

The Antianginal Drug Trimetazidine Shifts Cardiac Energy Metabolism From Fatty Acid Oxidation to Glucose Oxidation by Inhibiting Mitochondrial Long-Chain 3-Ketoacyl Coenzyme A Thiolase

Paul F. Kantor, Arnaud Lucien, Raymond Kozak, Gary D. Lopaschuk

Abstract—Trimetazidine is a clinically effective antianginal agent that has no negative inotropic or vasodilator properties. Although it is thought to have direct cytoprotective actions on the myocardium, the mechanism(s) by which this occurs is as yet undefined. In this study, we determined what effects trimetazidine has on both fatty acid and glucose metabolism in isolated working rat hearts and on the activities of various enzymes involved in fatty acid oxidation. Hearts were perfused with Krebs-Henseleit solution containing 100 μ U/mL insulin, 3% albumin, 5 mmol/L glucose, and fatty acids of different chain lengths. Both glucose and fatty acids were appropriately radiolabeled with either ^3H or ^{14}C for measurement of glycolysis, glucose oxidation, and fatty acid oxidation. Trimetazidine had no effect on myocardial oxygen consumption or cardiac work under any aerobic perfusion condition used. In hearts perfused with 5 mmol/L glucose and 0.4 mmol/L palmitate, trimetazidine decreased the rate of palmitate oxidation from 488 ± 24 to 408 ± 15 nmol \cdot g dry weight $^{-1} \cdot$ minute $^{-1}$ ($P < 0.05$), whereas it increased rates of glucose oxidation from 1889 ± 119 to 2378 ± 166 nmol \cdot g dry weight $^{-1} \cdot$ minute $^{-1}$ ($P < 0.05$). In hearts subjected to low-flow ischemia, trimetazidine resulted in a 210% increase in glucose oxidation rates. In both aerobic and ischemic hearts, glycolytic rates were unaltered by trimetazidine. The effects of trimetazidine on glucose oxidation were accompanied by a 37% increase in the active form of pyruvate dehydrogenase, the rate-limiting enzyme for glucose oxidation. No effect of trimetazidine was observed on glycolysis, glucose oxidation, fatty acid oxidation, or active pyruvate dehydrogenase when palmitate was substituted with 0.8 mmol/L octanoate or 1.6 mmol/L butyrate, suggesting that trimetazidine directly inhibits long-chain fatty acid oxidation. This reduction in fatty acid oxidation was accompanied by a significant decrease in the activity of the long-chain isoform of the last enzyme involved in fatty acid β -oxidation, 3-ketoacyl coenzyme A (CoA) thiolase activity (IC_{50} of 75 nmol/L). In contrast, concentrations of trimetazidine in excess of 10 and 100 μ mol/L were needed to inhibit the medium- and short-chain forms of 3-ketoacyl CoA thiolase, respectively. Previous studies have shown that inhibition of fatty acid oxidation and stimulation of glucose oxidation can protect the ischemic heart. Therefore, our data suggest that the antianginal effects of trimetazidine may occur because of an inhibition of long-chain 3-ketoacyl CoA thiolase activity, which results in a reduction in fatty acid oxidation and a stimulation of glucose oxidation. (*Circ Res.* 2000;86:580-588.)

Key Words: glycolysis ■ mitochondria ■ trimetazidine

Trimetazidine (1-[2,3,4-trimethoxybenzyl] piperazine dihydrochloride) is a clinically effective antianginal agent. In several double-blind trials, trimetazidine was shown to improve the ergometric exercise capacity and total work output of patients with effort angina,^{1,2} to reduce attack frequency and nitroglycerin requirement in patients with chronic stable angina,³ and to increase effort tolerance in angina patients.⁴ Multicenter trials of trimetazidine by a European collaborative working group have demonstrated that the antianginal efficacy of trimetazidine is equivalent to

that of propranolol but does not reduce cardiac rate-pressure product or coronary blood flow.⁵ A similar lack of alteration of hemodynamic parameters with comparable antianginal effect was noted in comparison with nifedipine.⁶ In combination with diltiazem, additive antianginal effects were also observed.^{7,8} Trimetazidine has thus been considered unique among antianginal agents for its lack of vasodilator activity and has been termed a “cellular anti-ischemic agent (p 292).”⁹ It is presently clinically used throughout Europe and in >80 countries worldwide.

Received December 2, 1999; accepted January 20, 2000.

From the Cardiovascular Research Group and the Division of Pediatric Cardiology (P.F.K., R.K., G.D.L.), University of Alberta, Edmonton, Canada, and Institut De Recherches Internationales Servier (A.L.), Courbevoie, France.

Correspondence to Gary D. Lopaschuk, 423 Heritage Medical Research Center, University of Alberta, Edmonton, Canada, T6G 2S2. E-mail gary.lopaschuk@ualberta.ca

© 2000 American Heart Association, Inc.

Circulation Research is available at <http://www.circresaha.org>

Despite these clinical successes, the understanding of trimetazidine's mechanism of action remains incomplete. It is proposed to act by directly improving myocardial energy metabolism, resulting in cytoprotective effects in several models of myocardial ischemia.^{9–11} How could trimetazidine improve myocardial energy balance? Switching energy substrate preference from fatty acid oxidation to glucose oxidation is one possible means of improving cardiac function during ischemia and reperfusion, as well as improving both cardiac energetics and cardiac efficiency (see References 12 through 15 for reviews). A study by Fantini et al¹⁶ has shown in isolated rat heart mitochondria that trimetazidine has a potent inhibitory effect on palmitoylcarnitine oxidation, with no significant effect on pyruvate oxidation. This indirect evidence suggests that trimetazidine may act to inhibit fatty acid oxidation in the heart. Consistent with this, we have recently shown that another structurally similar piperazine derivative, ranolazine, also increases glucose oxidation in isolated working rat hearts, secondary to an inhibition of fatty acid oxidation.¹⁷

This indirect evidence suggests that trimetazidine may exert its antianginal effects by inhibiting fatty acid oxidation. High fatty acid oxidation rates are detrimental in the setting of ischemia and reperfusion because of an inhibition of glucose oxidation.^{18,19} This leads to an increase in proton production due to an uncoupling of glycolysis from glucose oxidation.^{19,20} This increase in proton production has the potential to accelerate sodium and calcium overload in the heart,²¹ resulting in an exacerbation of ischemic injury and a decrease in cardiac efficiency during reperfusion.^{14,15,19,20} Despite the observation that trimetazidine both improves cardiac energetics^{10,11,16} and attenuates proton and sodium accumulation during and after ischemia,²² it has not been directly determined what effect trimetazidine has on myocardial energy metabolism.

The purpose of this study was to determine whether trimetazidine exerts a direct effect on glycolysis, glucose oxidation, or fatty acid oxidation in the heart and to determine at what site trimetazidine alters energy metabolism in the heart. We demonstrate that trimetazidine potently inhibits long-chain 3-ketoacyl coenzyme A (CoA) thiolase in the heart, resulting in a reduction in fatty acid oxidation and an increase in glucose oxidation. This stimulation of glucose oxidation may explain the cardioprotective effect of trimetazidine seen in fatty acid-perfused ischemic rat hearts.

Materials and Methods

Heart Perfusions

Isolated working hearts from male Sprague-Dawley rats were subjected to a 60-minute aerobic perfusion period with a modified Krebs-Henseleit solution containing 5 mmol/L glucose; 100 μ U/mL insulin; 3% BSA; and (in mmol/L) palmitate (16-carbon) 0.4, octanoate (8-carbon) 0.8, or butyrate (4-carbon) 1.6. Working hearts were used in these studies to approximate the metabolic demand of the heart seen *in vivo*.

An additional series of hearts perfused with 5 mmol/L glucose, 100 μ U/mL insulin and 0.4 mmol/L palmitate were subjected to a 30-minute perfusion, after which they were switched to the Langendorff mode and subjected to a 60-minute period of low-flow ischemia (coronary flow=0.5 mL/min). Hearts were switched to the

Langendorff mode during ischemia to obtain a constant coronary flow rate during ischemia.

Another series of hearts were perfused with Krebs-Henseleit perfusate containing 11 mmol/L glucose, 100 μ U/mL insulin, and 1.2 mmol/L palmitate.

Spontaneously beating hearts were perfused at an 11.5-mm Hg left atrial preload and an 80-mm Hg aortic afterload. Heart rate, peak systolic pressure, developed pressure, cardiac output, aortic flow, coronary flow, cardiac work, and O₂ consumption were measured as described previously.^{18,19,23}

Trimetazidine, when present, was added 5 minutes into the perfusion period at the concentrations indicated in the tables and figures.

Glycolysis and glucose oxidation rates were determined simultaneously by the quantitative collection of ³H₂O and ¹⁴CO₂ produced by hearts perfused with buffer containing [5-³H/U-¹⁴]glucose.¹⁹ Rates of fatty acid oxidation were assessed during identical parallel heart perfusions using [1-¹⁴C]palmitate, [1-¹⁴C]octanoate, or [1-¹⁴C]butyrate. Palmitate, octanoate, or butyrate oxidation was determined by quantitatively collecting ¹⁴CO₂ production as described above for glucose oxidation.

The rate of acetyl CoA production calculated from tricarboxylic acid (TCA)-cycle activity was as described previously.¹⁹ TCA-cycle efficiency was defined as the ratio of cardiac work to TCA-cycle acetyl CoA produced.

Biochemical Analysis

Analysis of active and total pyruvate dehydrogenase (PDHa and PDHt, respectively) was as described previously.^{23,24} Activities of long-chain, medium-chain, and short-chain acyl CoA dehydrogenase in isolated rat heart mitochondria were measured as described by Grimbert et al.²⁵ The long-, medium- and short-chain enoyl CoA hydratase activities were assayed as described by Wanders et al.²⁶ Long-, medium, and short-chain L-3-hydroxyacyl CoA dehydrogenase and 3-ketoacyl CoA thiolase were measured in membrane and soluble mitochondrial fractions as described by Venizelos et al.²⁷

CoA esters were extracted from the frozen powdered heart tissue and quantified by high-performance liquid chromatography (HPLC).^{28,29} Myocardial AMP-activated protein kinase (AMPK), acetyl CoA carboxylase (ACC), malonyl CoA carboxylase, and triacylglycerols were measured as described previously.^{29–31}

Statistical Analysis

All data are represented as the group mean \pm SEM. Data were analyzed using the statistical program Instat. One-way ANOVA was used to compare values among groups. A Tukey-Grammar post hoc test was used to confirm intergroup differences. $P < 0.05$ was considered significant.

