manner (Kantor et al., 2000; Lopaschuk et al., 2003), whose inhibition of 3-keto-acyl-CoA thiolase may be overcome by high supraphysiologic concentrations of substrate (MacInnes et al., 2003). As a consequence, trimetazidine reduces rates of fatty acid oxidation leading to a compensatory stimulation of glucose oxidation in the heart (Kantor et al., 2000; Lopaschuk et al., 2003).

In the experiments described in this report, we tested the hypothesis that trimetazidine improves postischemic function of ischemic-reperfused isolated working hypertrophied rat hearts by stimulating glucose oxidation and improving the fractional oxidation of glucose. Function, glycolysis, and oxidation of glucose, lactate, and palmitate were measured before and after global no-flow ischemia in isolated working hearts from sham-operated and aortic-constricted male Sprague-Dawley rats in the presence or absence of trimetazidine.

Materials and Methods

Animal Model. A mild pressure overload cardiac hypertrophy (25 to 30% increased heart weight) was produced by constriction of the suprarenal abdominal aorta of halothane-anesthetized 3-week-old male Sprague-Dawley rats by means of a metallic clip (Allard et al., 1994). Sham-operated nonhypertrophied rats had the aorta isolated but not clipped. Hearts were studied 8 weeks after surgery. Food and water were provided ad libitum. These experiments were approved by the institutional committee on the use of laboratory animals in research and were in accordance with the Canadian Council on Animal Care and the Guide for Care and Use of Laboratory Animals as adopted by the U.S. National Institutes of Health.

Isolated Heart Preparation and Perfusion Protocol. Isolated, working hearts from halothane (2–3%) anesthetized sham-operated and aortic-constricted rats were perfused with Krebs-Henseleit solution in the working heart mode at a left atrial preload of 11.5 mm Hg and an aortic afterload of 80 mm Hg, as previously described (Allard et al., 1994). Hearts in both groups were perfused under similar conditions to avoid potentially confounding effects of exposure to different afterloads. Although hearts from aortic-constricted rats are exposed to elevated afterloads in vivo, we have

shown that adjusting afterload to normalize coronary flow per gram in isolated working hypertrophied hearts does not influence the functional or metabolic outcomes observed after ischemia (Wambolt et al., 2001). The Krebs-Henseleit solution (100 ml), which was oxygenated with 95% O₂/5% CO₂ and maintained at 37°C throughout the perfusion, was continuously circulated through the closed perfusion system and contained 1.2 mM palmitate prebound to fatty acid-free albumin (3%), 5.5 mM glucose, 0.5 mM lactate, 2.0 mM calcium, and 100 mU/l insulin. In all heart perfusions, we simultaneously measured rates of myocardial substrate utilization and heart function, including heart rate, peak systolic pressure, and cardiac output, as described (Allard et al., 1994; Wambolt et al., 2000). A pressure transducer (Viggo-Spectramed, Oxnard, CA) inserted in the afterload line was used to measure heart rate and peak systolic pressure. Cardiac output and aortic flow were measured via external flow probes (Transonic Systems, Ithaca, NY) on the left atrial preload and aortic afterload lines, respectively. Coronary flow was calculated as the difference between cardiac output and aortic flow. Rate-pressure product, the product of heart rate and peak systolic pressure, and hydraulic work, the product of cardiac output and peak systolic pressure, were used to measure external work performed by the heart (Allard et al., 1994; Wambolt et al., 2000; Burelle et al., 2004).

Hearts were initially perfused under normoxic nonischemic conditions for 30 min followed by a 20-min global no-flow ischemia (induced by clamping both the left atrial preload and aortic afterload lines). At the end of ischemia, the clamps were removed and the hearts were reperfused for 40 min. Vehicle or 1 μ M trimetazidine was added to the perfusate at the onset of nonischemic perfusion. This concentration of TMZ was chosen because it has been shown to stimulate glucose oxidation in normal rat hearts and is comparable with the clinically efficacious concentration range (Kantor et al., 2000). Heart function and samples for determination of substrate utilization were taken every 10 min, except after ischemia when function was assessed at 2 and 5 min as well. Hearts were freeze-clamped by tongs chilled to the temperature of liquid nitrogen at the end of reperfusion and weighed with a portion of ventricular tissue taken to determine the dry-to-wet weight tissue ratio.

