Effect of Gender and Fatty Acids on Ischemic Recovery of Contractile and Pump Function in the Rat Heart

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ABSTRACT

Background: Clinical studies have shown that the incidence of heart disease is lower in premenopausal women compared with men. However, women are at increased risk of developing cardiac dysfunction after myocardial infarction. During a myocardial infarction, plasma levels of free fatty acids increase and contribute to postischemic left ventricular dysfunction.

Objective: The purpose of this study was to examine the effect of increasing concentrations of fatty acids on recovery of contractile parameters and pump function after 20 minutes of global ischemia and 30 minutes of reperfusion in male and female rat hearts.

Methods: Hearts were isolated and perfused with 5.5 mM glucose and 50 μU/mL insulin alone or in the presence of 0.4 or 1.2 mM palmitate. To determine whether inhibition of fatty acid metabolism was accompanied by an improvement in recovery of cardiac function after ischemia, the inhibitor of mitochondrial palmitate uptake, oxfenicine (2 mM), was used in female hearts perfused with 1.2 mM palmitate.

Results: Twenty-two female and 21 age-matched male rats were used. In hearts perfused under normoxic conditions, 1.2 mM palmitate reduced cardiac output and systolic pressure in female rat hearts. Heart rate, ventricular contraction, and ventricular relaxation were similar between male and female hearts and were not altered by fatty acids. After transient ischemia, all contractile parameters in male hearts returned to preischemic levels, regardless of the level of fatty acids in the perfusate. Recovery of female hearts, however, was inhibited by fatty acids. Aortic flow, ventricular contraction, ventricular relaxation, and systolic pressure were significantly lower in female hearts compared with male hearts in the presence of 1.2 mM palmitate (P < 0.05). In female hearts perfused with oxfenicine, however, recovery of systolic pressure, cardiac output, and ventricular contraction was significantly increased compared with control hearts (P < 0.05).

Conclusions: Our data indicate that the female myocardium is more sensitive to the effects of fatty acids after global ischemia compared with male hearts. This confirms that a gender effect exists in the recovery of heart function after ischemia, which can be accounted for by differences in ventricular contraction and relaxation. (Gender Med. 2004;1:86–99) Copyright © 2004 Excerpta Medica, Inc.

Key words: gender, cardiac function, fatty acids, ischemia, reperfusion.

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INTRODUCTION

Gender differences in cardiovascular function in response to experimental interventions have been reported. These include differential responses to adrenergic,1 vasoactive, and calcium stimulation,2-4 physical training,5 and gonadectomy,6,7 as well as after hormone replacement therapy.8-11 Gender differences are also evident in the incidence and severity of heart failure, which increases dramatically in women as they age during their postmenopausal years.12-15

The energy necessary for left ventricular (LV) contractility comes from adenosine triphosphate (ATP). In the heart, oxidation of fatty acids provides most of the ATP required for myocyte function.16 The heart can and will also generate ATP from glucose, lactate, and other energy substrates, depending on substrate availability and the metabolic demand of the heart.16 Although the oxidation of fatty acid is necessary for adequate ATP production, under conditions where fatty acid concentrations are elevated or their oxidation is increased—such as in the ischemic reperfused heart—deleterious consequences have been observed. These consequences include oxygen wasting,17,18 toxic accumulation of lipids,19 excessive proton production leading to Na+ and Ca2+ overload,20,21 and arrhythmias.22,23 Support for the role of fatty acids in the pathogenesis of the dysfunctional myocardium is evident in which inhibition of fatty acid use was associated with an improved recovery of mechanical function of the reperfused heart.24

Studies from our laboratory have shown marked gender differences in the extent of recovery of cardiac function after transient ischemia.25 Functional evidence of a greater sensitivity to ischemia was observed in the female heart, an effect dependent on the type of substrate oxidized by the myocardium. Indeed, recovery of mechanical function after ischemia was depressed in the female heart perfused with relevant ischemic levels of fatty acids, suggesting that the female myocardium is more sensitive to the effects of fatty acids compared with male hearts. This detrimental effect of fatty acids can explain, in part, the reason that women in their premenopausal years, despite experiencing a lower incidence of cardiovascular disease, are more susceptible than men to developing cardiac dysfunction after myocardial infarction.26,27