An expanded Materials and Methods section is available online at <http://www.circresaha.org>.

Results

Effect of Trimetazidine on Cardiac Function

Table 1 online (see <http://www.circresaha.org>) shows the effect of trimetazidine (1 μ mol/L) on cardiac function in isolated working rat hearts perfused in the presence of glucose as well as palmitate, octanoate, or butyrate. Regardless of the type of fatty acid substrate used, the presence of 1 μ mol/L trimetazidine did not cause any significant change in heart rate, peak systolic pressure, developed pressure, cardiac output, or cardiac work. This confirms earlier studies demonstrating that trimetazidine is not an inotropic agent and demonstrates that any changes in energy substrate metabolism in the heart are not occurring secondary to effects of trimetazidine on cardiac function. Trimetazidine also did not significantly alter O₂

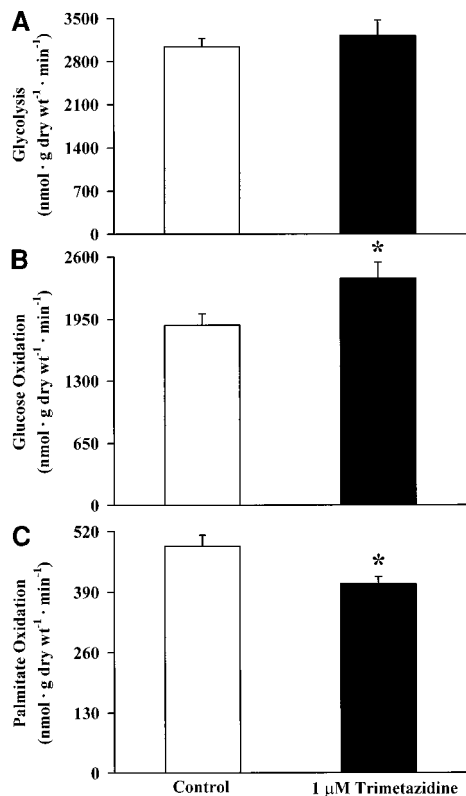


Figure 1. Effect of trimetazidine ($1 \mu\text{mol/L}$) on metabolic flux through glycolysis (A), glucose oxidation (B), and fatty acid oxidation (C) in hearts perfused with 5 mmol/L glucose and 0.4 mmol/L palmitate as substrate. $*P < 0.05$ vs control. $n = 10$ hearts in each group.

consumption in any of the perfusion groups. Trimetazidine also did not have any effect on coronary artery flow either, which argues against a primary vasodilatory effect of this drug in the normoxic setting.

Effects of Trimetazidine on Energy Metabolism

The effect of trimetazidine on glucose and fatty acid utilization rates in the presence of a physiological level of long-chain fatty acid substrate (palmitate 0.4 mmol/L) is demonstrated in Figure 1A through 1C. Rates of glycolysis were unaltered by trimetazidine, although there was a significant increase in glucose oxidation rates. In these hearts, glycolytic rates were significantly greater than glucose oxidation rates (Figure 1A and 1B). As a result, the selective increase in glucose oxidation rates resulted in an improved coupling between glycolysis and glucose oxidation. The increase in glucose oxidation rates in the trimetazidine hearts was accompanied by a significant decrease in palmitate oxidation rates (Figure 1C).

The effects of trimetazidine on glycolysis and glucose oxidation in hearts subjected to low-flow ischemia (coronary flow = 0.5 mL/min) are shown in Figure 2. During ischemia, glycolytic rates in control hearts were only slightly lower than rates observed under aerobic conditions (Figure 1A). However, as expected, a substantial decrease in glucose oxidation rates was observed during ischemia (Figures 2 and 1B). Addition of trimetazidine had no effect

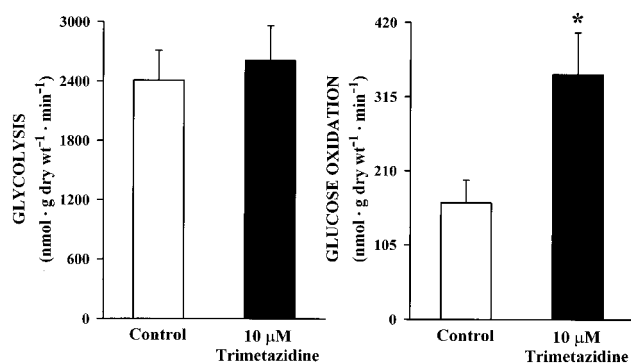


Figure 2. Effect of trimetazidine on metabolic flux through glycolysis (A) and glucose oxidation (B) in ischemic hearts perfused with 5 mmol/L glucose and 0.4 mmol/L palmitate. Ischemia was produced by switching hearts to the Langendorff mode and perfusing hearts at a coronary flow rate of 0.5 mL/min for 60 minutes. $*P < 0.05$ vs control. $n = 10$ hearts in each group.

on glycolytic rates but did result in a significant increase in glucose oxidation rates.

To determine whether the inhibitory effects of trimetazidine on fatty acid oxidation were specific for long-chain fatty acids, energy metabolism was also measured in hearts perfused with octanoate (an 8-carbon fatty acid) (Figure 3) and butyrate (a 4-carbon fatty acid) (Figure 4). In octanoate-perfused hearts, trimetazidine did not have any significant effect on glycolysis, glucose oxidation, or octanoate oxidation. Similarly, in the presence of butyrate,

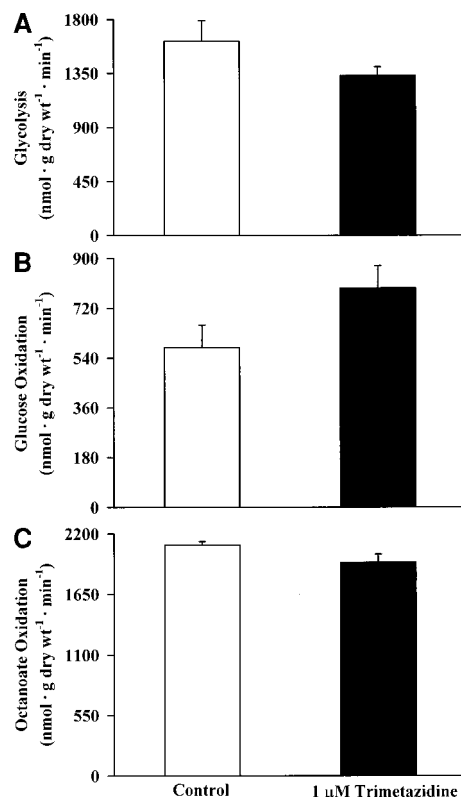


Figure 3. Effect of trimetazidine ($1 \mu\text{mol/L}$) on metabolic flux through glycolysis (A), glucose oxidation (B), and fatty acid oxidation (C) in hearts perfused with 5 mmol/L glucose and 0.8 mmol/L octanoate as substrate. $n = 10$ hearts in each group.

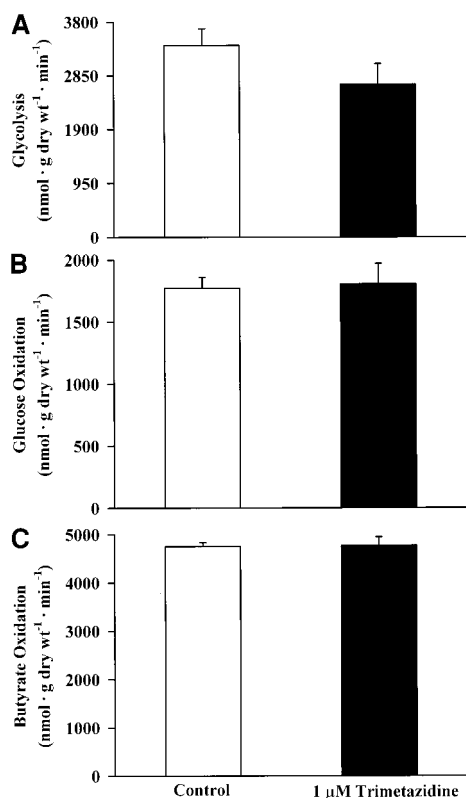


Figure 4. Effect of trimetazidine (1 $\mu\text{mol/L}$) on metabolic flux through glycolysis (A), glucose oxidation (B), and fatty acid oxidation (C) in hearts perfused with 5 mmol/L glucose and 1.6 mmol/L butyrate as substrate. $n=10$ hearts in each group.

trimetazidine was without effect on glycolysis, glucose oxidation, or butyrate oxidation. This suggests that trimetazidine specifically inhibits long-chain fatty acid oxidation and that the stimulation of glucose oxidation does not occur in the presence of shorter-chain fatty acids.

The effects of trimetazidine on cardiac work/TCA-cycle activity were also calculated to determine whether trimetazidine alters TCA-cycle efficiency in aerobically perfused hearts. As shown in Figure 1 online (see <http://www.circresaha.org>), no effect of trimetazidine on cardiac work/TCA-cycle activity was observed regardless of whether hearts were perfused with palmitate, octanoate, or butyrate. This suggests that the decrease in palmitate oxidation by trimetazidine is accompanied by a parallel increase in glucose oxidation.

We also determined whether trimetazidine was able to stimulate glucose oxidation in hearts perfused with high levels of fatty acids (Table 1). As expected, rates of glucose oxidation in untreated hearts were much lower in hearts perfused with 1.2 mmol/L palmitate ($347 \pm 58 \text{ nmol} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$) compared with hearts perfused with 0.4 mmol/L palmitate ($1889 \pm 119 \text{ nmol} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$) (Figure 1B). In contrast, rates of glycolysis were not affected by fatty acid concentration, confirming that fatty acids are much more potent inhibitors of glucose oxidation than of glycolysis. As shown in Table 1, even with glucose oxidation rates inhibited by high levels of fatty acids, trimetazidine was able to significantly stimulate glucose oxidation.

