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Partial inhibition of fatty acid oxidation increases regional contractile power and efficiency during demand-induced ischemia

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Abstract

Objective: Clinical trials in patients with stable angina show that drugs that partially inhibit myocardial fatty acid oxidation reduce the symptoms of demand-induced ischemia, presumably by reducing lactate production and improving regional systolic function. We tested the hypothesis that partial inhibition of fatty acid oxidation with oxfenicine (a carnitine palmitoyl transferase-I inhibitor) reduces lactate production and increases regional myocardial power during demand-induced ischemia. **Methods:** Demand-induced ischemia was produced in anesthetized open-chest swine by reducing flow by 20% in the left anterior descending coronary artery and increasing heart rate and contractility with dobutamine (15 μg kg^{-1} min^{-1} i.v.) for 20 min. Glucose and fatty acid oxidation were measured with an intracoronary infusion of [U-^{14}C] glucose and [9,10-^{3}H] oleate, and hearts were treated with oxfenicine (2 mmol l^{-1}; $n=7$) or vehicle ($n=7$). Regional anterior wall power was assessed from the left ventricular pressure–anterior free wall segment length loops. **Results:** During demand-induced ischemia, the oxfenicine group had a higher rate of glucose oxidation (6.9±1.1 vs. 4. 7±0.8 μmol min^{-1}; $P<0.05$), significantly lower fatty acid uptake, but no change in total or active PDH activity. The oxfenicine group had significantly lower lactate output integrals (1.11±0.23 vs. 0.60±0.11 mmol) and glycogen depletion (66±6 vs. 43±8%), and higher anterior wall power index (0.95±0.17 vs. 1.30±0.11%) and anterior wall energy efficiency index (91±17 vs. 129±10%). **Conclusions:** Partial inhibition of fatty acid oxidation reduced non-oxidative glycolysis and improved regional contractile power and efficiency during demand-induced ischemia.

Keywords: Angina; Dobutamine; Heart; Energy metabolism

1. Introduction

Coronary artery disease patients commonly have decreased coronary flow reserve, thus, in response to stress and a greater demand for oxygen, they fail to sufficiently increase coronary flow and myocardial oxygen consumption (MVO_2) resulting in 'demand-induced' ischemia [26,32]. The normal healthy heart gets approximately 2/3 of its energy from fatty acid oxidation, even during partial reductions in flow that result in lactate production [17,18,22,26,35]. Fatty acid oxidation strongly inhibits the mitochondrial enzyme pyruvate dehydrogenase and oxidation of the glycolytic intermediate pyruvate. Pharmacological inhibition of fatty acid oxidation increases pyruvate oxidation and the uptake and oxidation of glucose and lactate under aerobic conditions in humans and animals [16,24,34]. It has been proposed that agents that partially inhibit myocardial fatty acid oxidation are efficacious during demand-induced ischemia in stable angina patients because they reduce lactate production, prevent intracellu-

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lar metabolic acidosis resulting in improved regional systolic function. This mechanism, however, has never been demonstrated.

We have recently developed a model of demand-induced ischemia in pigs, where ischemia is the result of flow restriction and dobutamine stimulation of heart rate and contractility, with no change in MVO_2 [5]. Despite no change in MVO_2, there was a switch from lactate uptake to production, glycogen depletion and increased glucose uptake, but no change in anterior wall power or the anterior wall energy efficiency index. Furthermore, there was no change in fatty acid uptake, yet exogenous fatty acid oxidation was decreased. Thus, demand-induced ischemia stimulated non-oxidative glycolysis and lactate production, but did not affect fatty acid uptake or anterior wall power for a given MVO_2.

Traditional therapies for myocardial ischemia act by improving oxygen delivery to the ischemic cardiac muscle or by decreasing the requirement of the myocardium for oxygen by decreasing the heart rate, blood pressure or the inotropic state of the muscle. These therapies lessen the degree of ischemia by better matching the delivery of oxygen to the amount of work the myocardium performs [32]. An alternative approach to these therapies is pharmacological optimization of cardiac energy metabolism. The advantage of these therapies over traditional therapies is that they would increase the mechanical efficiency of the ischemic region without an increase in myocardial blood flow or MVO_2, and without suppressing cardiac contractility. There is no direct evidence, however, that these metabolic agents reduce lactate production or improve regional myocardial contractile function or mechanical efficiency during demand-induced ischemia. Therefore, we tested the hypothesis that partial inhibition of fatty acid oxidation with oxfenicine (a carnitine palmitoyl transferase-I (CPT-I) inhibitor) would reduce the production of lactate and improve regional myocardial contractile function during demand-induced ischemia.

2. Methods

Experiments were performed on 14 domestic pigs (vehicle, $n=7$ mean weight 40.5 ± 0.9 kg; oxfenicine, $n=7$, mean weight 39.3 ± 1.5 kg). Experiments with vehicle and oxfenicine treated animals were performed concurrently, however, the data from the vehicle group were published separately to present this novel model of experimental demand-induced ischemia [5]. Studies were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication Number 85-23, revised 1996) and the Institutional Animal Care and Use Committee at Case Western Reserve University.