Although the relationship of substrate provision to the cardiac outcome after ischemia is the focus of intense investigation, to our knowledge no studies to date have addressed the effects of a range of fatty acids on intrinsic cardiac work, expressed as either contractility or pump function, simultaneously in male and female hearts subjected to ischemia and subsequent reperfusion. Therefore, the purpose of the current study was to examine and compare LV function, using the isolated working heart technique, in the male and female rat heart perfused under physiologic workload conditions. The metabolic substrates supplied to the hearts included glucose alone, glucose in the presence of normal physiologic levels of fatty acids,28 and glucose in combination with a level of fatty acids that mimics the clinical setting of ischemia.29

MATERIALS AND METHODS

Materials

Bovine serum albumin (fraction V) and dialysis tubing (6-8000 molecular weight cutoff) were purchased from Sigma-Aldrich (St. Louis, Missouri). Insulin was purchased from Henry Schein, Inc. (Melville, New York). All other common laboratory chemicals were obtained from Sigma-Aldrich and were of analytic grade.

Animals

Age-matched male and female Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, Massachusetts). Animals were housed in groups of 2 and provided with food and water ad libitum until the time of experimentation. All rats received humane care according to the guidelines set forth in the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources.30

Heart Perfusions

Rats were killed using CO2 gas followed by decapitation. Hearts were removed and perfused
retrogradely with warm (37°C) Krebs-Henseleit buffer, pH 7.4, containing NaCl 118 mM, KCl 4.7 mM, KH$_2$PO$_4$ 1.2 mM, MgSO$_4$ 1.2 mM, CaCl$_2$ 2.5 mM, NaHCO$_3$ 25 mM, and glucose 5.5 mM and gassed with 95% O$_2$/5% CO$_2$. During this perfusion, the hearts were trimmed of excess tissue, the pulmonary artery was cut, and the opening to the left atrium cannulated. After a 10-minute washout period in the retrograde mode, hearts were switched to the working mode and perfused at a constant left atrial preload pressure of 15 cm H$_2$O and constant afterload in which the left ventricle was ejecting against an aortic resistance of 100 cm H$_2$O. Heart rate, contractility, and pressure development were monitored using a transducer inserted into the aortic line (Micro-Med, Inc., Louisville, Kentucky). Contractility measurements were expressed as ventricular contraction (positive dP/dt: maximum rate of rise in intraventricular pressure during contraction) and ventricular relaxation (negative dP/dt: maximum rate of decrease in intraventricular pressure during relaxation). Cardiac output and aortic flow were measured using flow probes inserted into the left atrial preload and aortic afterload lines, respectively (Transonic Systems Inc., Ithaca, New York). Coronary flow was calculated as the difference between cardiac output and aortic flow. Heart function was measured every 5 minutes throughout the working heart perfusion. Throughout the entire perfusion, the temperature of the perfusate was kept constant at 37°C.

**Perfusion Protocol**

Cardiac function was obtained from 3 series of perfusions in the male and female rats. All working hearts were perfused with Krebs-Henseleit buffer containing 5.5 mM glucose and 50 µU/mL insulin (termed “glucose only”); 5.5 mM glucose, 50 µU/mL insulin, and 0.4 mM palmitate (termed “low fat”); or 5.5 mM glucose, 50 µU/mL insulin, and 1.2 mM palmitate (subsequently termed “high fat”). Bovine serum albumin (3%) was added to all working heart buffers. High levels of palmitate (“high fat”) were used to mimic concentrations observed clinically under conditions of postischemic stress. Working hearts were initially perfused under aerobic conditions for 15 minutes and then subjected to 20 minutes of global ischemia. Global ischemia was induced by clamping the left atrial and aortic afterload lines. After ischemia, flow was restored and hearts were reperfused for 30 minutes. In a separate series of perfusions, the effects of the fatty acid blocking agent oxfenicine (4-hydroxyphenylglycine, Sigma-Aldrich), an inhibitor of palmitate uptake (carnitine palmitoyltransferase-1 inhibitor) into the mitochondria, were determined only in female hearts perfused in the presence of high fat. In these experiments, oxfenicine was added directly into the reservoir, at the concentration of 2 mM, in the working heart buffer 5 minutes before the onset of reperfusion. This concentration of oxfenicine was selected based on the observation that it produced a dramatic decrease in palmitate oxidation and a concomitant increase in glucose oxidation and cardiac efficiency.