Measurement of Myocardial Substrate Utilization. Myocardial substrate utilization was measured in two parallel series of experiments. In one series, rates of glycolysis and glucose oxidation were determined simultaneously by perfusing the hearts with [5-³H]glucose and [U-¹⁴C]glucose, respectively, as previously described (Allard et al., 1994). In a second series, palmitate and lactate oxidation were measured simultaneously by perfusing the hearts with solution containing [9, 10-³H]palmitate and [U-¹⁴C]lactate, respectively (Allard et al., 1994). Rates of glucose and lactate oxidation were measured by quantitative collection of ¹⁴CO₂ released as a gas and dissolved in the perfusate as [¹⁴C]bicarbonate. Rates of glycolysis and palmitate oxidation were determined by quantitatively measuring the rate of ³H₂O production. Perfusate and gaseous samples were taken from perfusion and were ultimately placed in vials containing scintillation cocktail and counted by standard techniques. Rates of substrate utilization were normalized to heart weight. Preischemic and postischemic values for all metabolic rates were calculated based on data collected between 10 and 30 min of normoxia and between 10 and 40 min of reperfusion. Fractional oxidation of glucose (or the percentage of glucose passing through the glycolysis that is oxidized) was calculated as the quotient of glucose oxidation and glycolysis multiplied by 100. Rates of nonoxidative glycolysis (i.e., glucose catabolized to lactate) were calculated as the difference between rates of glycolysis and glucose oxidation.

Proton Production from Glucose Metabolism. The stoichiometry of net proton (H⁺) production during nonoxidative glycolysis, as described by Hochachka and Mommsen (1983), indicates that 2 mol of H⁺ are produced per mole of glucose when the ATP formed is hydrolyzed. In contrast, glucose passing through glycolysis and glucose oxidation to form CO₂ yields 0 mol of H⁺ per mole of glucose.

Therefore, the overall rate of H^+ ion production derived from glucose catabolism was determined as follows (Lopaschuk et al., 1993; Burrelle et al., 2004):

$$J_{H^+} = 2 \times (J_{\text{glycolysis}} - J_{\text{GO}}) \quad (1)$$

Where J_{H^+} is the net rate of proton production from glucose utilization expressed in $\text{nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ dry wt., $J_{\text{glycolysis}}$ is the glycolytic flux, and J_{GO} is the rate of glucose oxidation.

Data Analysis. Values are expressed as mean \pm S.E.M. Weight data were analyzed using a one-way analysis of variance. Function and metabolism were examined using the two-way repeated measures analysis of variance. Post hoc tests with Bonferroni adjustments were applied to determine the source of differences among groups. A corrected p value of > 0.05 was considered as nonsignificant.

Results

Morphological Data

Morphological data are summarized in Table 1. Heart weight and the ratio of heart weight to body weight were significantly increased approximately 20% in aortic-constricted rats compared with sham-operated rats, indicative of a mild degree of cardiac hypertrophy. Body weight did not differ among the groups.

Contractile Function and Coronary Flow

In the absence of trimetazidine, hypertrophied hearts did not function as well as control hearts prior to ischemia. Specifically, cardiac output, hydraulic work, and rates of coronary flow in hypertrophied hearts were lower than in control hearts (Table 2). After ischemia, all parameters of function except for heart rate in hypertrophied hearts and heart rate and coronary flow in control hearts were significantly lower than corresponding values before ischemia in both groups (Table 2). Postischemic functional parameters of untreated hypertrophied hearts were lower than in untreated control hearts, but only cardiac output, hydraulic work, and coronary flow were significantly lower (Table 2).

Trimetazidine had beneficial effects in hypertrophied hearts prior to ischemia, causing a significant increase in cardiac output (Table 2). Trimetazidine had no measurable effect on coronary flow in either group before ischemia but increased coronary flow after ischemia in hypertrophied hearts. Trimetazidine also had significant beneficial effects on postischemic function in hypertrophied hearts (Table 2). Of note, parameters of function in hypertrophied hearts treated with trimetazidine no longer differed significantly from those in untreated control hearts (Table 2).

Myocardial Substrate Utilization

Glycolysis. Rates of glycolysis were significantly greater in hypertrophied hearts than in control hearts before and

after ischemia in the absence of trimetazidine (Fig. 1A). Trimetazidine significantly reduced rates of glycolysis before and after ischemia, but did so only in hypertrophied hearts (Fig. 1A). Postischemic glycolytic rates were lower than rates before ischemia in both groups in the presence or absence of trimetazidine.

Glucose Oxidation. In the absence of trimetazidine, glucose oxidation rates did not differ between control and hypertrophied hearts before or after ischemia (Fig. 1B). Glucose oxidation rates after ischemia did not differ from those before ischemia in either group. Compared with rates in corresponding, untreated control hearts, trimetazidine increased rates of glucose oxidation in both groups (Fig. 1B). However, this increase was significant only after ischemia.