2.1. Surgical preparation

Overnight fasted animals were sedated with Telazol, masked down with isoflurane, intubated, ventilated, and anesthesia was maintained with isoflurane. The heart was exposed via a midline sternotomy [27], the animal heparinized and a 20% triglyceride emulsion was infused to increase plasma free fatty acids to approximately 0.6 mmol l^{-1} [31]. Left anterior descending coronary artery (LAD) blood flow was controlled through an extra-corporeal perfusion circuit via a roller pump with blood taken from the femoral artery, as previously described in detail [20,27,31]. Arterial blood samples were obtained from a constant flow (10 ml min^{-1}) withdrawal loop from the LAD perfusion circuit so that blood sampling would not disturb coronary artery blood flow. A dual-transducer catheter was used to assess left ventricular (LV) pressure. A cannula was placed in the anterior interventricular vein to collect venous blood samples from the perfusion zone of the LAD. Regional segment length was measured at approximately midwall depth in the LAD bed using sonomicrometry as previously described [11,20]. This preparation allowed us to subject the LAD perfusion bed to demand-induced ischemia by infusing dobutamine while decreasing LAD flow by 20%. During demand-induced ischemia, anterior wall contractile dysfunction was assessed from the decrease in the LV pressure–segment length loop area from normal conditions to demand-induced ischemia. The right main and circumflex coronary artery blood flows were not restricted [10,11].

2.2. Experimental protocol

Following completion of the instrumentation, a continuous infusion of [U-^{14}C] glucose (0.2 μCi min^{-1}) and [9,10-^{3}H] oleate (0.2 μCi min^{-1}) was introduced into the proximal end of the coronary perfusion line at a rate of 0.1 ml min^{-1}. The radioactivity dose was greatly reduced, as was the extent of secondary labeling, by infusing tracer directly into the coronary perfusion circuit. After 30 min of tracer infusion, vehicle or 2 mmol l^{-1} oxfenicine were infused directly into the coronary perfusion circuit and arterial and interventricular venous samples were drawn 10 min apart (40, 50 and 60 min after tracer infusion). Sixty minutes after initiating the tracer infusion, demand-induced ischemia was initiated with an infusion of dobutamine (15 μg kg^{-1} min^{-1}) to increase myocardial oxygen demand and by reducing LAD blood flow by 20% for a period of 20 min. Arterial and anterior interventricular venous blood samples were then taken at 3, 6, 10 and 20 min of demand-induced ischemia. Blood samples were analyzed for the concentrations of oxygen, lactate and glucose in blood and plasma free fatty acids. In addition, samples were analyzed for ^{14}C-glucose, $^{14}CO_2$, ^{3}H-oleate and $^{3}H_2O$ for calculation of the rates of glucose and oleate oxidation, as described below. Heart rate, left ventricular pressure (LVP), peak positive and negative dP/dt, and segment length were continuously recorded using a commercial on-line data acquisition system. Small myocardial biopsies (10–20 mg) were taken from the anterior LV free wall

with a 14-gauge biopsy needle 5 min before initiating demand-induced ischemia, and at 8 and 18 min of demand-induced ischemia, and were immediately freeze-clamped (3–5 s) on aluminum blocks pre-cooled in liquid nitrogen and stored at −80 °C for subsequent analysis. Tissue lactate was assayed in these samples. After 20 min of demand-induced ischemia, two large (~3 g) punch biopsies were rapidly excised from the anterior and posterior LV free wall and freeze-clamped in large steel tongs pre-cooled in liquid nitrogen. These samples were assayed for concentrations of tissue glycogen and triglycerides and for PDH activities.

2.3. Analytical methods

Detailed analytical methods have been previously cited in the literature [5]. Blood samples for glucose, lactate and ^{14}C-glucose were deproteinized and analyzed for glucose and lactate using enzymatic spectrophotometric assays. ^{14}C-glucose and ^{14}C-lactate were measured using ion-exchange chromatography. Plasma ^{3}H-oleate concentration was measured by extracting the fatty acids from plasma in heptane/isopropanol and counting the organic phase. $^{3}H_2O$ concentration was measured by distilling plasma in modified Hickman stills. Blood $^{14}CO_2$ concentration was measured by expelling $^{14}CO_2$ with the addition of concentrated lactic acid and trapping it in hyamine hydroxide. Plasma free fatty acids were measured using a commercially available enzymatic spectrophotometric kit. Tissue concentrations of ATP were measured using the ATP Bioluminescent Assay Kit (Sigma Aldrich). Tissue lactate and triglyceride concentrations were measured using enzymatic spectrophotometric methods. Tissue glycogen was assayed using the amyloglucosidase method.