**Statistical Analysis**

Statistical analysis was performed using GraphPad InStat, version 2.0 (GraphPad Software, Inc., San Diego, California). All values are reported as mean (SEM). A one-way analysis of variance for repeated measures, followed by the Student-Newman-Keuls test, was used to determine differences in heart function between 0, 0.4, and 1.2 mM palmitate for each sex. The unpaired Student t test was applied to determine the group mean differences in heart function between male and female hearts. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

The physical characteristics of 22 female rats and 21 male rats are listed in the table. As expected and consistent with earlier observations for age-matched rats, body weight and heart weight were significantly higher ($P < 0.05$) in male animals compared with female animals. However, there were no significant differences in the heart-weight to body-weight ratio between male and female rats.
The cardiac functional consequences of altered substrate supply to male and female hearts perfused under aerobic conditions and after ischemia are shown in Figures 1 through 4. For Figures 1 through 3, the aerobic heart data represent the recordings obtained just before ischemia, whereas the reperfusion data are shown after 30 minutes of reperfusion. In hearts perfused under aerobic conditions, there were no significant differences in heart rate between male and female hearts regardless of the substrate used (Figure 1A). Systolic pressure was significantly decreased in female hearts compared with male hearts but only in the presence of high fat (P < 0.05) (Figure 1B). At this concentration of fatty acid, however, diastolic pressure was significantly increased in male hearts compared with female hearts (P < 0.05) (Figure 1C).

Figures 2 and 3 illustrate the effects of fatty acids on flow and contractility parameters. In aerobically perfused hearts, the only parameter altered by fatty acids was cardiac output in female hearts. Cardiac output was significantly decreased in the presence of high fat compared with glucose only (Figure 2A). Both ventricular contraction (Figure 3A) and ventricular relaxation (Figure 3B), although not significantly different between male and female hearts, tended to decrease in high-fat perfusions.

After a 20-minute period of global no-flow ischemia, recovery of mechanical function was monitored for 30 minutes. Recovery of heart rate was significantly increased in male hearts even in the presence of 1.2 mM palmitate, whereas this was not the case in female hearts (Figure 1A). At this concentration of fatty acid, recovery of systolic pressure was depressed in female hearts compared with male hearts, an event also observed when hearts were perfused with glucose only (Figure 1B). Also at this concentration of fatty acid (Figure 2B), aortic flow in female hearts was significantly lower compared with male hearts, although coronary flow was similar (Figure 2C). In female hearts, the reduction in aortic flow was associated with disturbances in ventricular contraction and relaxation. Both ventricular contraction (Figure 3A) and ventricular relaxation (Figure 3B) were significantly lower in female hearts compared with male hearts perfused with high fat.

The rate of recovery during reperfusion was also monitored. In hearts reperfused with either glucose alone or in the presence of low fat, the rate of recovery was not significantly different between male and female hearts (data not provided). However, in hearts reperfused with high fat, the rate of recovery remained depressed in the female hearts. As shown in Figure 4, recovery of systolic pressure (Figure 4A), ventricular contraction (Figure 4B), and ventricular relaxation (Figure 4C) was depressed in the female heart at all time points throughout the reperfusion period.

To determine whether increased levels of fatty acids were contributing to postischemic LV dysfunction in the female heart, the effects of inhibition of fatty acid uptake using oxfenicine was determined. Oxfenicine, when added to the perfusate of female hearts containing high fat, resulted in a dramatic improvement in heart function. As shown in Figure 5, recovery of systolic pressure at 10 minutes into the reperfusion period was significantly increased compared with control hearts (P < 0.05) (Figure 5A). At 30 minutes into the reperfusion, ventricular contraction was also increased in female hearts treated with oxfenicine (Figure 5B). In the same

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Table. Physical characteristics of age-matched male and female rats. Values are given as mean (SEM).