Palmitate Oxidation. Palmitate oxidation rates were lower in hypertrophied hearts than in control hearts before and after ischemia in the absence of trimetazidine (Fig. 1C), achieving statistical significance only prior to ischemia. Although trimetazidine appeared to lower rates of palmitate oxidation in control hearts, this was not statistically significant (Fig. 1C). Trimetazidine did not significantly alter oxidation of palmitate in hypertrophied hearts before or after ischemia (Fig. 1C). In the presence of trimetazidine, palmitate oxidation in hypertrophied hearts was significantly lower than in control hearts, but only before ischemia (Fig. 1C). Rates of palmitate oxidation after ischemia did not differ significantly from rates before ischemia in the presence or absence of trimetazidine in either group.

Lactate Oxidation. Lactate oxidation rates in hypertrophied hearts were lower than rates in control hearts before and after ischemia in the presence or absence of trimetazidine (Fig. 1D). Rates of lactate oxidation before ischemia did not differ from rates after ischemia in either group, and trimetazidine did not alter rates of lactate oxidation significantly in either group before or after ischemia.

ATP Production from Substrate Catabolism. The relative contribution of individual substrates to ATP production was similar before and after ischemia in both groups (Fig. 2). Fatty acid oxidation was the predominant source of ATP, contributing slightly but insignificantly less in hypertrophied hearts than in control hearts, whereas ATP production from glycolysis was accelerated in hypertrophied hearts. Trimetazidine reduced the contribution of fatty acid oxidation slightly in both groups, but this difference was not statistically significant. In contrast, the contribution of glycolysis to ATP production in hypertrophied hearts was significantly reduced by trimetazidine. The contribution to ATP production by oxidation of glucose and lactate was not significantly altered by trimetazidine in either group. The tendency of an increased contribution of carbohydrate oxidation to ATP production in hearts exposed to trimetazidine may be a reflex-

TABLE 1

Morphologic data of hearts from sham-operated control and aortic-constricted rats

Values are mean \pm S.E.M.

	Control (n = 36)	Control + TMZ (n = 34)	Hypertrophy (n = 33)	Hypertrophy + TMZ (n = 33)
Heart weight (g)	1.86 \pm 0.02	1.86 \pm 0.04	2.23 \pm 0.04 ^{a,b}	2.18 \pm 0.04 ^{a,b}
Body weight (g)	451 \pm 5	456 \pm 7	453 \pm 10	451 \pm 7
Heart weight/body weight (g/kg)	4.12 \pm 0.04	4.09 \pm 0.08	4.95 \pm 0.09 ^{a,b}	4.83 \pm 0.05 ^{a,b}

^a Versus control, $p < 0.05$.

^b Versus control + TMZ, $p < 0.05$.

TABLE 2
Pre- and postischemic function of control and hypertrophied hearts in the presence and absence of trimetazidine
Values expressed as mean \pm S.E.M. are taken at 30' preischemia (Pre-I) and 40' postischemia (Post-I).

	Control		Control + TMZ		Hypertrophy		Hypertrophy + TMZ	
	Pre-I (n = 36)	Post-I (n = 27)	Pre-I (n = 34)	Post-I (n = 28)	Pre-I (n = 33)	Post-I (n = 21)	Pre-I (n = 21)	Post-I (n = 17)
Heart rate (bpm)	253 \pm 4	261 \pm 5	254 \pm 5	260 \pm 4	248 \pm 5	242 \pm 10	241 \pm 5	258 \pm 7
Peak systolic pressure (mm Hg)	111.2 \pm 1.4	99.9 \pm 1.6 ^d	114.3 \pm 1.2	104.4 \pm 1.3 ^{a,d}	109.5 \pm 1.5 ^b	95.2 \pm 1.9 ^d	111.6 \pm 2.1	103.4 \pm 1.6 ^{c,d}
Cardiac output (ml/min)	68.9 \pm 2.1	47.3 \pm 3.4 ^d	74.9 \pm 2.1	57.0 \pm 3.3 ^{a,d}	56.2 \pm 2.0 ^{a,b}	30.6 \pm 3.8 ^{a,b,d}	63.8 \pm 2.3 ^{b,c}	48.6 \pm 3.1 ^{c,d}
Coronary flow (ml/min/g wet wt.)	15.8 \pm 1.0	12.6 \pm 1.1 ^d	15.0 \pm 0.7	13.1 \pm 0.7 ^d	10.5 \pm 0.4 ^{a,b}	6.2 \pm 0.7 ^{a,b,d}	10.5 \pm 0.4 ^{a,b}	8.9 \pm 0.6 ^{a-d}
Rate pressure product (bpm \times mm Hg $\times 10^{-3}$)	28.2 \pm 0.6	26.1 \pm 0.7 ^d	28.9 \pm 0.5	27.1 \pm 0.6 ^d	27.1 \pm 0.6 ^b	23.1 \pm 1.1 ^{a,b,d}	26.8 \pm 0.5 ^b	26.6 \pm 0.7 ^c
Hydraulic power (mW)	12.9 \pm 0.4	8.6 \pm 0.6 ^d	14.1 \pm 0.4 ^a	10.4 \pm 0.6 ^a	10.8 \pm 0.4 ^{a,b}	5.3 \pm 0.7 ^{a,b,d}	12.0 \pm 0.4 ^{b,c}	9.0 \pm 0.7 ^{c,d}