Active and total PDH activity was determined using a newly developed radiochemical assay [33]. The assay is based on the production of [1-^{14}C] acetyl-CoA from [2-^{14}C] pyruvate, which is converted to [1-^{14}C] acetylcarnitine in the presence of excess L-carnitine and carnitine acetyltransferase. The positively charged product, [1-^{14}C] acetylcarnitine is then separated from the negatively charged radiolabeled substrate by exclusion chromatography.

2.4. Calculations

The net uptake (μmol min^{-1}) for glucose and free fatty acids were calculated as the product of the arterial and coronary venous substrate concentration difference and myocardial blood flow. The rate of glucose and fatty acid oxidation (μmol min^{-1}) were calculated as the product of myocardial blood flow (ml min^{-1}) and the release of either $^{14}CO_2$ or $^{3}H_2O$ (dpm ml^{-1}) into the coronary vein, divided by the arterial specific radioactivity of glucose or free fatty acids (dpm μmol^{-1}) [36]. The interventricular venous concentrations of $^{14}CO_2$ and $^{3}H_2O$ were corrected for dilution of blood (~10%) derived from coronary arteries other than the LAD by multiplying the measured values by the concentration of green dye in venous plasma divided by the concentration in arterial plasma [29]. Myocardial blood flow (ml min^{-1}) was measured from the calibrated pump flow of the coronary perfusion line. Myocardial oxygen consumption was calculated as the product of the arterial and venous oxygen concentration difference times the myocardial blood flow.

The LV pressure (LVP)-segment length loop area was used as an index of external wall work (expressed as mmHg mm beat^{-1}) of the anterior free wall. Anterior wall external power index was calculated as the LVP-segment length loop area times heart rate (expressed as mmHg mm s^{-1}). An index of anterior wall energy efficiency was taken as anterior wall power index divided by the estimated ATP production. An estimation of total ATP production (μmol s^{-1}) was calculated from the MV_{O_2} in μmol s^{-1} times 6 ATP/O_2 consumed, plus the total lactate production (blood and tissue; μmol s^{-1}) times 1 ATP/lactate [5].

2.5. Statistical analysis

All hemodynamic parameters, rates of substrate and oxidation, tissue concentrations of lactate, triglyceride and glycogen, and regional anterior wall work and power index were compared between normal conditions and demand-induced ischemia using two-way ANOVA. Lactate production, MV_{O_2} and arterial and venous differences over time were analyzed using a two-way repeated measure ANOVA, using a Student–Neuman–Keuls test for post-hoc comparisons. All values are reported as mean±S.E with a 0.05 level of significance.

3. Results

3.1. Hemodynamics

All hemodynamic variables are listed in Table 1. In both the vehicle and oxfenicine groups, heart rate (48±8 and 47±10%), maximal positive dP/dt (85±9 and 82±14%) and maximal negative dP/dt (29±9 and 37±9%) were all significantly increased during demand-induced ischemia (Table 1 and Fig. 1). MV_{O_2} did not increase in the LAD perfusion bed in either group during the period of demand-induced ischemia (Table 1 and Fig. 1).

3.2. Substrate metabolism

Arterial concentrations of lactate and glucose were unchanged in both groups during the course of the experiment. Arterial concentrations for glucose were 3.99±0.04 and 4.01±0.06 mM for vehicle and oxfenicine groups, respectively. Arterial–venous concentration difference (a–v difference) for free fatty acids was not different between the two groups, however, the a–v difference

Table 1
Hemodynamic variables in vehicle and oxfenicine groups during normal flow and demand-induced ischemia conditions

	Vehicle		Oxfenicine	
	Normal conditions	Demand-induced ischemia	Normal conditions	Demand-induced ischemia
Peak LV pressure (mmHg)	86±2	84±5	90±3	89±5
Mean heart rate (beats min^{-1})	119±5	175±4*	126±10	180±4*
Peak LV+dP/dt (mmHg s^{-1})	2027±131	3725±246*	2238±112	4015±200*
Peak LV−dP/dt (mmHg s^{-1})	−1478±144	−1893±210*	−1670±81	−2302±211*
Anterior wall MV_{O_2} (μmol min^{-1})	115±12	109±11	116±5	112±9
Anterior wall work index (mmHg mm $beat^{-1}$)	169±35	109±28*	150±40	135±39

Values are means±S.E. in seven vehicle and seven oxfenicine pigs. dP/dt, first derivative of pressure. *$P<0.05$, demand-induced ischemia vs. normal conditions.

increased throughout demand-induced ischemia and was significantly greater at 80 min of demand-induced ischemia compared to normal conditions (Fig. 2). Similarly, the a–v difference for glucose increased significantly (70 and 80 min) but was not different between the two groups. However, the uptake of lactate converted to production in