TABLE 1. Effect of Various Doses of Trimetazidine (TMZ) on Glycolysis and Glucose Oxidation in Isolated Working Rat Hearts Perfused With 1.2 mmol/L Palmitate

| Condition | Glycolysis ($\text{nmol} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$) | Glucose Oxidation ($\text{nmol} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$) |
|---------------------------|---|--|
| Control | 2976 ± 371 | 347 ± 58 |
| 0.1 $\mu\text{mol/L}$ TMZ | 3003 ± 282 | $520 \pm 61^*$ |
| 1 $\mu\text{mol/L}$ TMZ | 2919 ± 325 | $673 \pm 72^{*\dagger}$ |
| 10 $\mu\text{mol/L}$ TMZ | 2665 ± 252 | $697 \pm 98^{*\dagger}$ |

Values are mean \pm SEM of 7 hearts.

*Significantly different from control values.

†Significantly different from 0.1 $\mu\text{mol/L}$ TMZ values.

A significant stimulation of glucose oxidation was observed in the presence of 0.1 $\mu\text{mol/L}$ trimetazidine. A maximal stimulation of glucose oxidation was observed in the presence of 1 $\mu\text{mol/L}$ trimetazidine.

The heart contains a sizeable triacylglycerol pool that can contribute as a source of fatty acids for β -oxidation. We therefore measured triacylglycerol levels in the palmitate-, octanoate-, and butyrate-perfused hearts at the end of the 60-minute perfusion period. Trimetazidine had no effect on triacylglycerol levels in the palmitate-perfused hearts (145.3 ± 15.6 versus $150.6 \pm 38.4 \mu\text{mol fatty acid/g dry weight}$, in control and trimetazidine-treated hearts, respectively; $P=\text{NS}$). Under control conditions, triacylglycerol levels in octanoate- and butyrate-perfused hearts (88.1 ± 10.2 and $99.2 \pm 6.3 \mu\text{mol fatty acid/g dry weight}$, respectively) were lower than levels seen in palmitate-perfused hearts ($P<0.05$). The oxidation of unlabeled fatty acids released from triacylglycerol hydrolyzed from these groups would explain the slightly higher cardiac work/TCA-cycle activity observed in the octanoate- and butyrate-perfused hearts compared with the palmitate-perfused hearts (Figure 1 online, see <http://www.circresaha.org>). However, similar to the palmitate-perfused hearts, trimetazidine had no effect on triacylglycerol levels in the octanoate- and butyrate-perfused hearts (83.4 ± 6.6 and $104.9 \pm 17.2 \mu\text{mol fatty acid/g dry weight}$, respectively; $P=\text{NS}$ compared with the appropriate control perfusion group).

Effects of Trimetazidine on Pyruvate Dehydrogenase (PDH) Activity

PDH is the rate-limiting step for glucose oxidation and is inhibited when fatty acid oxidation rates are high (see Reference 14 for review). We therefore measured PDH activity in hearts perfused with 5 mmol/L glucose and 0.4 mmol/L palmitate (Table 2). In control hearts, the active unphosphorylated form of the enzyme (PDHa) was 18% as active as PDHt. These values are expected in hearts perfused with normal levels of fatty acids. In hearts perfused with 1 $\mu\text{mol/L}$ trimetazidine, a significant increase in PDHa was observed, with no difference in PDHt. As a result, a significant increase in the active-to-total ratio was observed. In hearts perfused with octanoate, in which no increase in glucose oxidation was observed, no significant difference in the PDHa (4.12 ± 0.48 and

TABLE 2. Effects of Trimetazidine (TMZ) on PDHa, PDHt activity, and Percentage of PDHa to PDHt in Hearts Perfused With 0.4 mmol/L Palmitate

| Condition | Active (PDHa) Acetyl CoA ($\mu\text{mol} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$) | Total (PDHt) Acetyl CoA ($\mu\text{mol} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$) | % Active of Total |
|-------------------------|---|--|-------------------|
| Control | 6.01 \pm 0.69 | 32.56 \pm 2.94 | 19 \pm 2 |
| 1 $\mu\text{mol/L}$ TMZ | 8.19 \pm 0.50* | 32.35 \pm 1.51 | 26 \pm 2* |

Values are mean \pm SEM of 10 hearts per group.

* P <0.05 vs control.

5.16 \pm 0.56 $\mu\text{mol acetyl CoA} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$, in control and trimetazidine-treated hearts, respectively; $n=10$ in each group) or PDHt was observed (23.9 \pm 2.1 and 22.4 \pm 2.6 $\mu\text{mol acetyl CoA} \cdot \text{g dry weight}^{-1} \cdot \text{minute}^{-1}$ in control and trimetazidine-treated hearts, respectively; $n=10$ in each group).

To determine whether the effects of trimetazidine were due to direct actions on PDH itself, a large concentration of trimetazidine (100 $\mu\text{mol/L}$) was added directly to the incubation medium used to measure PDHa and PDHt. As shown in Table 2 online (see <http://www.circresaha.org>), trimetazidine had no direct effect on either PDHa or PDHt, suggesting that the stimulation of PDHa seen in Table 2 online was an indirect effect on trimetazidine and not due to a direct stimulation of PDHa activity.

Effect of Trimetazidine on Levels of Malonyl CoA and the Enzymes That Control Malonyl CoA Levels

Malonyl CoA is a potent inhibitor of fatty acid oxidation due to inhibition of carnitine palmitoyltransferase (CPT) 1, the rate-limiting step in fatty acid oxidation. We therefore determined what effect trimetazidine had on malonyl CoA levels, because measurements of energy metabolism suggest that trimetazidine may directly inhibit fatty acid oxidation. The effect of trimetazidine on malonyl CoA levels was measured in hearts perfused with palmitate, octanoate, or butyrate. As

shown in Table 3 online (see <http://www.circresaha.org>), trimetazidine had no effect on malonyl CoA levels in any of these 3 perfusion groups.

Malonyl CoA in the heart is synthesized by ACC and degraded by malonyl CoA decarboxylase (MCD).³¹ We therefore determined what effect trimetazidine had on enzyme activity in the hearts perfused with palmitate, as well as what effect trimetazidine had directly on enzyme activity. Addition of trimetazidine to palmitate-perfused hearts did not result in any change in either ACC activity (8.23 \pm 0.4 and 6.43 \pm 0.35 $\text{nmol} \cdot \text{mg protein}^{-1} \cdot \text{minute}^{-1}$ [P =NS] in control and trimetazidine-treated hearts, respectively; $n=9$ in each group). Trimetazidine also had no effect on citrate-stimulatable ACC activity. It also had no effect on MCD activity (8.01 \pm 0.46 and 6.87 \pm 0.29 $\mu\text{mol} \cdot \text{mg protein}^{-1} \cdot \text{minute}^{-1}$ [P =NS], in control and trimetazidine-treated hearts, respectively; $n=9$ in each group). Although the cellular location of MCD has yet to be established (ie, some is probably intramitochondrial), our data suggest that trimetazidine does not alter fatty acid oxidation by altering MCD activity. Because ACC is also inhibited by AMPK, we also determined what effect trimetazidine had on AMPK activity in the palmitate-perfused hearts. Similar to ACC and MCD, no significant effect of trimetazidine on AMPK activity was observed (852 \pm 111 and 1022 \pm 191 $\text{pmol} \cdot \text{mg protein}^{-1} \cdot \text{minute}^{-1}$ [P =NS] in control and trimetazidine-treated hearts, respectively, $n=9$ in each group).

TABLE 3. Effects of Trimetazidine on Enzymes of β -Oxidation (Acyl-CoA Dehydrogenase, Enoyl-CoA Hydratase, and L-3-Hydroxyacyl-CoA Dehydrogenase) in Isolated Heart Mitochondria

| Enzyme Substrate | Control | 10 $\mu\text{mol/L}$ Trimetazidine |
|--|---|---|
| Acyl-CoA dehydrogenase activity | ($\Delta\text{OD}_{290} \cdot \text{minute}^{-1} \cdot \text{mg protein}^{-1}$) | |
| Palmitoyl CoA | 0.54 \pm 0.02 | 0.69 \pm 0.06 |
| Octanoyl CoA | 0.54 \pm 0.04 | 0.63 \pm 0.02 |
| Butyryl CoA | 0.79 \pm 0.08 | 0.73 \pm 0.08 |
| Enoyl-CoA hydratase activity | ($\Delta\text{OD}_{365} \cdot \text{minute}^{-1} \cdot \text{mg protein}^{-1}$) | |
| Hexadecenoyl CoA | 0.42 \pm 0.02 | 0.44 \pm 0.01 |
| Octanoyl CoA | 1.44 \pm 0.12 | 1.50 \pm 0.01 (75 $\mu\text{mol/L}$) |
| Crotonyl CoA | 2.36 \pm 0.45 | 1.98 \pm 0.14 |
| L-3-Hydroxyacyl-CoA dehydrogenase activity | ($\Delta\text{OD}_{340} \cdot \text{minute}^{-1} \cdot \text{mg protein}^{-1}$) | |
| 3-keto-hexadecenoyl CoA | 0.42 \pm 0.02 | 0.45 \pm 0.01 (75 $\mu\text{mol/L}$) |
| 3-keto-octanoyl CoA | 2.13 \pm 0.12 | 2.12 \pm 0.18 (75 $\mu\text{mol/L}$) |
| Acetoacetyl CoA | 0.063 \pm 0.008 | 0.079 \pm 0.015 |

Values are mean \pm SEM of at least 3 values per group. β -Oxidation enzyme activities were determined as described previously. OD indicates optical density.

Direct addition of 10 $\mu\text{mol/L}$ trimetazidine to the incubation medium was also without effect on the activity of ACC, MCD, or AMPK (data not shown).

Effect of Trimetazidine on Myocardial CoA Ester Levels

The effects of trimetazidine on other measured short-chain CoA esters in the palmitate-, octanoate-, and butyrate-perfused hearts are also shown in Table 3 online (see <http://www.circresaha.org>). No effect of trimetazidine was observed on any of the measured CoA esters. The lack of effect on succinyl CoA levels suggests that, despite the inhibition of fatty acid oxidation in palmitate-perfused hearts, the supply of carbon for the TCA cycle was not compromised.