^a Versus control, $p < 0.05$.

^b Versus control + TMZ, $p < 0.05$.

^c Versus hypertrophy, $p < 0.05$.

^d Versus Pre-I value, $p < 0.05$.

tion of increased workload, as the modest elevation in glucose oxidation rates in postischemic trimetazidine-treated groups disappears when normalized for contractile work performed (data not shown).

Fractional Oxidation of Glucose and Nonoxidative Glycolysis. Fractional oxidation of glucose was lower in hypertrophied hearts than in control hearts before and after ischemia in the absence of trimetazidine (Table 3), achieving significance only after ischemia. The fraction of glucose oxidized after ischemia tended to be higher than the fraction oxidized before ischemia in both groups without trimetazidine but was significantly higher only in control hearts. Trimetazidine increased fractional oxidation of glucose in both groups before and after ischemia (Table 3). In keeping with the preceding data, nonoxidative glycolysis was higher in hypertrophied hearts than in control hearts before and after ischemia. Exposure to trimetazidine led to significant reductions in nonoxidative glycolysis, but only in hypertrophied hearts (Table 3). Of interest, rates of nonoxidative glycolysis showed a strong inverse relationship to postischemic function (Fig. 3), the only metabolic parameter to demonstrate such a relationship (data not shown).

Myocardial Proton Production from Glucose Catabolism

Rates of proton production were significantly and substantially higher in hypertrophied hearts than in control hearts in the absence of trimetazidine before (8447 ± 463 versus 5007 ± 383 nmol/min/g dry wt.; $p < 0.05$) and after ischemia (5496 ± 374 versus 2590 ± 218 nmol/min/g dry wt.; $p < 0.05$). Proton production after ischemia was lower than that before ischemia in both groups ($p < 0.05$). Trimetazidine significantly lowered proton production in hypertrophied hearts before (5475 ± 474 versus 8447 ± 463 nmol/min/g dry wt.; $p < 0.05$) and after (3496 ± 377 versus 5496 ± 374 nmol/min/g dry wt.; $p < 0.05$) ischemia. Proton production was not significantly altered by trimetazidine in control hearts before (4127 ± 491 nmol/min/g dry wt.) or after (2137 ± 307 nmol/min/g dry wt.).

Discussion

In this study, we show for the first time that trimetazidine normalized postischemic function of hypertrophied hearts. This effect occurred in association with relatively minor alterations in oxidative energy metabolism. However, trimetazidine unexpectedly inhibited glycolysis but did so only in hypertrophied hearts, representing a previously unrecognized action of trimetazidine that possibly contributed to the improved postischemic of hypertrophied hearts. The results of the current investigation extend the potential usefulness of trimetazidine in the treatment of ischemic heart disease to include the amelioration of postischemic left ventricular dysfunction in the setting of cardiac hypertrophy, a beneficial effect of significance because cardiac hypertrophy and coronary artery disease are both highly prevalent conditions that commonly coexist (Benjamin and Levy, 1999; Kannel, 2000).

Trimetazidine and Contractile Function. Postischemic function of hypertrophied hearts was improved by trimetazidine to a value comparable with that in untreated nonhypertrophied hearts (Table 2). In other words, postischemic function of hypertrophied hearts was normalized by trimetazidine. Beneficial effects of trimetazidine during isch-