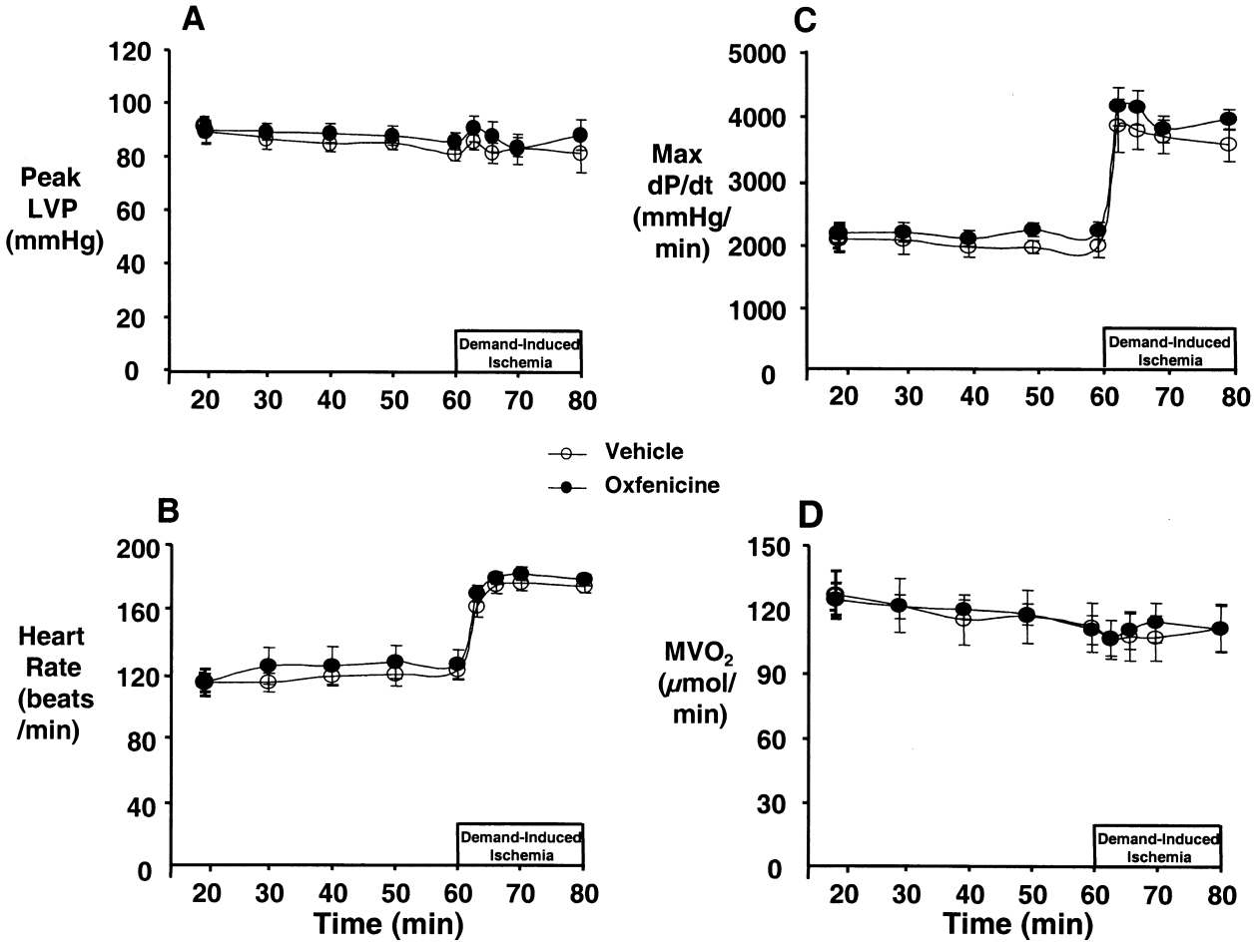

Fig. 1. Effect of oxfenicine on hemodynamic parameters under normal conditions and during demand-induced ischemia. There were no significant differences between groups as assessed by (A) peak left ventricular pressure (mmHg); (B) heart rate (beats min^{-1}); (C) maximal first derivative of left ventricular pressure with time or dP/dt (mmHg min^{-1}); or (D) MV_{O_2} (μmol min^{-1}) in the vehicle and oxfenicine groups.