The effects of trimetazidine on levels of long-chain CoA, acetyl CoA, and free CoA are shown in Table 4 online (see <http://www.circresaha.org>). As expected, levels of long-chain acyl CoA were highest in hearts perfused with palmitate, compared with either octanoate- or butyrate-perfused hearts. However, trimetazidine did not affect long-chain acyl CoA levels in any perfusion group. Similarly, no effect of trimetazidine on acetyl CoA levels was observed in any of the perfusion groups. Trimetazidine did result in a significant increase in free CoA in hearts perfused with palmitate, which would be expected if trimetazidine directly inhibited long-chain fatty acid β -oxidation. However, the ratio of acetyl CoA/CoA was not significantly affected by trimetazidine in any of the perfusion groups.

Effect of Trimetazidine on the Enzymes of Fatty Acid β -Oxidation

Because reduction of fatty acid oxidation by trimetazidine could not be explained by alterations in malonyl CoA control of mitochondrial fatty acid uptake, we directly measured the effects of trimetazidine on the enzymes of mitochondrial fatty acid β -oxidation. Table 3 summarizes the activities of long-, medium- and short-chain isoforms of acyl CoA dehydrogenase, enoyl CoA dehydrogenase, and L-3-hydroxyacyl CoA dehydrogenase activity. The presence of 10 or 75 $\mu\text{mol/L}$ trimetazidine did not have any significant effect on any of the isoforms for the first 3 enzymes involved in mitochondrial fatty acid β -oxidation. Concentration effects of trimetazidine on enzyme activities were also measured, which confirmed that trimetazidine did not alter the activity of any of these enzymes (data not shown).

The effect of trimetazidine on the long-, medium, and short-chain isoform of 3-ketoacyl CoA thiolase is shown in Figure 5. Trimetazidine caused a potent inhibition of long-chain 3-ketoacyl CoA thiolase, with an IC_{50} of ≈ 75 nmol/L. In contrast, a concentration of 10 $\mu\text{mol/L}$ trimetazidine was necessary to significantly inhibit the medium-chain 3-ketoacyl CoA thiolase, and concentrations in excess of 100 $\mu\text{mol/L}$ were necessary to inhibit the short-chain 3-ketoacyl CoA thiolase. In these experiments, 4-pentenoic acid, a nonselective inhibitor of 3-ketoacyl CoA thiolase, was used as a positive inhibitor control. As expected, 4-pentenoic acid inhibited the enzyme activities of all 3 isoforms of 3-ketoacyl CoA thiolase.

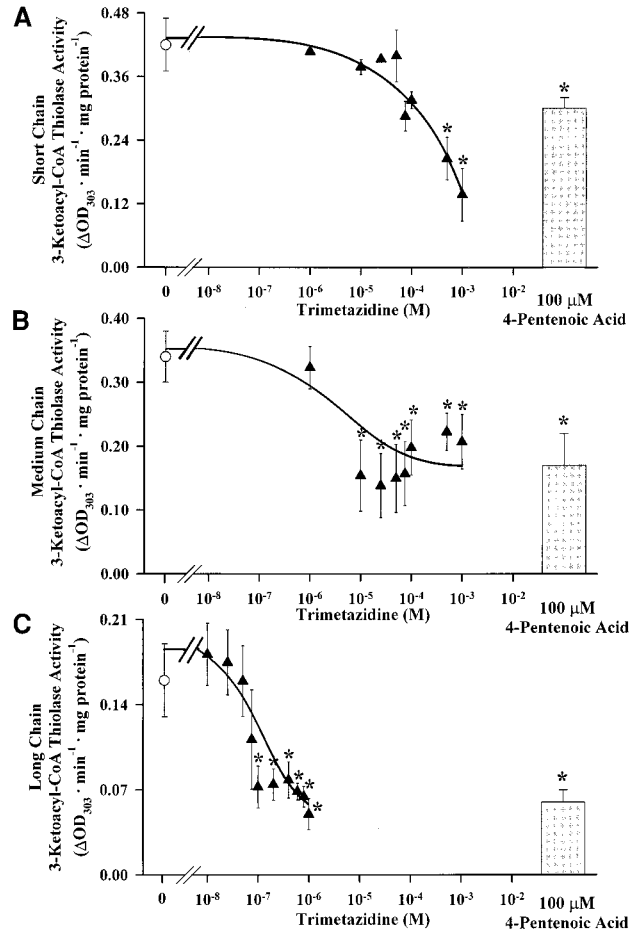


Figure 5. Direct effects of trimetazidine on rat heart mitochondrial short-chain (A), medium-chain (B), and long-chain (C) 3-ketoacyl CoA thiolase. Activity in the presence of 4-pentenoic acid (100 $\mu\text{mol/L}$), a nonselective 3-ketoacyl CoA thiolase inhibitor is shown in the shaded column ($n=5$ in each group). * $P<0.05$ vs no addition. $n=7$ determinations in each group.

Discussion

Although the clinical efficacy of trimetazidine as an anti-ischemic agent is undisputed, its cellular mechanism of action has not been previously delineated. In this study, we demonstrate that trimetazidine directly inhibits fatty acid oxidation in the heart, secondary to an inhibition of mitochondrial long-chain 3-ketoacyl CoA thiolase. The selective inhibition of the long-chain 3-ketoacyl CoA thiolase is consistent with a specific inhibition by trimetazidine of long-chain fatty acid oxidation and not medium- or short-chain fatty acids. This inhibition of long-chain fatty acid oxidation is accompanied by an increase in PDH activity, resulting in a significant increase in glucose oxidation rates. The increase in glucose oxidation is seen regardless of whether hearts are perfused with low or high concentrations of fatty acids and is also observed in hearts subjected to low-flow ischemia. Furthermore, trimetazidine is without effect on glycolytic rates, which will improve the coupling between glycolysis and glucose oxidation and a decrease in proton production from glucose metabolism. Because increasing glucose oxidation

and decreasing proton production from glucose metabolism is cardioprotective (see References 14 and 15 for reviews), we hypothesize that reduction in fatty acid oxidation is the mechanism by which trimetazidine exerts its antianginal effects. Our data also implicate inhibition of long-chain 3-ketoacyl CoA thiolase as the site of action for the beneficial anti-ischemic effects of trimetazidine. It also confirms that reduction in fatty acid oxidation is a novel approach to treating ischemic heart disease.

Trimetazidine and Myocardial Fatty Acid Oxidation

The demonstration that trimetazidine can inhibit fatty acid oxidation in palmitate-perfused hearts is consistent with the previous studies of Fantini et al¹⁶ using isolated mitochondrial preparations. These authors demonstrated that trimetazidine could markedly reduce *in vitro* mitochondrial respiratory activity but that this effect is dependent on the substrate offered to the mitochondria; ie, palmitoylcarnitine oxidation is markedly reduced, but not that of pyruvate, glutamate, or citrate.¹⁶ This suggests that trimetazidine is not inhibiting either TCA-cycle activity or mitochondrial oxidative phosphorylation, but rather is directly inhibiting fatty acid β -oxidation. This is supported by our data, in which we demonstrate that trimetazidine does not alter overall oxygen consumption in aerobically perfused hearts (Table 1 online, see <http://www.circresaha.org>), nor does it alter TCA-cycle activity or cardiac work/TCA-cycle activity (Figure 1 online, see <http://www.circresaha.org>). Rather, the effects of trimetazidine are associated with a switch in the source of acetyl CoA for the TCA cycle from fatty acid β -oxidation to glucose oxidation. This is not due to a direct stimulation of PDH, but rather an indirect stimulation of PDH secondary to a reduction in fatty acid oxidation. Because fatty acid β -oxidation rates are a key determinant of PDH activity,³² trimetazidine reduction in fatty acid oxidation was accompanied by an increase in glucose oxidation, with no overall decrease in the levels of TCA-cycle intermediates (Tables 3 and 4 online, see <http://www.circresaha.org>).

Mitochondrial uptake of fatty acids is another important site at which myocardial fatty acid oxidation can be inhibited. CPT 1 is an important enzyme in this process and is potently inhibited by malonyl CoA. Because long-chain fatty acid uptake by the mitochondria, and not medium- or short-chain fatty acid uptake, is dependent on CPT 1, it is possible that the effects of trimetazidine on fatty acid oxidation may be occurring secondary to an inhibition of CPT 1 activity. However, we believe this is unlikely for a number of reasons, as follows. (1) Previous studies have shown that trimetazidine does not directly inhibit CPT 1 activity.³³ (2) Inhibition of mitochondrial respiration of palmitoylcarnitine would not occur if trimetazidine were acting at the level of CPT 1.¹⁶ (3) CPT 1 inhibitors result in a decrease in long-chain acyl CoA levels, presumably because of a decrease in mitochondrial long-chain acyl CoA levels.³⁴ As shown in our studies, trimetazidine did not lower long-chain acyl CoA levels (Table 3 online [see <http://www.circresaha.org>]), which is consistent with a site of inhibition lower in the fatty acid β -oxidation pathway. (4) Trimetazidine did not alter the

levels of malonyl CoA under any perfusion condition, nor did it alter the activity of any of the enzymes involved in the regulation of malonyl CoA synthesis or degradation (ACC, MCD, or AMPK).

Combined, these data suggest that trimetazidine inhibits fatty acid oxidation directly in the β -oxidative pathway.

The β -oxidation of fatty acids utilizes 4 enzymes, including acyl CoA dehydrogenase, enoyl CoA hydratase, L-3-hydroxyacyl CoA dehydrogenase, and 3-ketoacyl CoA thiolase. Each of these enzymes exists as a number of different isoforms, with each isoform having a different specificity for fatty acyl-chain length (see Reference 35 for review). This allows complete oxidation of fatty acids as successive cycles shorten the fatty acyl esters. As shown in Table 3, trimetazidine did not inhibit any isoform of the first 3 β -oxidative enzymes, but was a potent inhibitor of long-chain 3-ketoacyl CoA thiolase (Figure 5), the enzyme catalyzing the last step in β -oxidation. The concentration of trimetazidine necessary to inhibit long-chain 3-ketoacyl CoA thiolase is consistent with the concentration of trimetazidine necessary to stimulate glucose oxidation in hearts perfused with high levels of fatty acids (Table 1) and is compatible with the clinically relevant plasma concentrations seen in patients taking trimetazidine for the treatment of angina.³⁶ Although trimetazidine also inhibited the medium- and short-chain isoforms of 3-ketoacyl CoA thiolase, the concentrations needed were much higher than clinically relevant concentrations. The observation that trimetazidine was ineffective at inhibiting either octanoate or butyrate oxidation is also consistent with an inhibition of long-chain 3-ketoacyl CoA thiolase, given that both of these substrates enter the β -oxidative pathway at a level beyond long-chain 3-ketoacyl CoA thiolase. The selective increase in free CoA by trimetazidine in palmitate-perfused hearts is also consistent with inhibition of long-chain 3-ketoacyl CoA thiolase.

