Fructose-Fed Rats Are Protected against Ischemia/Reperfusion Injury

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ABSTRACT
This study examines the relationship between insulin resistance (IR) induced by fructose feeding (FF) and susceptibility to myocardial ischemia/reperfusion injury (MI/R). Six-week-old male Sprague-Dawley rats were randomized into control (CON; n = 59) or FF (n = 58) groups. After 4 weeks, rats were further randomized into one of the following groups: placebo, ischemic preconditioning (IPC), 5-hydroxydecanoic acid (5-HD) (10 mg/kg), or 5-HD + IPC. Moreover, to determine the role of fructose, a second model of IR (Zucker obese) and rats fed fructose diet for 3 days (FF-3) were also subjected to MI/R. In all experiments, rats were subjected to 30 min of myocardial ischemia and 4 h of reperfusion. In rats randomized to placebo, infarct size was significantly reduced by FF (24 ± 5%) compared with CON (54 ± 1%, p < 0.05). Pretreatment with 5-HD did not alter the infarct size in CON (45 ± 5%) but inhibited the protection afforded by FF (53 ± 7%). IPC reduced the infarct size to an equivalent level in both groups, whereas 5-HD administration prior to IPC blunted the IPC effect. In Zucker obese rats, infarct size was significantly larger (57 ± 4%) compared with lean controls (37 ± 4%, p < 0.05). In FF-3 rats, infarct size was also decreased (20 ± 2%, p < 0.01) compared with CON. This study suggests that fructose feeding affords protection against MI/R that is related to or mimics preconditioning. This protection is not consistent with other models of IR and is likely related to the fructose diet itself.

Insulin resistance is defined as a defect in the ability of insulin to stimulate glucose uptake and results in a metabolic syndrome characterized by impaired glucose tolerance and hyperinsulinemia (Reaven, 2003). Without necessary diet and lifestyle changes, the insulin-resistant syndrome usually progresses to type II diabetes mellitus (Reaven, 2003). Both insulin resistance and type II diabetes are known risk factors for the development of atherosclerosis and myocardial infarction (Bressler et al., 1996; Haffner et al., 1998; Plutzky et al., 2002). However, in addition to being a risk for the development of ischemic heart disease, being diabetic is also a liability during an acute ischemic event, where these patients and animals have larger infarctions and decreased survival rates after myocardial infarction versus their nondiabetic counterparts (Nadeau et al., 1986; Haffner et al., 1998; Cho et al., 2002; Donnan et al., 2002; Marfella et al., 2002). The impact of insulin resistance without diabetes during an acute ischemic episode is not known.

Previous data from our laboratory and others’ have shown that the fructose-fed rat model develops the insulin-resistant syndrome with a very similar metabolic profile to the human condition, including hyperinsulinemia, insulin resistance, hypertriglyceridemia, and decreased HDL cholesterol (Hwang et al., 1987; Miller et al., 1999). Moreover, we have shown that rats fed this diet for a period of 4 weeks exhibit coronary vascular dysfunction, such that endothelium-dependent vasodilation is impaired (Miller et al., 1999).

In the current study, we hypothesized that based on the underlying metabolic derangements and coronary vascular dysfunction present in insulin resistance, greater myocardial damage injury would result following myocardial ischemia/reperfusion injury (MI/R). Therefore, we examined the effects of MI/R in two rodent models of insulin resistance: the fructose-fed rat and the Zucker obese rat. Furthermore, we examined the effects of fructose diet alone (without metabolic changes) on MI/R injury.

Materials and Methods

The protocol was approved by the Institutional Animal Care and Use Committee of Wake Forest University School of Medicine in August, 2001. All experiments complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats were obtained at 6 weeks of age and

ABBREVIATIONS: MI/R, myocardial ischemia/reperfusion injury; FF, fructose fed; IPC, ischemic preconditioning; 5-HD, 5-hydroxydecanoic acid; K_{ATP}, ATP-dependent potassium channel; TTC, triphenyltetrazolium chloride; MPO, myeloperoxidase.
randomized into one of the following two groups: 1) control (n = 59) or 2) fructose-fed (n = 58) rats. Animals in the fructose-fed group were fed a fructose-rich diet containing 66% fructose, 22% casein, and 12% lard plus essential vitamins and minerals (Harlan Teklad; Madison, WI), whereas control animals received standard rat chow. Each diet was continued for 4 weeks. Previous studies in our laboratory and others’ have shown that receiving the fructose-rich diet for a period of 4 weeks induces a state of insulin resistance, which is characterized by glucose intolerance, hyperinsulinemia, hypertriglyceridemia, and decreased HDL cholesterol (Hwang et al., 1987; Miller et al., 1999). Moreover, we and others have shown that this model of insulin resistance develops impaired function in several vascular beds, including the coronary, mesenteric, and cerebral arteries (Katakam et al., 1999; Miller et al., 1999; Erdos et al., 2002).

**Myocardial Ischemia and Reperfusion Studies.** After 4 weeks of their respective diets, the rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.), and the jugular vein and trachea were cannulated. The rats were ventilated with 100% oxygen by a rodent ventilator adjusted to maintain exhaled CO2 between 3.5 and 4.5%. Anesthesia was maintained with sodium pentobarbital (25 mg/kg i.v.) on an as-needed basis. A left thoracotomy was performed, and a suture was placed 3 to 4 mm from the origin of the left coronary artery. Index ischemia (30 min) was initiated by tightening of the suture around the coronary artery and was followed by 4 h of reperfusion.