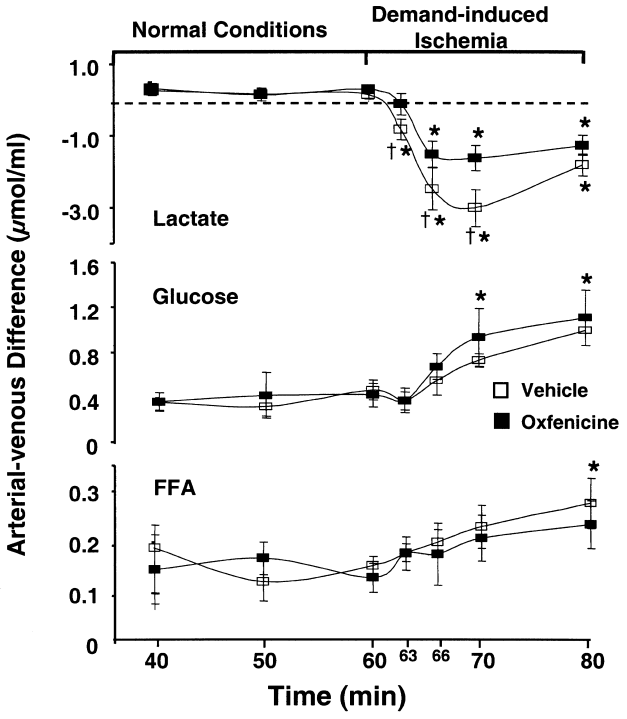

Fig. 2. Circulating arterial–venous concentration differences for lactate, glucose and free fatty acids plotted as a function of time for both the vehicle and oxfenicine groups during normal conditions and demand-induced ischemia. *$P<0.05$, demand-induced ischemia vs. normal conditions; †$P<0.05$, vehicle vs. oxfenicine.

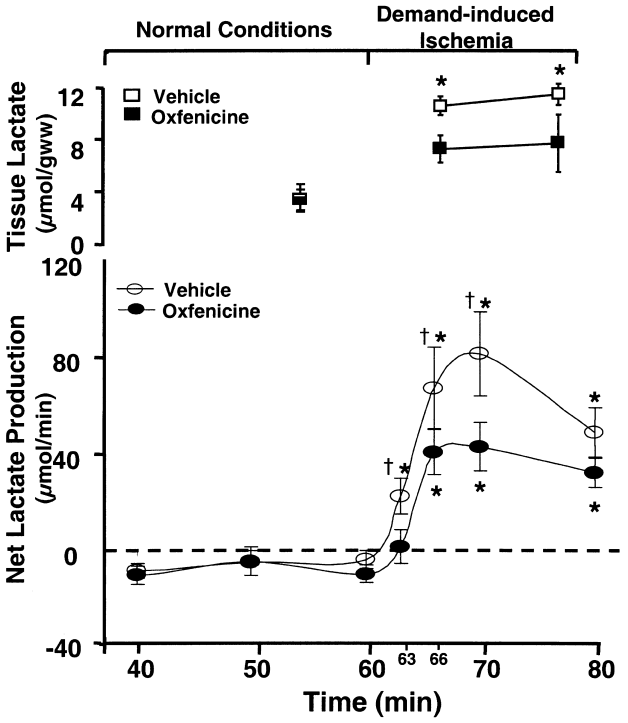

Fig. 3. Net myocardial lactate production plotted in the lower panel as a function of time for both groups during normal conditions and demand-induced ischemia. *$P<0.05$, demand-induced ischemia vs. normal conditions; †$P<0.05$, vehicle vs. oxfenicine. Tissue lactate concentrations for the vehicle and oxfenicine groups during normal conditions and at 8 and 18 min of demand-induced ischemia are presented in the upper panel. *$P<0.05$, oxfenicine vs. vehicle group.

both the vehicle and oxfenicine groups during demand-induced ischemia, but the a–v difference for lactate was significantly lower in the oxfenicine group (Fig. 2).

During normal flow conditions there was net lactate uptake, however, there was a dramatic switch to net lactate production in both groups during demand-induced ischemia (Table 2). The time course of lactate production (see Fig. 3, lower panel) indicates that a switch to lactate production occurred 3 min after the onset of demand-induced ischemia (63 min total protocol time), but was not significantly greater than normal conditions in the oxfenicine group until 6 min after the onset of demand-induced ischemia (or 66 min total protocol time). Furthermore, the net lactate output integral (a measure of total lactate production during demand-induced ischemia) was greater in the vehicle group compared to oxfenicine

Table 2
The rates of free fatty acid (FFA), glucose and lactate uptakes and FFA and glucose oxidation in the left anterior descending coronary artery bed in vehicle and oxfenicine groups during conditions of normal flow and demand-induced ischemia

	Vehicle		Oxfenicine	
	Normal conditions	Demand-induced ischemia	Normal conditions	Demand-induced ischemia
FFA tracer uptake (μmol min^{-1})	7.73±1.05	7.68±1.68	6.01±0.81	4.55±0.94
Glucose uptake (μmol min^{-1})	13.00±2.5	22.80±2.9*	15.40±3.1	25.0±4.4*
Lactate uptake (μmol min^{-1})	5.90±3.1	−74.50±16.3*	8.30±3.3	−38.7±5.5*†
FFA oxidation (μmol min^{-1})	6.16±1.48	1.07±0.34*	3.71±1.12†	0.75±0.39*
Glucose oxidation (μmol min^{-1})	2.91±0.77	4.05±0.58*	4.67±0.79†	6.87±1.13*†