In octanoate-perfused hearts, trimetazidine did result in a small, nonsignificant decrease in octanoate oxidation, as well as a small, nonsignificant increase in PDHa. A possible explanation for this may be that the long-chain 3-ketoacyl CoA thiolase may be metabolizing some of the octanoate. Although the long-chain 3-ketoacyl CoA thiolase prefers fatty acids of 10 carbons or greater in length, it can use 8-carbon-length fatty acids as a substrate (albeit with a lower affinity). Unfortunately, no study to date has clearly defined what proportion of medium-chain 3-ketoacyl CoAs are metabolized by the long- versus medium-chain 3-ketoacyl CoA thiolase *in vivo*. Therefore, it is possible that inhibition of the long-chain 3-ketoacyl CoA thiolase by trimetazidine could have some effect on overall octanoate oxidation rates (and therefore a minor effect on PDH activity).

Trimetazidine and Glucose Oxidation

Our data also show that stimulation of PDHa and glucose oxidation by trimetazidine does not occur by a direct effect on the PDH complex. This is because trimetazidine had no direct effect on PDHa, and in octanoate-perfused hearts trimetazidine had no effect on PDH activity. The PDH complex that activates and then translocates activated pyruvate into the mitochondrial matrix in the form of acetyl CoA is the

rate-limiting step for glucose oxidation by the TCA cycle. PDH activity itself is subject to various control mechanisms, including PDH kinase-mediated phosphorylation, which inactivates the enzyme, and PDH phosphatase-mediated dephosphorylation, which restores the active state.³² Flux of fatty acids through β -oxidation is closely linked to PDHa, because both reduced equivalents and accumulation of acetyl CoA from β -oxidation activate the kinase and decrease PDHa. In contrast, inhibition of fatty acid oxidation has the opposite effect of decreasing kinase activity, increasing PDHa, and stimulating glucose oxidation rates. As a result, trimetazidine reduction in fatty acid oxidation was accompanied by a significant increase in glucose oxidation rates.

Trimetazidine and Cardioprotection During and After Ischemia

Energy metabolism during ischemia and during reperfusion is closely linked to cardiac function, presenting the clinician an arena for possible intervention. Current approaches that are used to manipulate myocardial energy metabolism involve either stimulating glucose metabolism or inhibiting fatty acid metabolism. Numerous experimental studies have demonstrated that stimulation of glucose oxidation both during and after ischemia can benefit the ischemic heart (see References 14 and 15 for reviews). By improving the coupling of glycolysis to glucose oxidation, proton production is decreased, resulting in a decrease in tissue acidosis and an improvement in cardiac efficiency. As a result, selective stimulation of glucose oxidation by trimetazidine may explain the anti-ischemic effects of this agent, possibly by increasing the coupling between glycolysis and glucose oxidation, thereby decreasing proton production.

We propose that trimetazidine acts by reducing fatty acid oxidation, thereby increasing glucose oxidation before and during the ischemic event. This altered metabolic balance will improve the capacity of the ischemic heart to balance the dramatic increase in glycolytic flux with an increase in glucose oxidation. This strategy has been demonstrated to be effective in experimental as well as clinical settings using agents such as dichloroacetate or ranolazine, which stimulate glucose oxidation in the hearts.^{14,15,17}

In summary, we demonstrate that trimetazidine inhibits fatty acid oxidation secondary to an inhibition of long-chain 3-ketoacyl CoA thiolase, resulting in an increase in glucose oxidation. This results in an improved coupling of glycolysis with glucose oxidation, which has previously been shown to protect the ischemic heart. As a result, switching energy substrate preference from fatty acid oxidation to glucose oxidation may explain the antianginal properties of trimetazidine.

Acknowledgments

This work was funded by a grant from the Institut De Recherches Internationales Servier. P.F.K. is Clinical Fellow of the Heart and Stroke Foundation of Canada and the Alberta Heritage Foundation for Medical Research. G.D.L. is a Heritage Medical Scientist.

References

- Sellier P. The effects of trimetazidine on ergometric parameters in exercise-induced angina: controlled multicenter double blind versus placebo study. *Arch Mal Coeur Vaiss.* 1986;79:1331-1336.
- Sellier P, Audouin P, Payen B, Duong TC, Ourbak P. Ergometric effects of a single administration of trimetazidine. *Presse Med.* 1986;15:1771-1774.
- Passeron J. Effectiveness of trimetazidine in stable effort angina due to chronic coronary insufficiency: a double-blind versus placebo study. *Presse Med.* 1986;15:1775-1778.
- Gallet M. Clinical effectiveness of trimetazidine in stable effort angina: a double blind versus placebo controlled study. *Presse Med.* 1986;15:1779-1782.
- Detry JM, Sellier P, Pennaforte S, Cokkinos D, Dargie H, Mathes P. Trimetazidine: a new concept in the treatment of angina: comparison with propranolol in patients with stable angina. Trimetazidine European Multicenter Study Group. *Br J Clin Pharmacol.* 1994;3:279-288.
- Dalla-Volta S, Maraglino G, Della-Valentina P, Viena P, Desideri A. Comparison of trimetazidine with nifedipine in effort angina: a double-blind, crossover study. *Cardiovasc Drugs Ther.* 1990;4:853-859.
- Levy S, Group of South of France Investigators. Combination therapy of trimetazidine with diltiazem in patients with coronary artery disease. *Am J Cardiol.* 1995;76:12B-16B.
- Manchanda SC, Krishnaswani S. Combination treatment with trimetazidine and diltiazem in stable angina pectoris. *Heart.* 1997;78:353-357.
- Harpey C, Clauser P, Labrid C, Freyria JL, Poirier JP. Trimetazidine, a cellular anti-ischemic agent. *Cardiovasc Drug Rev.* 1989;6:292-312.
- Lavanchy N, Martin J, Rossi A. Anti-ischemic effects of trimetazidine: ³¹P NMR spectroscopy in the isolated rat heart. *Arch Int Pharmacodyn Ther.* 1987;286:97-110.
- Kay L, Finelli C, Aussedat J, Guarnieri C, Rossi A. Improvement of long term preservation of the isolated arrested rat heart by trimetazidine: effects of the energy state and mitochondrial function. *Am J Cardiol.* 1995;76:45B-49B.
- Opie LH. Metabolism of the heart in health and disease. *Am Heart J.* 1968;76:685-689.
- Taegetmeyer H. Energy metabolism in the heart: from basic concepts to clinical application. *Curr Prob Cardiol.* 1994;19:59-113.
- Lopaschuk GD, Belke DD, Gamble J, Itoi I, Schönekeess BO. Regulation of fatty acid oxidation in the mammalian heart in health and disease. *Biochim Biophys Acta.* 1994;1213:263-276.
- Stanley WC, Lopaschuk GD, Hall JL, McCormack JG. Regulation of myocardial carbohydrate metabolism under normal and ischemic conditions: potential for pharmacological interventions. *Cardiovasc Res.* 1997;33:243-257.
- Fantini E, Demaison L, Sentex E, Grynberg A, Athias P. Some biochemical aspects of the protective effect of Trimetazidine on rat cardiomyocytes during hypoxia and reoxygenation. *J Mol Cell Cardiol.* 1994;26:949-958.
- McCormack JG, Barr RL, Wolff AA, Lopaschuk GD. Ranolazine stimulates glucose oxidation in normoxic, ischemic and reperfused ischemic rat hearts. *Circulation.* 1996;93:135-142.
- Lopaschuk GD, Wambolt RB, Barr RL. An imbalance between glycolysis and glucose oxidation is a possible explanation for the detrimental effects of high levels of fatty acids during aerobic reperfusion of ischemic hearts. *J Pharmacol Exp Ther.* 1993;264:135-144.
- Liu B, el Alaoui-Talibi Z, Clanachan AS, Schulz R, Lopaschuk GD. Uncoupling of contractile function from mitochondrial TCA cycle activity and MVO₂ during reperfusion of ischemic hearts. *Am J Physiol.* 1996;270:H72-H80.
- Liu B, Clanachan AS, Schulz R, Lopaschuk GD. Cardiac efficiency is improved after ischemia by altering both the source and fate of protons. *Circ Res.* 1996;79:940-948.
- Pierce GN, Czubyrt MP. The contribution of ionic imbalance to ischemia/reperfusion-induced injury. *J Mol Cell Cardiol.* 1995;27:53-63.
- El Banani H, Bernard M, Baetz D, Cabanes E, Cozzone P, Lucien A, Feuvray D. Changes in nuclear magnetic resonance study of the effects of TMZ on intracellular Na⁺ and pH during ischemia and reperfusion. *J Mol Cell Cardiol.* 1999;31:A53. Abstract.
- Collins-Nakai RL, Noseworthy D, Lopaschuk GD. Epinephrine increases ATP production in hearts by preferentially increasing glucose metabolism. *Am J Physiol.* 1994;267:H1862-H1871.
- Constantin-Teodosiu D, Cederblad G, Hultman E. A sensitive radioisotopic assay of pyruvate dehydrogenase complex in human muscle tissue. *Anal Biochem.* 1991;198:347-351.
- Grimbert S, Fisch C, Deschamps D, Berson A, Fromenty B, Feldman G, Pessayre D. Effects of female sex hormones on mitochondria: possible role in acute fatty liver of pregnancy. *Am J Physiol.* 1995;268:G107-G115.
- Wanders RJA, Ijlst L, Poggi F, Bonnefont JP, Munnich A, Brivet M, Rabier D, Saudubray JM. Human trifunctional protein deficiency: a new