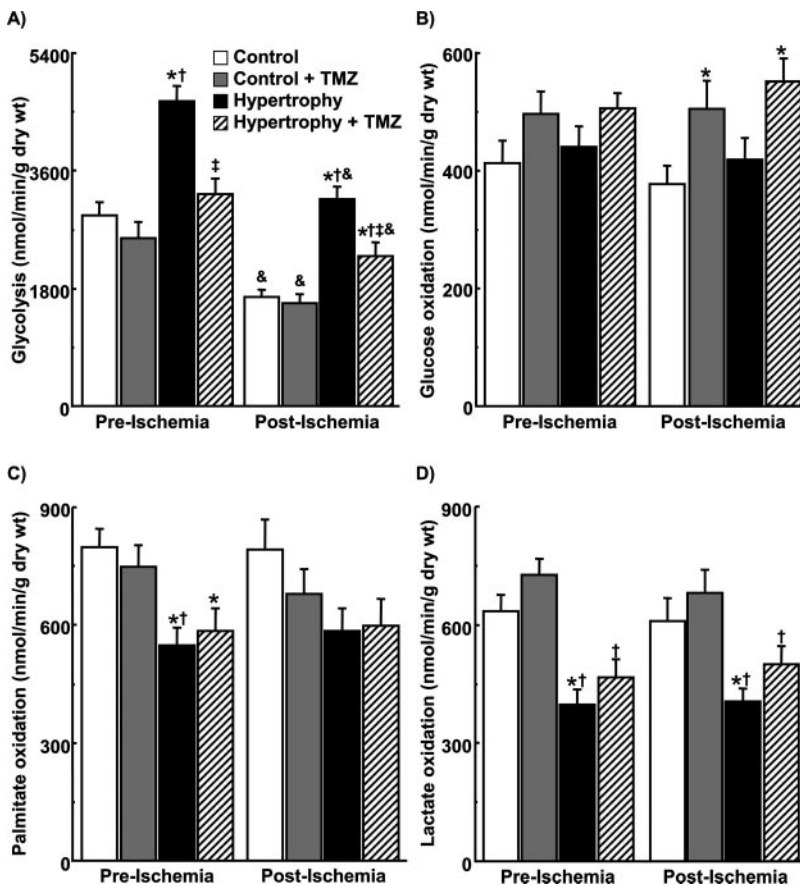


Fig. 1. Rates of glycolysis (A), glucose oxidation (B), palmitate oxidation (C), and lactate oxidation (D) in control and hypertrophied hearts before (preischemia) and after (postischemia) ischemia. Values are mean \pm S.E.M. $n = 9$ to 20 per group. *, versus control hearts, $p < 0.05$. †, versus control hearts + TMZ hearts, $p < 0.05$. ‡, versus hypertrophied hearts, $p < 0.05$. &, versus corresponding preischemic value, $p < 0.05$.

emia and reperfusion have been observed by others in experimental (Rahman et al., 1989; Guarnieri and Muscari, 1993; Boucher et al., 1994; Allibardi et al., 1998; El Banani et al., 1998, 2000) and clinical (Anonymous, 2000; Steg et al., 2001) studies. That significant functional improvement after ischemia was only observed in hypertrophied hearts suggests that trimetazidine is especially beneficial to the hypertrophied heart. This view is consistent with that of others (Guarnieri and Muscari, 1993) who found that the protective effects of trimetazidine against ischemia-induced damage to mitochondria and oxidative injury were more prominent in hypertrophied hearts.

Trimetazidine and Myocardial Substrate Utilization.

Changes in myocardial substrate utilization accompanied the functional improvements produced by trimetazidine in hypertrophied hearts (Fig. 1, A–D). To our surprise, changes in absolute rates of oxidative metabolism produced by trimetazidine were relatively minor. Compared with untreated nonhypertrophied hearts, oxidation of glucose was stimulated in both hypertrophied and nonhypertrophied hearts after ischemia (Fig. 1B). In the absence of significant changes in fatty acid oxidation rates, it is possible that the enhanced oxidation of glucose observed in trimetazidine-treated hearts during reperfusion was a consequence of the increased workload accompanying improved functional recovery.

Palmitate oxidation was not elevated during reperfusion in either hypertrophied or nonhypertrophied hearts, a finding that may be due to presence of high concentrations of palmitate in the perfusate before and after ischemia. Rates of palmitate oxidation in hypertrophied and nonhypertrophied hearts were not significantly altered by trimetazidine (Fig.

1C). This finding differs from that of others (Kantor et al., 2000) who found that palmitate oxidation was decreased in response to a comparable dose of trimetazidine. The reason for this discrepancy is unclear but may be related to differing experimental conditions. In the study by Kantor et al., palmitate oxidation was reduced by trimetazidine in hearts perfused with a lower concentration of palmitate (0.4 mM) than that used in the present study (1.2 mM). The effect of trimetazidine on palmitate oxidation in hearts perfused with 1.2 mM palmitate was not assessed in their study. That trimetazidine had a functionally beneficial effect in hypertrophied hearts is somewhat surprising since fatty acid oxidation is already suppressed in this setting (Fig. 1C). Further suppression of fatty acid oxidation by trimetazidine could potentially have had detrimental functional consequences, given that impaired fatty acid oxidation is a characteristic feature of failing hearts (Sack et al., 1996; Davila-Roman et al., 2002). However, our data indicate that trimetazidine is not detrimental to contractile function of hypertrophied hearts, possibly because fatty acid oxidation was not significantly suppressed further by trimetazidine.