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>HW (g)</th>
<th>HW/BW Ratio (x10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (n = 22)</td>
<td>255 (5)*</td>
<td>1.30 (0.03)*</td>
<td>5.1 (1.6)</td>
</tr>
<tr>
<td>Males (n = 21)</td>
<td>369 (12)</td>
<td>1.73 (0.08)</td>
<td>4.6 (1.2)</td>
</tr>
</tbody>
</table>

BW = body weight; HW = heart weight.
*P < 0.05 versus males.
Figure 1. (A) Heart rate, (B) systolic pressure, and (C) diastolic pressure in isolated working hearts from male and female rats perfused with glucose alone or in the presence of either 0.4-mM palmitate or 1.2-mM palmitate concentrations. Aerobic heart function data represent the recordings obtained at 15 minutes into the aerobic period; reperfusion data represent those at 30 minutes of reperfusion. Values are given as mean (SEM) for 10 male and 10 female hearts perfused with glucose; 9 male and 9 female hearts perfused with 0.4 mM palmitate; and 10 male and 10 female hearts perfused with 1.2 mM palmitate. *P < 0.05 compared with male hearts perfused without palmitate; †P < 0.05 compared with male hearts perfused with the same substrate.
Figure 2. (A) Cardiac output, (B) aortic flow, and (C) coronary flow in isolated working hearts from male and female rats perfused with glucose alone or in the presence of either 0.4-mM palmitate or 1.2-mM palmitate concentrations. Aerobic heart function data represent the recordings obtained at 15 minutes into the aerobic period; reperfusion data represent those at 30 minutes of reperfusion. Values are given as mean (SEM) for 10 male and 10 female hearts perfused with glucose; 9 male and 9 female hearts perfused with 0.4 mM palmitate; and 10 male and 10 female hearts perfused with 1.2 mM palmitate. *P < 0.05 compared with hearts perfused without palmitate; †P < 0.05 compared with male hearts perfused with the same substrate.
hearts (Figure 6), there was a beneficial effect of oxfenicine on both aortic flow and coronary flow (Figures 6B and 6C), resulting in a significant increase in cardiac output compared with control hearts ($P < 0.05$) (Figure 6A). However, the effect of oxfenicine on diastolic pressure during reperfusion was not significant. Heart rate tended to be greater in female hearts treated with oxfenicine but failed to reach statistical significance (data not provided).

**DISCUSSION**

In a previous report, we demonstrated that female rat hearts perfused with a level of fatty acids reflecting the clinical setting of ischemia are more sensitive to ischemic injury compared with male rat hearts. This difference in sensitivity to ischemic insult was reflected by a depression in pressure development during reperfusion in the female heart. This gender dimorphism is consistent with the current findings of reduced...
Figure 4. Recovery of (A) systolic pressure, (B) ventricular contraction, and (C) ventricular relaxation after ischemia in isolated working hearts from male and female rats perfused with glucose in the presence of 1.2 mM palmitate. Values are given as mean (SEM) for 10 male and 10 female hearts. *P < 0.05 compared with male hearts.
Figure 5. Recovery of (A) systolic pressure, (B) ventricular contraction, and (C) ventricular relaxation after ischemia in isolated working hearts from female rats perfused with glucose in the presence of 1.2 mM palmitate. Oxfenicine (2 mM), when used, was added directly into the working heart perfusate at 5 minutes before reperfusion. Values are given as mean (SEM) for 3 female control hearts and 3 female oxfenicine-treated hearts. *P < 0.05 compared with control hearts.
Figure 6. Recovery of (A) cardiac output, (B) aortic flow, and (C) coronary flow after ischemia in isolated working hearts from female rats perfused with glucose in the presence of 1.2 mM palmitate. Oxfenicine (2 mM), when used, was added directly into the working heart perfusate at 5 minutes before reperfusion. Values are given as mean (SEM) for 3 female control hearts and 3 female oxfenicine-treated hearts. *P < 0.05 compared with control hearts.
contractile function in the female heart under these conditions of ischemia and reperfusion. However, in our earlier work, a lack of measurable intrinsic cardiac parameters and adequate substrate conditions precluded us from confirming the role of fatty acids in the female myocardium. For this reason, we chose to address these limitations in the current study by the inclusion of additional substrate conditions and the measurement of more sensitive intrinsic contractile and pump function parameters. In addition, to determine whether ischemic levels of fatty acids contribute to the LV dysfunction in the female heart, we examined the effects of drug-induced inhibition of fatty acid uptake, using oxfenicine, on recovery of cardiac function after ischemia.