- disorder of mitochondrial fatty acid β -oxidation. *Biochem Biophys Res Commun*. 1992;188:1139–1145.
27. Venizelos N, Ijlst L, Wanders RJA, Hagenfeldt L. β -Oxidation enzymes in fibroblasts from patients with 3-hydroxydicarboxylic aciduria. *Pediatr Res*. 1994;36:111–114.
 28. Saddik M, Gamble J, Witters LA, Lopaschuk GD. Acetyl CoA carboxylase regulation of fatty acid oxidation in the heart. *J Biol Chem*. 1993;268:25836–25845.
 29. Kudo N, Barr A, Barr R, Desai S, Lopaschuk GD. High rates of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA levels due to an increase in 5'AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. *J Biol Chem*. 1995;270:17513–17520.
 30. Hardie DG, Salt IP, Hawley SA, Davies SP. AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. *Biochem J*. 1999;338:717–722.
 31. Dyck JRB, Barr AJ, Barr RL, Kolattukudy PE, Lopaschuk GD. Characterization of cardiac malonyl CoA decarboxylase and its putative role in regulating fatty acid oxidation. *Am J Physiol*. 1998;275:H2122–H2129.
 32. Randle PJ, Hales CN, Garland PB, Newsholme EA. The glucose fatty-acid cycle: its role in insulin sensitivity and the metabolic disturbances of diabetic mellitus. *Lancet*. 1963;i:785–789.
 33. Willoughby SR, Chirkov YY, Kennedy JA, Murphy GA, Chirkova LP, Horowitz JD. Inhibition of long-chain fatty acid metabolism does not effect platelet aggregation responses. *Eur J Pharmacol*. 1998;356:207–213.
 34. Lopaschuk GD, Spafford MA, Davies NJ, Wall SR. Glucose and palmitate oxidation in isolated working rat hearts reperfused following a period of transient global ischemia. *Circ Res*. 1989;66:546–553.
 35. Schulz H. Beta oxidation of fatty acids. *Biochim Biophys Acta*. 1991;1081:109–120.
 36. Sellier P, Audouin P, Payen B, Corona P, Duong TC, Ourbak P. Acute effects of trimetazidine evaluated by exercise testing. *Eur J Clin Pharmacol*. 1987;33:205–207.

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



The Antianginal Drug Trimetazidine Shifts Cardiac Energy Metabolism From Fatty Acid Oxidation to Glucose Oxidation by Inhibiting Mitochondrial Long-Chain 3-Ketoacyl Coenzyme A Thiolase

Paul F. Kantor, Arnaud Lucien, Raymond Kozak and Gary D. Lopaschuk

Circ Res. 2000;86:580-588

doi: 10.1161/01.RES.86.5.580

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2000 American Heart Association, Inc. All rights reserved.

Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://circres.ahajournals.org/content/86/5/580>

Data Supplement (unedited) at:

<http://circres.ahajournals.org/content/suppl/2000/03/13/86.5.580.DC1>

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Circulation Research* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the [Permissions and Rights Question and Answer](#) document.

Reprints: Information about reprints can be found online at:
<http://www.lww.com/reprints>

Subscriptions: Information about subscribing to *Circulation Research* is online at:
<http://circres.ahajournals.org/subscriptions/>

The anti-anginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long chain 3-ketoacyl CoA thiolase

Paul F. Kantor¹, Arnaud Lucien², Raymond Kozak¹, and Gary D. Lopaschuk¹.

1, Cardiovascular Research Group, and the Division of Pediatric Cardiology

University of Alberta, Edmonton, Canada

2, Institut De Recherches Internationales Servier (France).

Materials: Trimetazidine was supplied by Institut De Recherches Internationales Servier (France). Radiolabelled substrates ([U- ^{14}C], [5- ^3H]-glucose, [1- ^{14}C]-palmitate, [1- ^{14}C]-octanoate and [1- ^{14}C]-butyrate) were purchased from ICN Radiochemicals. Hyamine hydroxide (methylbenzethonium 1 Mol/L in methanol) was purchased from ICN Pharmaceuticals. Bovine serum albumin (fraction V) was obtained from Boehringer Mannheim. Dowex 1-X4 anion exchange resin (200-400 mesh chloride form) was purchased from Biorad Laboratories. Ecolite and ACS counting scintillation fluid were obtained from ICN Biomedicals Canada and Amersham Canada, respectively. All other chemicals were reagent grade.

Heart perfusions: Hearts were excised from pentobarbital (60 mg·kg $^{-1}$) anesthetized male Sprague-Dawley rats (250-300g). The aorta was quickly cannulated and a retrograde perfusion with Krebs'-Henseleit solution (pH 7.4, containing 5 mM glucose and 2.5 mM free calcium, gassed with a 95% O $_2$ - 5% CO $_2$) was initiated. During a 10 min stabilization period the heart was trimmed of excess tissue, and the pulmonary artery and left atrium were cannulated. Following equilibration, hearts were switched to the working mode for a 60 min aerobic perfusion period with a modified Krebs'-Henseleit solution containing 5 mM glucose, 2.5 mM free calcium, 100 $\mu\text{U}/\text{ml}$ insulin, 3% bovine serum albumin and either 0.4 mM palmitate (16-carbon), 0.8 mM octanoate (8-carbon) or 1.6 mM butyrate (4 carbon). Concentrations of palmitate, octanoate, and butyrate were chosen so that hearts were exposed to equicarbon concentrations of fatty acids. Working

hearts were used in these studies to approximate the metabolic demand of the heart seen *in vivo*.

An additional series of isolated working hearts perfused with 5 mM glucose, 100 μ U/ml insulin and 0.4 mM palmitate were subjected to a 30 min perfusion, following which they were switched to the Langendorff mode and subjected to a 60 min period of low-flow ischemia (coronary flow = 0.5 ml/min). Another series of hearts were perfused under similar conditions, except that the Krebs'-Henseleit perfusate contained 11 mM glucose, 100 μ U/ml insulin, and 1.2 mM palmitate bound to 3% bovine serum albumin. Hearts were switched to the Langendorff mode during ischemia in order to obtain a constant coronary flow rate during ischemia.

Hearts were allowed to beat spontaneously and were perfused at a constant left atrial preload (11.5 mm Hg) and a constant aortic afterload (80 mm Hg). Mechanical function (heart rate, peak systolic pressure, and maximum developed pressure) was measured using a Gould P21 (Gould Inc., Valley View, OH) pressure transducer in the aortic outflow line. Cardiac output and aortic flow were measured with Transonic ultrasound flow probes (Transonic Systems, Ithaca NY) in the preload and afterload lines. Coronary flow was calculated as the difference between cardiac output and aortic flow. Myocardial O₂ consumption (MVO₂) was determined with YSI micro oxygen probes (Yellow Springs Company, Yellow Springs ID) in the preload and the cannulated pulmonary arterial line. Cardiac work was calculated as the product of peak systolic pressure and cardiac output, while rate-pressure product was determined as the product of

heart rate and peak systolic pressure. Hearts were maintained at 37°C during the entire perfusion period.

Trimetazidine, when present, was added 5 min into the perfusion period. A final concentration of 1 µM trimetazidine was used in most studies, except in hearts perfused with 11 mM glucose and 1.2 mM palmitate, in which a concentration of 0.1, 1 and 10 µM trimetazidine were used.

Measurements of glycolysis and glucose oxidation: Glycolysis and glucose oxidation rates were determined simultaneously by the quantitative collection of $^3\text{H}_2\text{O}$ and $^{14}\text{CO}_2$, respectively, produced by hearts perfused with buffer radiolabelled with [5- ^3H /U- 14]-glucose. Tritiated water production from steady state glycolysis was determined from coronary effluent samples by separating the unmetabolized [^3H]-glucose in Dowex 1-X4 columns, as previously described (1). Glucose oxidation rates were determined by quantitative collection of $^{14}\text{CO}_2$ production from the gaseous and aqueous phase (1, 2). $^{14}\text{CO}_2$ was extracted from perfusate samples as described earlier (1). Total myocardial $^3\text{H}_2\text{O}$ and $^{14}\text{CO}_2$ production were determined at 10 minute intervals throughout the aerobic perfusion period.

Measurement of fatty acid oxidation: Rates of fatty acid oxidation were assessed during identical parallel heart perfusions using either [1- ^{14}C]palmitate, [1- ^{14}C]octanoate, or [1- ^{14}C]butyrate. Palmitate, octanoate or butyrate oxidation was determined by

quantitatively collecting $^{14}\text{CO}_2$ production as described above for glucose oxidation. The sampling of total $^{14}\text{CO}_2$ production was conducted at 10 minute intervals throughout the aerobic perfusion period.

After the 60 min perfusion period, hearts were quickly frozen with Wollenberger clamps pre-cooled to the temperature of liquid N_2 . The frozen ventricular tissue was weighed and powdered in a mortar and pestle cooled to the temperature of liquid N_2 and a portion of the powdered ventricle were used to determine the dry/wet ratio. The atria were dried, weighed and incorporated into the calculation of the total dry weight of the heart. The remaining ventricular tissue was stored at -80°C to be used for subsequent biochemical analysis.

Calculation of tricarboxylic acid cycle activity rates: The rate of acetyl CoA production calculated from TCA cycle activity assumed a net yield of 2 moles of acetyl CoA from each mole of glucose oxidized, 8 moles of acetyl CoA from each mole of palmitate oxidized, 4 moles of acetyl CoA produced from each mole of octanoate oxidized, and 2 acetyl CoA produced from each mole of butyrate oxidized. TCA cycle efficiency was defined as the ratio of cardiac work to TCA cycle acetyl CoA produced.

Pyruvate dehydrogenase complex assay: Analysis of PDH complex activity utilized a modified protocol (3) based on the radiometric assay originally described by Constantin-Teodosiu *et al* (4). Dual samples of frozen ventricular tissue (20 mg) were weighed for

active pyruvate dehydrogenase (PDHa) and for total pyruvate dehydrogenase (PDHt) determination.

Measurements of β -oxidation enzymes: Activities of long-chain, medium-chain, and short-chain acyl CoA dehydrogenase were measured basically as described by Grimbert *et al* (5). Mitochondria were isolated by the method of Grimbert *et al* (5), sonicated on ice, centrifuged at 100,000 g for 60 min, and the soluble fractions used for acyl CoA dehydrogenase measurements. The activities of long chain acyl CoA dehydrogenase were measured as described by Grimbert *et al* (5), using palmitoyl-CoA as a substrate, while medium chain acyl CoA dehydrogenase was measured using octanoyl-CoA as a substrate, and short chain acyl CoA dehydrogenase measured using butyryl CoA as a substrate.