Trimetazidine did not significantly alter glycolytic rates in nonhypertrophied hearts (Fig. 1A), in keeping with previous results in isolated nonhypertrophied rat hearts (Boucher et al., 1994; Kantor et al., 2000). In contrast, trimetazidine significantly reduced rates of glycolysis in hypertrophied hearts (Fig. 1A), a finding that, to our knowledge, has not yet been reported. The mechanistic explanation for this observation is unknown at this time and remains a challenge for future investigations. It is worth noting that dichloroacetate, an agent that stimulates myocardial glucose oxidation in

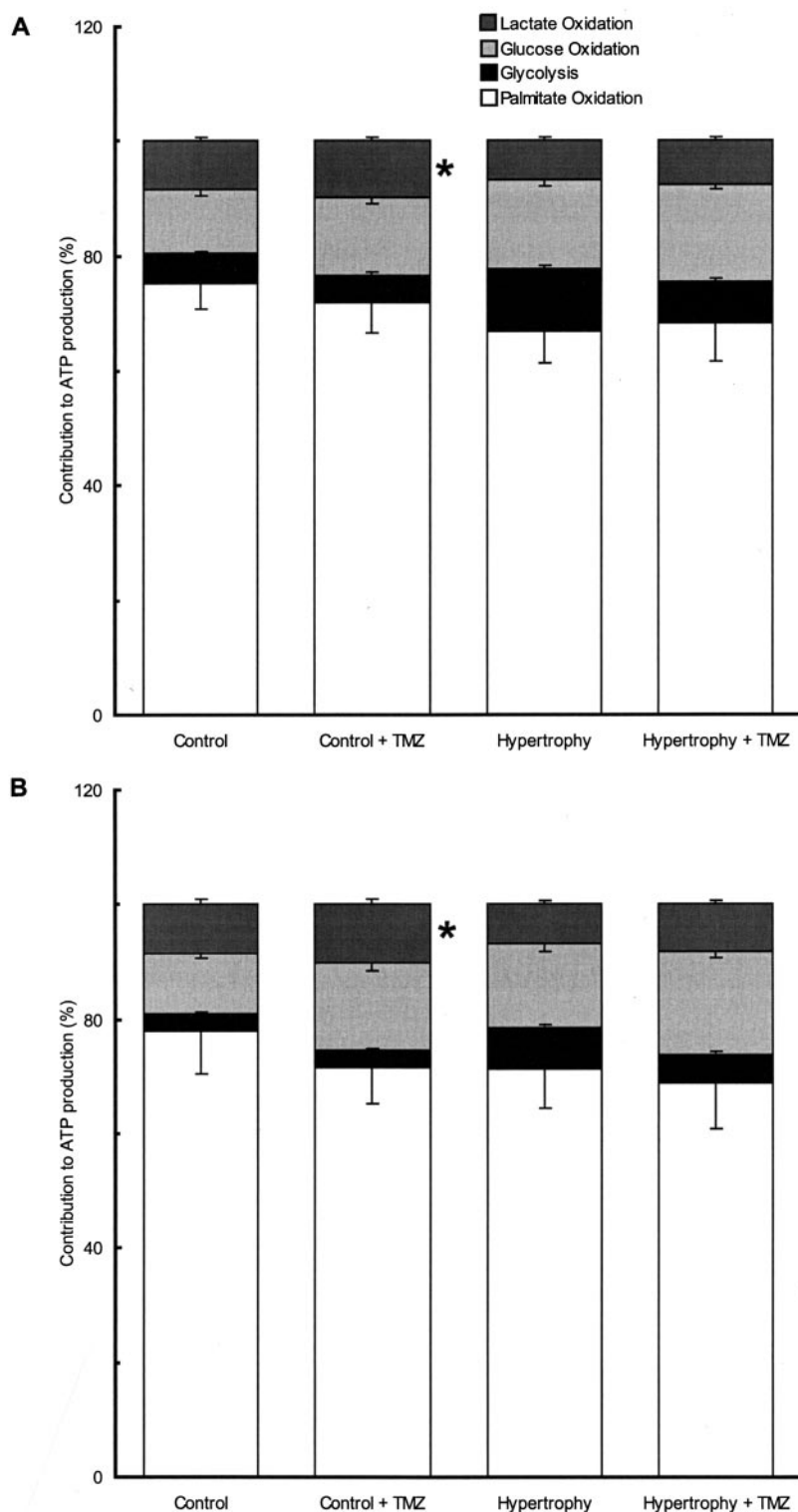


Fig. 2. Relative contribution of substrate catabolism to ATP production in control and hypertrophied hearts in the presence or absence of TMZ before (A) and after (B) ischemia. Rates of ATP production were calculated by assuming that 129 mol of ATP were produced per mole of palmitate oxidized, 36 mol of ATP were produced per mole of glucose oxidized, 18 mol of ATP were produced per mole of lactate oxidized, and 2 mol were produced for each mole of glucose catabolized by glycolysis. *, versus relative ATP production from glycolysis in control hearts, $p < 0.05$.