Values are means±S.E. Substrate uptakes were taken as the product of myocardial blood flow (ml min^{-1}) and arteriovenous differences for each substrate (average of 50 and 60 min during normal flow conditions, and 70 and 80 min during demand-induced ischemia). *$P<0.05$ demand-induced ischemia vs. normal conditions; †$P<0.05$ oxfenicine vs. vehicle

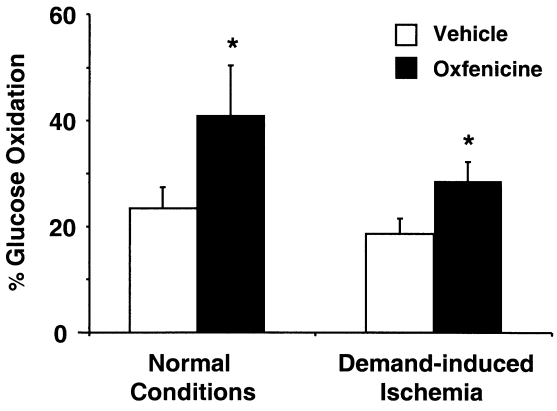

Fig. 4. Effect of oxfenicine on the percent glucose oxidation measured as the percent of glucose uptake (μmol min^{-1}) that was oxidized to produce $^{14}CO_2$ during normal conditions and demand-induced ischemia. *$P<0.05$, oxfenicine vs. vehicle group.

(1.1±0.25 vs. 0.60±0.11 mmol; $P<0.05$). The switch from lactate uptake to lactate production was accompanied by no change in MVO_2 in either group. Demand-induced ischemia caused a greater increase in myocardial tissue lactate content in the vehicle group compared to the oxfenicine group (371±199 vs. 212±166%, respectively; average of two time points during demand-induced ischemia compared to normal conditions) (Fig. 3, upper panel).

Free fatty acid, glucose and lactate uptake and exogenous glucose and free fatty acid oxidation measurements are presented in Table 2. Demand-induced ischemia resulted in significantly lower rates of fatty acid oxidation in both the oxfenicine and vehicle groups despite no significant decrease in tracer measured fatty acid uptake within each group. However, the oxfenicine group had significantly lower tracer-measured fatty acid uptake compared to the vehicle group when the two conditions were combined (mean of normal conditions and demand-induced ischemia combined; 7.68±1.3 vs. 5.3±0.7 μmol min^{-1} for the vehicle and oxfenicine groups, respectively, $P<0.05$).

Myocardial glucose uptake was significantly increased during demand-induced ischemia in both groups and was accompanied by significant increases in the rate of glucose oxidation in both groups. However, the rates of glucose oxidation were significantly higher in the oxfenicine group compared to the vehicle group under both normal conditions (80±24%) and during demand-induced ischemia (93±50%) (Table 2). Furthermore, the percent of glucose taken up that was subsequently oxidized was greater in the oxfenicine group compared to the vehicle group under both normal conditions (23±4 vs. 41±9%, $P<0.05$) and during demand-induced ischemia (19±3 vs. 29±4%, $P<0.05$) (Fig. 4). Greater rates of glucose oxidation during demand-induced were not accompanied by differences in either total PDH activity (2.52±0.11 vs. 2.74±0.15 μmol min^{-1} gww^{-1}) or the percent of active PDH (74±5 vs. 69±9%) in the LAD bed between the two groups, suggesting that an increase in active PDH was not responsible for the increased glucose oxidation.

Tissue triglyceride stores in the ischemic LAD bed relative to the non-ischemic posterior left ventricular free wall (CFX bed) were not different between the two groups. The reduction in tissue glycogen content in the ischemic LAD bed relative to the non-ischemic CFX bed was significantly less in the oxfenicine group compared to the vehicle group (43±8% vs. 66±6%, respectively) suggesting a glycogen sparing effect of oxfenicine during demand-induced ischemia (Table 3). Tissue ATP contents were not different between the vehicle and oxfenicine groups under both normal conditions and during demand-induced ischemia.

3.3. Regional left ventricular wall work and power

Anterior wall work and power index were calculated from the LVP-segment length loop area using sonomicrometry [5]. Demand-induced ischemia resulted in a significant reduction in anterior wall work index (169±35 vs. 109±28 mmHg mm^{-1} $beat^{-1}$; $P<0.05$) (Table 1) and power index was unchanged (299±47 vs. 311±86 mmHg mm^{-1} s^{-1}) in the vehicle group (Fig. 5). Oxfenicine prevented a significant reduction in anterior wall work index (150±40 vs. 135±9 mmHg mm^{-1} $beat^{-1}$) (Table 1) and increased the anterior wall power index (320±105 vs. 395±107 mmHg mm^{-1} s^{-1}) (Fig. 5).