The long-, medium- and short-chain enoyl CoA hydratase activities were assayed as described by Wanders *et al* (6). Sonicated mitochondria were prepared as described above, centrifuged, and both the membrane and soluble fractions collected. Long-chain enoyl CoA hydratase was measured using the membrane fraction and *trans*-2-hexadecanoyl-CoA as a substrate as described by Wanders *et al* (6). Medium- and short-chain enoyl CoA hydratase were measured in the soluble fraction using *trans*-2-octenoyl CoA and crotonyl CoA as substrates, respectively.

L-3-hydroxyacyl CoA dehydrogenase was measured in membrane and soluble mitochondrial fractions as described by Venizelos *et al* (7). Long-chain L-3-hydroxyacyl

CoA dehydrogenase in the membrane fraction was measured using 3-keto-palmitoyl CoA as a substrate, while medium- and short-chain L-3-hydroxyacyl CoA dehydrogenase were measured using 3-keto-octanoyl CoA and acetoacetyl-CoA as substrates, respectively.

3-ketoacyl CoA thiolase was measured in membrane and soluble mitochondrial fractions as described by Venizelos *et al* (8). Long-chain 3-ketoacyl CoA thiolase in the membrane fraction was measured using 3-ketohexadecanoyl CoA as a substrate, while medium- and acetoacetyl 3-ketoacyl CoA thiolase were measured using 3-keto-octanoyl CoA and acetoacetyl CoA as substrates, respectively.

Measurement of malonyl CoA levels, AMP-activated protein kinase activity, acetyl-CoA carboxylase activity, and malonyl-CoA decarboxylase activity: CoA esters were extracted from the frozen powdered heart tissue using 6% perchloric acid, as described previously (8). The CoA esters were separated and quantified using a previously described high performance liquid chromatography procedure (9).

AMP-activated protein kinase (AMPK) and acetyl CoA carboxylase were extracted from approximately 200 mg of frozen heart tissue, as described previously (9). ACC activity in the PEG 6000 fractions was measured using the CO₂ fixation method (10). AMPK activity was measured as described previously (9), except that the synthetic peptide AMARAASAAALARRR was used as a substrate for the kinase (10). MCD activity was measured in heart extracts by detecting acetyl-CoA, the product of the MCD reaction, as described previously (9).

Statistical Analysis: All data are represented as the group mean \pm S.E.M. Data was analyzed using the statistical program Instat[®]. One-way ANOVA was used to compare values between control and TMZ-treated hearts, as well as between different control groups. A Tukey-Grammar post hoc test was used to confirm inter-group differences. A p value <0.05 was considered significant.

Figure 1 online.

Effect of trimetazidine (1 μ M) on TCA cycle efficiency (cardiac work/ TCA cycle activity) in hearts perfused with 5 mM glucose and either 0.4 mM palmitate (**A**), 0.8 mM octanoate (**B**), or 1.6 mM butyrate (**C**).

TCA cycle activity was calculated, as described in the “Methods”, from glucose oxidation and fatty acid oxidation values presented in Figures 1, 2 and 3.

References:

1. Liu B, el Alaoui-Talibi Z, Clanachan AS, Schulz R, Lopaschuk GD. Uncoupling of contractile function from mitochondrial TCA cycle activity and MVO₂ during reperfusion of ischemic hearts. *Am J Physiol* 1996;270:H72-H80.
2. Liu B, Clanachan AS, Schulz R, Lopaschuk GD. Cardiac efficiency is improved after ischemia by altering both the source and fate of protons. *Circ Res* 1996;79:940-948.
3. Collins-Nakai RL, Noseworthy D, Lopaschuk GD. Epinephrine increases ATP production in hearts by preferentially increasing glucose metabolism. *Am J Physiol* 1994;267:H1862-H1871.
4. Constantin-Teodosiu D, Cederblad G, Hultman E. A sensitive radioisotopic assay of pyruvate dehydrogenase complex in human muscle tissue. *Anal Biochem* 1991;198:347-351
5. Grimbert, S. Fisch, C., Deschamps, D., Berson, A., Fromenty, B., Feldman, G., and Pessayre, D. Effects of female sex hormones on mitochondria: possible role in acute fatty liver of pregnancy. *Am J Physiol* 1995;268:G107-G115
6. Wanders, R.J.A., Ijlst L., Poggi, F., Bonnefont, J.P., Munnich, A., Brivet, M., Rabier, D., Saudubray, J.M. Human trifunctional protein deficiency: a new disorder of mitochondrial fatty acid β -oxidation. *Biochem Biophys Res Commun* 192;188:1139-1145.

7. Venizelos, N., Ijlst, L., Wanders, R.J.A., and Hagenfeldt, L. β -Oxidation enzymes in fibroblasts from patients with 3-Hydroxydicarboxylic aciduria. *Pediatr Res* 194;36:111-114.
8. Saddik M, Gamble J, Witters LA, Lopaschuk GD. Acetyl CoA carboxylase regulation of fatty acid oxidation in the heart. *J Biol Chem* 1993;268:25836-25845.
9. Kudo N, Barr A, Barr R, Desai S, Lopaschuk GD. High rates of fatty acid oxidation during reperfusion of ischemic hearts are associated with a decrease in malonyl-CoA levels due to an increase in 5'AMP-activated protein kinase inhibition of acetyl-CoA carboxylase. *J Biol Chem* 1995;270:17513-17520.
10. Hardie DG, Salt IP, Hawley SA, Davies SP. AMP-activated protein kinase: an ultrasensitive system for monitoring cellular energy charge. *Biochem J* 1999;338:717-722.

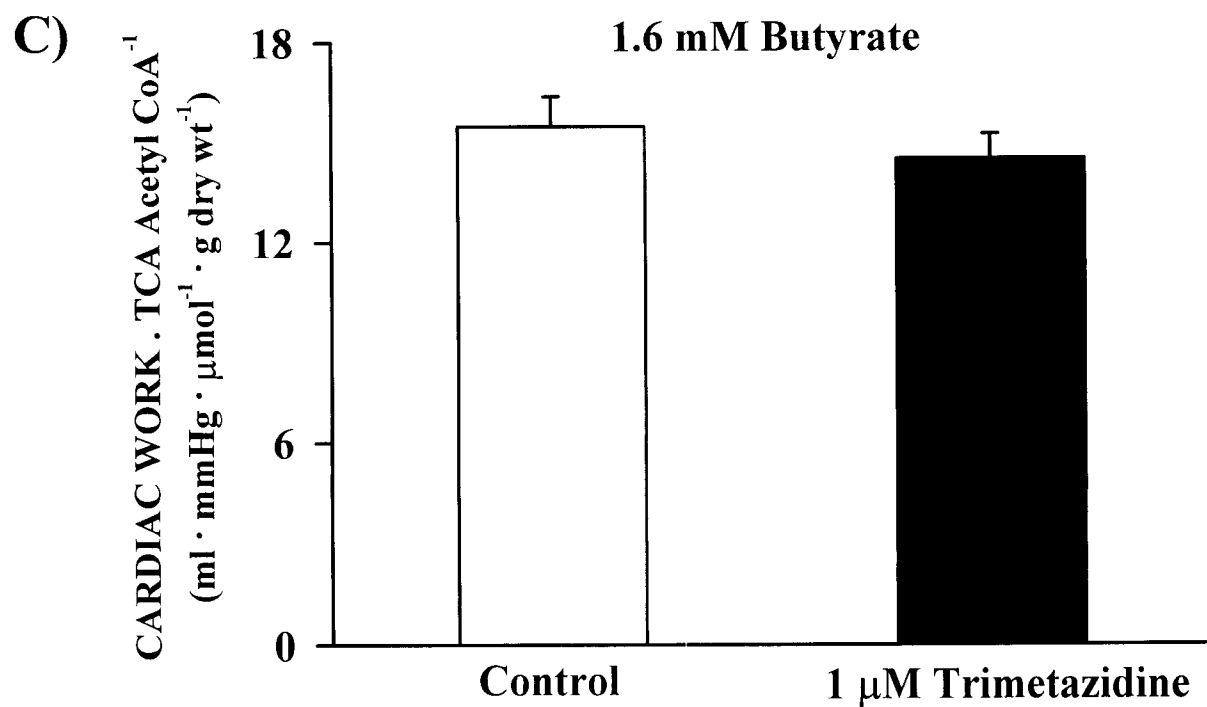
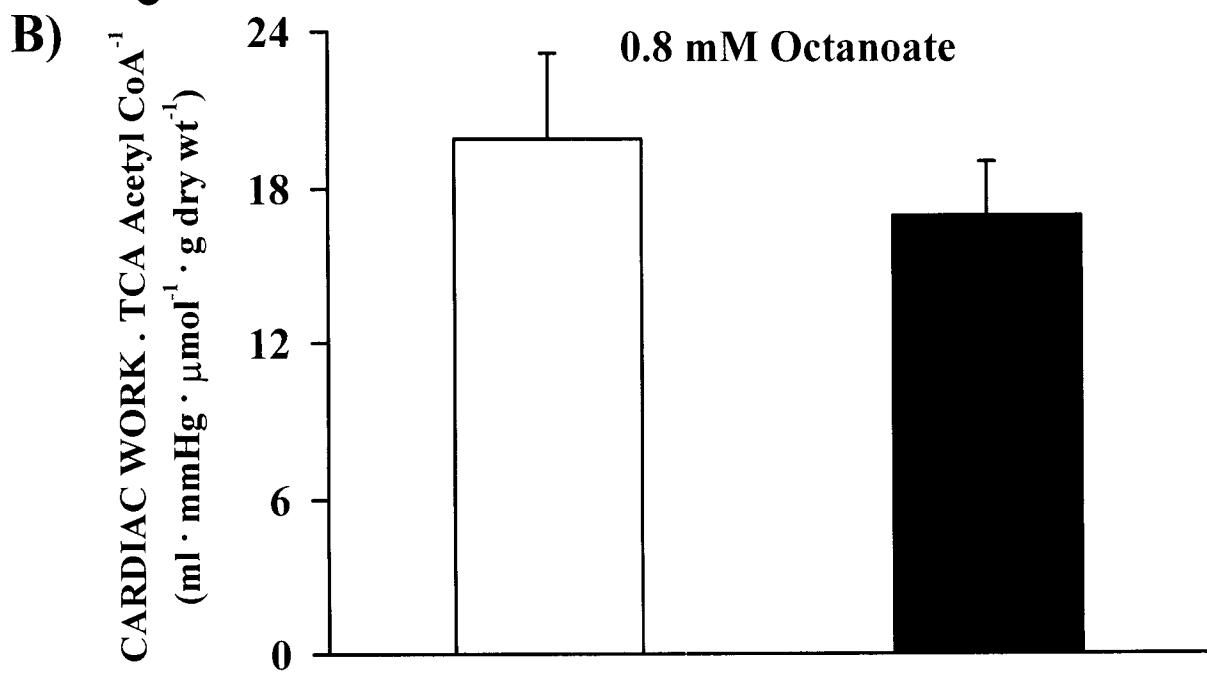
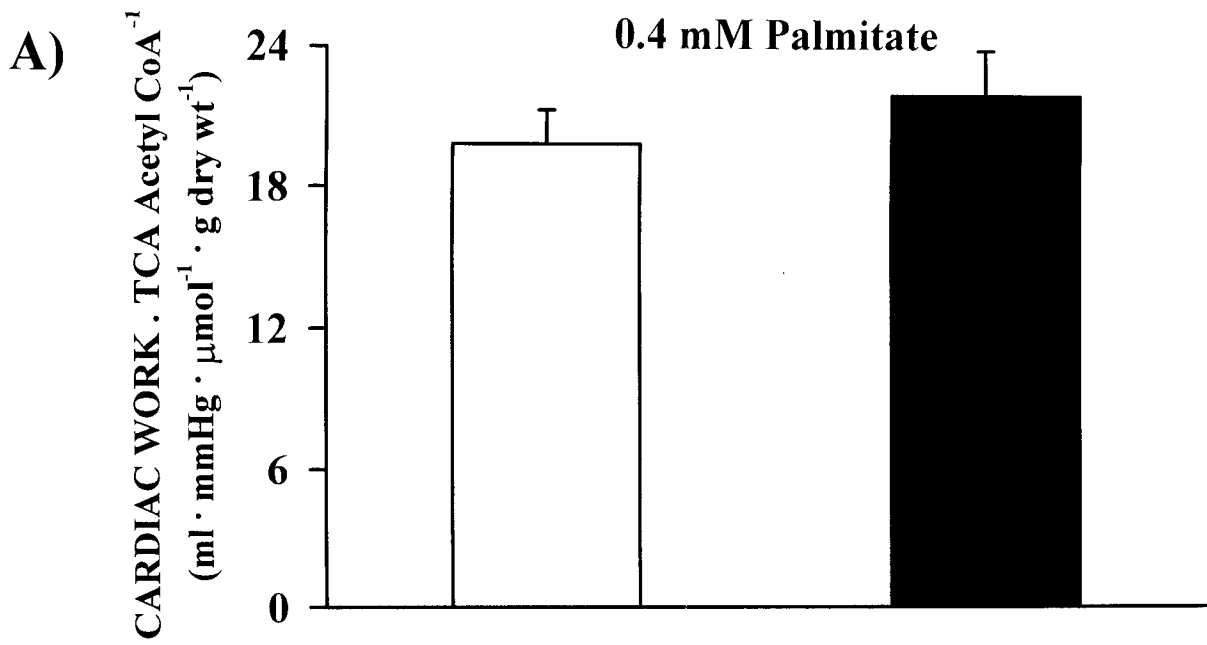