hypertrophied (Wambolt et al., 2000) and nonhypertrophied hearts by a different mechanism (Stanley et al., 1997), also inhibits myocardial glycolysis but does so only in hypertrophied hearts (Wambolt et al., 2000). As with trimetazidine, the mechanisms responsible are not yet known.

Trimetazidine, Myocardial Glucose Metabolism, and Contractile Function. In the absence of trimetazidine, the fraction of glucose passing through glycolysis that was oxidized was substantially lower in hypertro-

phied hearts than in nonhypertrophied hearts, even though glycolysis is increased (Table 3). This finding is characteristic of hypertrophied hearts, regardless of whether or not glucose oxidation rates are significantly lower than nonhypertrophied hearts (for review, see Sambandam et al., 2002). A corollary of this observation is that nonoxidative catabolism of glucose is accelerated in hypertrophied hearts (Table 3). Evidence from experimental and clinical studies suggests that the catabolic fate of glucose

TABLE 3
Fractional glucose oxidation and nonoxidative glycolysis
Values are mean \pm S.E.M.

	Control		Control + TMZ		Hypertrophy		Hypertrophy + TMZ	
	Pre-I (n = 16)	Post-I (n = 12)	Pre-I (n = 15)	Post-I (n = 11)	Pre-I (n = 14)	Post-I (n = 8)	Pre-I (n = 11)	Post-I (n = 8)
Nonoxidative glycolysis (nmol/min/g dry wt.)	2504 \pm 191	1295 \pm 109 ^d	2063 \pm 245	1069 \pm 154 ^d	4224 \pm 231 ^{a,b}	2748 \pm 187 ^{a,b,d}	2737 \pm 237 ^b	1748 \pm 188 ^{b,c,d}
Fractional glucose oxidation (%)	15 \pm 1	24 \pm 2 ^d	21 \pm 2 ^a	34 \pm 3 ^{a,d}	10 \pm 1 ^{a,b}	14 \pm 2 ^{a,b}	16 \pm 1 ^{b,c}	25 \pm 2 ^{b,c,d}

Pre-I, preischemia; Post-I, postischemia.

^a Versus control, $p < 0.05$.

^b Versus control + TMZ, $p < 0.05$.

^c Versus hypertrophy, $p < 0.05$.

^d Versus Pre-I value, $p < 0.05$.

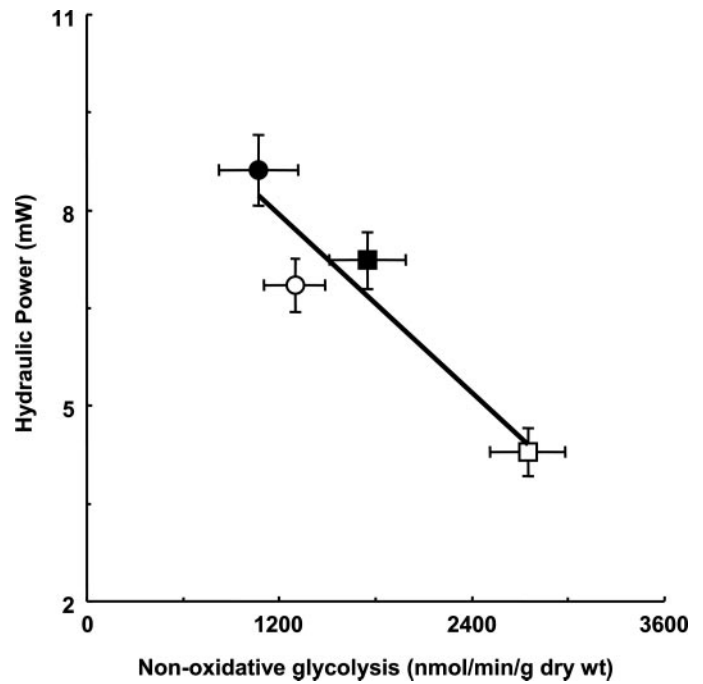


Fig. 3. Relationship of postischemic contractile function to rates of non-oxidative glycolysis in control (circle) and hypertrophied (square) hearts in the presence (solid) or absence (open) of trimetazidine. Nonoxidative glycolysis was calculated as the difference between rates of glycolysis and glucose oxidation. Regression analysis was performed using data from individual hearts ($n = 39$ hearts; $R = -0.60$, $p < 0.05$) and is expressed as mean data per group ($n = 8$ to 16 per group).