The estimated rate of ATP production was constant between normal conditions and demand-induced ischemia in both the vehicle (11.5±1.2 and 12.4±1.3 μmol ATP s^{-1}) and oxfenicine groups (11.7±0.5 and 12.0±1.0 μmol

Table 3
Tissue concentrations for glycogen and triglycerides following 20 min of demand induced ischemia in the non-ischemic posterior left ventricular free wall (CFX) and ischemic anterior left ventricular free wall (LAD) beds

	Vehicle		Oxfenicine	
	CFX	LAD	CFX	LAD
Tissue glycogen (μmol gww^{-1})	31.10±3.2	9.80±1.3*	31.40±4.5	17.80±3.8*†
Tissue triglycerides (μmol gww^{-1})	3.12±0.54	3.91±0.44	3.14±0.43	3.56±0.37

Values are means±S.E. *$P<0.05$ LAD vs. CFX bed; †oxfenicine vs. vehicle.

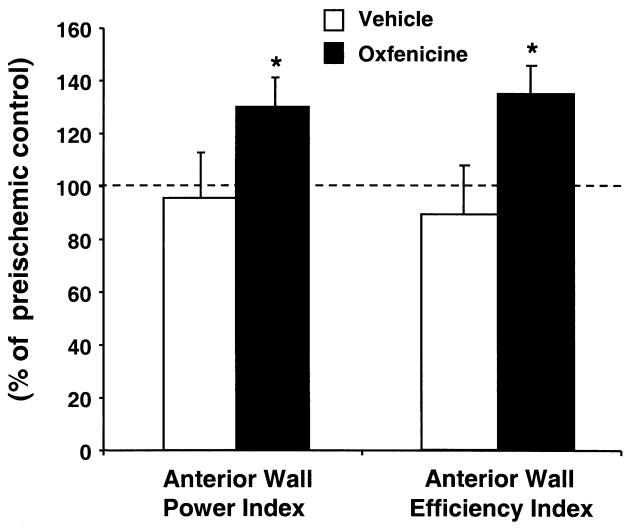

Fig. 5. Anterior wall power index and myocardial energy efficiency index in the anterior left ventricular free wall. Anterior wall power index was calculated as the rate of work production. Myocardial energy efficiency is calculated as the ratio of the change in anterior wall power index to the change in myocardial oxygen consumption during demand-induced ischemia. Vertical axis is anterior wall power and efficiency index during demand-induced ischemia expressed as a percent of normal conditions; *$P<0.05$, oxfenicine vs. vehicle group.

ATP s^{-1}). However, the switch to lactate production that occurred during demand-induced ischemia, which contributed 1.5 ± 0.3 μmol ATP s^{-1} or approximately $11.9\pm1.4\%$ of the total energy expenditure during demand-induced ischemia, was significantly reduced in the oxfenicine group to 0.8 ± 0.2 μmol ATP s^{-1} or $6.5\pm1.2\%$ of the total energy expenditure. Furthermore, as a result of an increase in the anterior wall power index with no change in the estimated total ATP production, the anterior wall energy efficiency index was increased in the oxfenicine group during demand-induced ischemia (Fig. 5).

4. Discussion

The results of the present investigation show that partial inhibition of fatty acid oxidation with the CPT-I inhibitor oxfenicine reduced the rate of non-oxidative glycolysis and improved regional mechanical function during demand-induced ischemia. During demand-induced ischemia, oxfenicine resulted in less lactate production, glycogen depletion and fatty acid uptake and oxidation, while glucose oxidation, anterior wall power and myocardial energy efficiency were all enhanced. Furthermore, inhibition of fatty acid oxidation with oxfenicine, which has been shown to result in removal of fatty acid inhibition of pyruvate dehydrogenase [12,13], increased pyruvate oxidation during demand-induced ischemia. However, the increased pyruvate oxidation we observed in this study was not due to a removal of phosphorylation inhibition of PDH as there was no increase in either total PDH activity or the percent of active PDH. While previous studies have shown improved cardiac function during low-flow ischemia/reperfusion [30] with partial inhibition of myocardial fatty acid oxidation, the present investigation was the first to demonstrate improved regional myocardial systolic function and mechanical efficiency during demand-induced ischemia.

In the present investigation we observed that partial inhibition of fatty acid oxidation with oxfenicine reduces lactate production and tissue lactate accumulation during demand-induced ischemia. In our swine model of demand-induced ischemia there is a dramatic switch from lactate uptake to production and marked lactate accumulation in the tissue, consistent with studies in coronary artery disease patients, which showed stimulation of lactate production when the heart was stressed by atrial pacing [4,28,37]. An increased efflux and accumulation of lactate in the tissue with no change in oxygen consumption, reflects an increased activation of non-oxidative glycolysis under these conditions. In vitro studies indicate that lactate and H^+ accumulation and efflux during ischemia have adverse effects on the capacity of cardiac muscle to maintain Ca^{2+} homeostasis and to utilize the energy released from ATP breakdown to perform contractile work [9,23]. Furthermore, drugs such as trimetazidine [15] or ranolazine [21], that decrease non-oxidative glycolysis by partial inhibition of fatty acid oxidation, improve the symptoms of patients with exercise induced angina, most likely through increasing mitochondrial pyruvate oxidation resulting in a reduction of lactate efflux and/or accumulation. Our results show in a clinically relevant model of demand-induced ischemia that oxfenicine reduced this high rate of non-oxidative glycolysis as demonstrated by less glycogen depletion and lactate accumulation and efflux, and greater glucose oxidation.