Table 1 online

Effects of trimetazidine (TMZ) (1 μ M) on various parameters of heart function, oxygen consumption, and cardiac efficiency after 60 minutes of aerobic perfusion in medium containing palmitate (0.4 mM), octanoate (0.8 mM), or butyrate (1.6 mM).

| PERFUSION CONDITION | Palmitate | | Octanoate | | Butyrate | |
|---|-------------|-------------|-------------|-------------|-------------|-------------|
| | Control | TMZ | Control | TMZ | Control | TMZ |
| Heart Rate (beats \cdot min ⁻¹) | 284 \pm 8 | 285 \pm 7 | 280 \pm 9 | 297 \pm 7 | 278 \pm 5 | 275 \pm 7 |
| Peak Systolic Pressure (mm Hg) | 127 \pm 3 | 127 \pm 2 | 118 \pm 2 | 115 \pm 2 | 125 \pm 2 | 119 \pm 2 |
| Developed Pressure (mm Hg) | 51 \pm 4 | 48 \pm 2 | 44 \pm 2 | 41 \pm 3 | 47 \pm 2 | 45 \pm 2 |
| Cardiac Output (ml \cdot min ⁻¹) | 58 \pm 2 | 60 \pm 2 | 59 \pm 3 | 63 \pm 2 | 55 \pm 2 | 56 \pm 2 |
| Coronary Flow (ml \cdot min ⁻¹) | 21 \pm 1 | 24 \pm 1 | 23 \pm 2 | 22 \pm 1 | 20 \pm 1 | 23 \pm 1 |
| Cardiac Work (ml \cdot mm Hg \cdot min ⁻¹) | 74 \pm 4 | 76 \pm 3 | 70 \pm 4 | 72 \pm 3 | 69 \pm 3 | 67 \pm 4 |
| O ₂ Consumption (μ mol O ₂ \cdot min ⁻¹ \cdot g dry wt ⁻¹) | 43 \pm 3 | 45 \pm 4 | 44 \pm 3 | 43 \pm 3 | 35 \pm 3 | 43 \pm 3 |

-Values are the mean \pm SEM of 14-19 control and trimetazidine-treated hearts. Hearts were perfused for a 60 minute aerobic period. Trimetazidine was added at 5 minutes of the perfusion. Values shown above were at 60 minutes of the perfusion period. CW=cardiac work. Developed pressure = difference between systolic aortic pressure and peak diastolic aortic pressure.

Table 2 online

Effects of 100 μM trimetazidine on pyruvate dehydrogenase (PDH) levels when added directly into the incubation media using aerobic rat hearts perfused with 1.2 mM palmitate.

| Condition | Active | Total | % Active of Total |
|---|--|--|--------------------------|
| | Acetyl CoA ($\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1}$) | Acetyl CoA ($\mu\text{mol} \cdot \text{g dry wt}^{-1} \cdot \text{min}^{-1}$) | (%) |
| Control | 3.57 ± 0.50 | 21.91 ± 1.52 | 17 ± 3 |
| 100 μM TMZ | 3.31 ± 0.45 | 19.33 ± 1.41 | 18 ± 3 |

-Values are the mean \pm SEM of at least 6 hearts/group. 100 μM Trimetazidine was added during the incubation phase. PDH levels were determined as described previously.

Table 3 online

Effects of trimetazidine on short chain CoA levels (malonyl, glutathione, succinyl, propionyl, and butyryl, hexanyl, and octanyl) in aerobic hearts perfused with fatty acids of graded carbon chain lengths (palmitate, 16; octanoate, 8; and butyrate, 4).

| Condition | Control | 1 μ M Trimetazidine | Control | 1 μ M Trimetazidine | Control | 1 μ M Trimetazidine |
|------------------------|--|----------------------------|--|----------------------------|---|----------------------------|
| | 0.4 mM Palmitate (nmol · g dry wt ⁻¹) | | 0.8 mM Octanoate (nmol · g dry wt ⁻¹) | | 1.6 mM Butyrate (nmol · g dry wt ⁻¹) | |
| <i>Malonyl CoA</i> | 5.0 ± 0.5 | 4.5 ± 0.4 | 4.1 ± 0.2 | 4.8 ± 0.3 | 3.4 ± 0.4 | 3.6 ± 0.3 |
| <i>Glutathione CoA</i> | 4.3 ± 0.4 | 4.2 ± 0.6 | 4.8 ± 0.4 | 4.8 ± 0.5 | 4.7 ± 0.7 | 5.3 ± 0.9 |
| <i>Succinyl CoA</i> | 9.3 ± 1.0 | 11.4 ± 1.0 | 11.4 ± 1.2 | 13.3 ± 1.7 | 11.6 ± 0.5 | 13.0 ± 1.2 |
| <i>Propionyl CoA</i> | 4.1 ± 0.3 | 4.4 ± 0.1 | N.D. | N.D. | N.D. | N.D. |
| <i>Butyryl CoA</i> | 5.3 ± 0.9 | 5.6 ± 0.4 | 4.3 ± 0.4 | 5.4 ± 0.8 | 4.4 ± 0.5 | 6.1 ± 0.8 |
| <i>Hexanyl CoA</i> | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |
| <i>Octanyl CoA</i> | N.D. | N.D. | N.D. | N.D. | N.D. | N.D. |

-Values are the mean ± SEM of at least 6 hearts/group. Trimetazidine (if present) was added at 5 minutes into the aerobic perfusion. Short chain CoA levels were determined as described previously. N.D. = not detected

Table 4 online
 Effects of 1 μ M trimetazidine on long chain CoA, Acetyl CoA, and CoASH levels in aerobic hearts perfused with fatty acids of graded carbon chain lengths (palmitate, 16; octanoate, 8; and butyrate, 4).

| Condition | 1 μ M | | 1 μ M | | 1 μ M | |
|--|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | Control | Trimetazidine | Control | Trimetazidine | Control | Trimetazidine |
| <i>Long Chain CoA</i> | 191 \pm 18 | 223 \pm 20 | 92 \pm 8 | 93 \pm 5 | 139 \pm 30 | 127 \pm 12 |
| <i>Acetyl CoA</i> | 16.6 \pm 0.9 | 18.4 \pm 0.9 | 18.5 \pm 0.8 | 18.0 \pm 0.9 | 15.3 \pm 0.7 | 17.9 \pm 1.2 |
| <i>CoASH</i> | 227.3 \pm 7.4 | 258.3 \pm 7.0* | 274.6 \pm 8.8 | 285.0 \pm 15.0 | 281.9 \pm 10.6 | 313.0 \pm 20.6 |
| <i>Acetyl CoA · CoASH⁻¹</i> | 0.074 \pm 0.005 | 0.072 \pm 0.004 | 0.068 \pm 0.003 | 0.064 \pm 0.003 | 0.057 \pm 0.003 | 0.057 \pm 0.002 |

-Values are the mean \pm SEM of at least 6 hearts/group. Trimetazidine (if present) was added at 5 minutes into the aerobic perfusion. Long and short chain CoA levels were determined as described previously.

*, significantly different as compared to control values