is an important determinant of postischemic myocardial function in nonhypertrophied and hypertrophied hearts (Stanley et al., 1997; Wambolt et al., 2000; Sambandam et al., 2002). Our data, which demonstrate a significant inverse relationship between nonoxidative glycolysis and function during reperfusion (Fig. 3), are consistent with this concept and implicate changes in glycolysis induced by trimetazidine in hypertrophied hearts as contributing to the beneficial effects on function observed.

That a reduction in glycolysis during reperfusion is beneficial is inconsistent with the results of others who found pharmacologic inhibition of glycolysis to be detrimental during reperfusion after ischemia (Jeremy et al., 1993), presumably because glycolytic ATP is important for maintenance of sarcolemmal ion pumps (Dizon et al., 1998). On the other hand, the beneficial effect of dichloroacetate to postischemic function of pressure overload hypertrophied hearts (Wambolt et al., 2000) and the cardioprotective effect of exercise training (Burrelle et al., 2004) were both associated with mild to moderate reductions in glycolysis, observations in keeping with those in the current investigation. Taken together, these data indicate that a reduction in glycolysis during reperfusion is not always detrimental and may in fact be associated with a beneficial outcome.

How a decrease in nonoxidative glycolysis improves contractile function during reperfusion of hypertrophied hearts is not immediately apparent. However, based upon the different proton stoichiometry of glucose fermentation compared with oxidation (Hochachka and Mommsen, 1983; Lopaschuk et al., 1993), one possibility is that it is related to alteration in net proton production (Stanley et al.,

1997). Higher rates of proton production during reperfusion lead to increased myocyte calcium overload caused by successive *trans*-sarcolemmal H^+/Na^+ and Na^+/Ca^{2+} exchanges, which in turn causes increased energetic cost associated with the maintenance of ion homeostasis and in doing so, leads to decreased postischemic contractile function and efficiency (Hata et al., 1994; Stanley et al., 1997; Liu et al., 2002). Such changes could also enhance the depression of contractile function due to pH-dependent alterations in the sensitivity of contractile proteins to Ca^{2+} (Liu et al., 2002). By contributing to alterations in ion homeostasis as described above, accelerated proton production may be a factor contributing to the poor outcome of hypertrophied hearts after ischemia in the absence of trimetazidine (Sambandam et al., 2002).

Proton production in the hypertrophied heart was dramatically reduced by trimetazidine, in large part because glycolysis was reduced (Fig. 2A). This reduction in H^+ production, and its resultant effects on myocardial ion homeostasis, might have a role, in part, in the functional improvements observed in trimetazidine-treated hypertrophied hearts, particularly during reperfusion. In keeping with this concept, trimetazidine has been shown to reduce acidosis and sodium ion accumulation in isolated rat hearts exposed to ischemia and reperfusion (Lavanchy et al., 1987; El Banani et al., 2000). Further support comes from the finding that stimulation of glucose oxidation during reperfusion in working non-hypertrophied rat hearts reduces calculated net proton production from glucose catabolism, causes a more rapid recovery of intracellular pH as measured by ^{31}P NMR, and improves postischemic recovery (Liu et al., 2002). However, it is important to recognize that proton production was calculated and not directly measured in the current investigation. Direct measurement of changes in H^+ as well as Na^+ and Ca^{2+} concentrations during reperfusion of hypertrophied hearts in the presence and absence of trimetazidine will be required in future studies to determine whether this view is correct.

Methodological Considerations. It is important to recognize that the findings in this study pertain specifically to the substrate and hormonal milieu used and that extrapolation to other settings such as patients with ischemic heart disease must be made with caution. In the current investigation, higher than normal concentrations of long-chain fatty acid, which is the major energy substrate of the heart, were used in an attempt to at least partially reproduce conditions present during and after myocardial ischemia. However, it should be recognized that concentrations of this key energy substrate vary widely under these conditions (Lopaschuk et al., 1994) and that fatty acids are not the only substrate whose concentration changes. For instance, concentration of glucose and lactate has also been observed to change during acute myocardial ischemia and after cardiac surgery, especially when cardiac dysfunction is severe (Chiolero et al., 2000; Schreiber et al., 2002). Additionally, elevations in hormones, whose actions can alter myocardial substrate utilization, may occur under the same conditions.

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