The consequence of the greater glucose oxidation and reduction in lactate production during demand-induced ischemia was a significant increase in external power and mechanical efficiency in the ischemic zone (Table 1 and Fig. 5). Traditional therapies for myocardial ischemia (e.g. beta blockers, calcium antagonists, or long acting nitrates), improve oxygen delivery to the ischemic cardiac muscle or they reduce the oxygen requirement of the myocardium by decreasing heart rate, blood pressure or inotropy [19,32]. While these therapies effectively lessen the degree of ischemia by better matching the delivery of oxygen to the amount of myocardial external power, they do not improve myocardial mechanical efficiency. Although some studies in the literature have demonstrated improvements in myocardial energetics with beta blocker therapy, these studies were conducted in patients with idiopathic cardiomyopathy [8] or left ventricular dysfunction [1]. To our knowledge, this is the first study to demonstrate that partial inhibition of fatty acid oxidation improves myocardial energy efficiency during demand-induced ischemia by

increasing myocardial contractile function at no additional energy cost.

It is important to note that the high rate of non-oxidative glycolysis observed during demand-induced ischemia in the vehicle group accounts for only 11.9±1.4% of the total energy expenditure, but it clearly reflects a dramatic disruption to normal myocardial metabolism as seen in the marked lactate efflux and accumulation in the tissue. However, the contribution of non-oxidative glycolysis during demand-induced ischemia was significantly reduced in the oxfenicine group to 6.5±1.2% of the total energy expenditure. These results clearly demonstrate a reduction in the contribution of non-oxidative glycolysis to the total energy expenditure of the myocardium during demand-induced ischemia.

During demand-induced ischemia, tracer measured fatty acid uptake and oxidation were both decreased and glucose oxidation was increased in the oxfenicine group. These changes in myocardial substrate use were accompanied by enhanced anterior wall power index. Furthermore, this switch in myocardial substrate use occurred despite no activation of PDH, since neither total PDH activity or the percent active PDH were increased in the oxfenicine group. Thus, during demand-induced ischemia, oxfenicine effectively enhanced glucose oxidation at the expense of fatty acid oxidation that resulted in improved myocardial contractile function. Pre-clinical and clinical studies have demonstrated that with myocardial ischemia or ischemia/reperfusion, interventions that increase myocardial carbohydrate metabolism benefit heart function or lessen tissue injury, whereas increases in fatty acid availability and oxidation can lead to a worsening of outcome [2,6,7,21,25]. Oxfenicine, a CPT-I inhibitor, has been shown to inhibit fatty acid oxidation and stimulate carbohydrate oxidation in rat heart and diaphragm [3,12,13] and human hearts [14]. Furthermore, oxfenicine attenuated cell damage in working rat hearts subjected to brief global ischemia [13] and increased the pacing time to angina in patients with obstructive coronary artery disease [2]. Thus, our findings are in agreement with previous studies suggesting that shifting energy substrate preference away from fatty acid oxidation towards glucose oxidation is an effective approach to improving myocardial contractile function during conditions of demand-induced ischemia.

It has been suggested that improvement in cardiac function with agents that switch myocardial substrate oxidation from fatty acids to carbohydrate is due to the greater theoretical ATP/O_2 ratio for glucose and lactate (6.0) than for long-chain fatty acids (5.2) [26,32]. In the present investigation oxfenicine resulted in partial inhibition of fatty acid oxidation and thus likely caused only a minor change in the ATP/O_2 ratio that would not explain the large increase in mechanical power without an increase in MVO_2. This strongly suggests that the greater power was not due to greater ATP production, but rather to more efficient ATP use at the level of the sarcoplasmic reticulum Ca^{2+} pump and myosin ATPase due to less lactate production and more optimal pH [9,23].

In conclusion, the results of the present investigation show that partial inhibition of fatty acid oxidation with the CPT-I inhibitor oxfenicine resulted in decreased lactate production, glycogen utilization and fatty acid uptake and oxidation, and greater glucose oxidation, anterior wall power and myocardial energy efficiency. This is the first direct evidence that partial inhibition of myocardial fatty acid oxidation will reduce lactate production and improve regional myocardial contractile function and mechanical efficiency during demand-induced ischemia. Our findings suggest that this mechanism is responsible for the improvement in symptoms of exercise-induced ischemia in patients with chronic stable angina treated with partial inhibitors of myocardial fatty acid oxidation.

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