In the current study, the response of isolated working hearts from female and male rats was compared under normal aerobic conditions and during reperfusion after severe ischemia in the presence of various metabolic fuels. Substrate for the supply of energy to the myocardium was provided by glucose solely, glucose with a physiologic level of fatty acids (0.4 mM palmitate), or glucose in the presence of an elevated level of fatty acid representative of the clinical scenario of ischemia (1.2 mM palmitate). The results of our study indicate that altering substrate availability produced marked differences in cardiac function between male and female hearts; these differences are particularly evident after a period of transient ischemia as the heart recovers during reperfusion. Recovery of LV function in female hearts, compared with male hearts, was clearly a function of the level of fatty acids. In fact, at the highest concentration of the fatty acid palmitate used (1.2 mM), systolic pressure, aortic flow, and both ventricular contraction and ventricular relaxation were significantly lower in female hearts compared with male hearts. These significant differences in function were not seen when female hearts were perfused at the lower concentration of 0.4 mM palmitate. Our results also indicate that a gender difference exists in the rate of recovery of cardiac function after ischemia with a 1.2 mM concentration of palmitate. Recovery of systolic pressure, as well as recovery of ventricular contraction and ventricular relaxation during the early stages of reperfusion, was compromised in female hearts compared with male hearts, indicating that a greater sensitivity to ischemia with this concentration of fatty acid occurs in the female heart. However, in response to inhibition of fatty acid uptake using oxfenicine, recovery of systolic pressure, ventricular contraction, and cardiac output was improved in the female heart perfused under conditions of high fat.

The mechanisms by which elevated concentrations of fatty acids are detrimental to the recovery of cardiac performance during reperfusion in the heart have been well documented. What warrants further investigation is whether these mechanisms can account for the reported greater sensitivity to ischemia in the female heart. It is possible that the requirement for oxygen from fatty acid esterification into the triacylglycerol pool is greater in the female heart. The complete oxidation of palmitate requires typically ~11% more oxygen per carbon unit than complete oxidation of glucose. The female myocardium may also be more sensitive to the uncoupling effect of fatty acids on mitochondrial respiratory chain phosphorylation, as well as to the production of reactive oxygen species mediated by this substrate. The accumulation of intermediates of fatty acid metabolism, which inhibit high-energy phosphate transfer across mitochondrial membranes and thus impair the efficient energy use, may also be implicated, although this relationship is not always consistent.

A mechanism more likely contributing to postischemic LV dysfunction in the female myocardium is the effect fatty acids exert on pyruvate metabolism. During reperfusion, rates of fatty acid oxidation are elevated, which in turn inhibit pyruvate oxidation contributing to LV dysfunction. Overcoming the inhibitory effects of fatty acids can be accomplished by pharmacologic stimulation of glucose oxidation or by inhibition of fatty acid oxidation at the level of beta-oxidation or uptake by the mitochondria. In rat and pig hearts, inhibition of carnitine palmitoyltransferase-1 (the key enzyme
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for mitochondrial fatty acid uptake), using oxfenicine, produced a dramatic decrease in palmitate oxidation and increased glucose oxidation and cardiac efficiency.\textsuperscript{30,37,39} In the current study, treating female hearts previously subjected to ischemia with this inhibitor resulted in an improvement in cardiac function in the presence of elevated levels of fatty acids. This improvement presumably occurred as a result of this inhibition usage, a mechanism consistent with energy optimization, and clearly indicates that exposure of female hearts to elevated concentrations of fatty acids is a contributing factor to the development of the dysfunctional myocardium.

**CONCLUSIONS**

This study has confirmed that a gender difference exists in the sensitivity of the rat myocardium to a period of transient severe global ischemia. This sexual dimorphism is manifested by a depression in the mechanical recovery, expressed as contractility and pump function, of the myocardium during reperfusion in the female rat heart. Moreover, although these differences are observed only in the presence of ischemic levels of fatty acids rather than at physiologic levels, it is clear that the contributory role of this substrate deserves further attention for the design of gender-specific therapeutic interventions for impaired cardiac function after ischemia.

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