Following the reperfusion period, infarct size was determined by differential staining and triphenyltetrazolium chloride (TTC) as described previously, with minor modifications (Vakeva et al., 1998). Briefly, the coronary ligature was retightened, and an intravenous injection of patent blue violet (0.5%) was given to stain the normally perfused regions of the heart. The heart was then removed and bathed in ice-cold saline before removal of the atria, great vessels, and right ventricle. The left ventricle was sliced into thin sections, and the unstained area at risk was separated from the normally perfused blue sections, cut into 1- to 2-mm3 pieces, incubated with TTC (1% in phosphate buffer) for 5 min, and then fixed with 10% formaldehyde for 5 min. With a dissecting microscope, the necrotic areas (area of necrosis, pale) were separated from the TTC-positive (brick red-staining) areas. All areas of the myocardium were then weighed individually, and infarct size was calculated (area of necrosis/area at risk × 100).

Based on our initial findings of a protective effect of fructose feeding, we further randomized control and fructose-fed rats to receive one of the following treatments (Fig. 1): 1) ischemic preconditioning (IPC) (5 min of coronary occlusion followed by 5 min of reperfusion), 2) 5-hydroxydecanoic acid (5-HD) (10 mg/kg i.v.), or 3) 5-HD + IPC. Following each of the above treatments, the aforementioned ischemia/reperfusion protocol ensued followed by infarct size measurement via differential staining.

**Myeloperoxidase Activity.** In a separate set of experiments, rats were randomized to the same groups as above, including placebo (saline), and underwent the same ischemia/reperfusion protocol. After reperfusion, the hearts were removed and rinsed in ice-cold saline, and the left ventricular free wall was frozen. Frozen samples were analyzed for myeloperoxidase (MPO) activity as described previously (Vakeva et al., 1998). Briefly, the tissue was homogenized in 5 ml of phosphate buffer, pelleted by centrifugation, and rehomogenized in 5 mM phosphate buffer with 0.5% hexadecyltrimethylammonium bromide (10% w/v). The samples were then subjected to three freeze/thaw cycles and brief sonication. The suspension was then centrifuged to remove cellular and membrane debris. The supernatant was analyzed spectrophotometrically in the presence of hydrogen peroxide and o-dianisidine and reported as units/100 mg tissue.

**Consistency among Models of Insulin Resistance.** To ascertain whether the protective effect afforded by fructose feeding was consistent among models of insulin resistance, we determined the effect of M/R injury in the Zucker obese rat, another commonly used model of insulin resistance. Zucker lean (controls) (n = 8) and obese (n = 8) rats were anesthetized and instrumented as described above and underwent 30 min of index ischemia followed by 4 h of reperfusion. Fasting insulin, glucose, and body weights for all experimental groups were located in Table 1. Fasting glucose was not significantly different in either of the fructose-fed groups compared with the saline control. Moreover, fasting glucose was not significantly elevated in the Zucker obese rats compared with their Zucker lean control. Conversely, fasting insulin was markedly elevated in both the fructose-fed (4-week) group and in the Zucker obese group compared with their respective controls. It should be noted, however, that in the 5-day fructose-fed rats, insulin concentrations were almost identical to the saline control. There were no differences in weight, with the exception of the
TABLE 1
Fasting glucose and insulin for all groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose</th>
<th>Insulin</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td>pg/ml</td>
<td>g</td>
</tr>
<tr>
<td>Control (Sprague-Dawley)</td>
<td>101\pm 3</td>
<td>771 \pm 140</td>
<td>324 \pm 8</td>
</tr>
<tr>
<td>Fructose-fed (4-week)</td>
<td>107 \pm 7</td>
<td>2279 \pm 152$^*$</td>
<td>331 \pm 7</td>
</tr>
<tr>
<td>Fructose-fed (3-day)</td>
<td>101 \pm 4</td>
<td>514 \pm 175</td>
<td>344 \pm 6</td>
</tr>
<tr>
<td>Zucker lean</td>
<td>125 \pm 3</td>
<td>895 \pm 209</td>
<td>304 \pm 3</td>
</tr>
<tr>
<td>Zucker obese</td>
<td>144 \pm 21</td>
<td>11039 \pm 1135$^*$</td>
<td>441 \pm 6$^*$</td>
</tr>
</tbody>
</table>

$^*$ p < 0.05 vs. respective control.

Zucker obese rat being significantly heavier than its Zucker lean control.

**Effect of Fructose Feeding on Infarct Size.** All groups studied had a similar area of the left ventricle made ischemic (area at risk) by occlusion of the left coronary artery (Table 2). Infarct size, expressed as the area of necrosis as a percentage of area at risk, is shown in Fig. 2. In the initial groups studied, which received no active treatment (i.e., saline/placebo), the area of necrosis was significantly smaller in the fructose-fed rats (24 \pm 5\% versus those on control diet (54 \pm 1\%, p < 0.01). Ischemic preconditioning decreased the area of necrosis to similar levels in both groups (11 \pm 3\% for fructose-fed and 11 \pm 1\% for control). Administration of 5-HD to each group prior to the induction of the index ischemia reversed the protection afforded by fructose feeding to control levels (53 \pm 7\% for fructose-fed, p < 0.01 versus placebo-treated, fructose-fed group), whereas it did not alter the area of necrosis for the control group (45 \pm 5\%). Administration of 5-HD to both groups of rats prior to ischemic preconditioning partially inhibited the protective effect of IPC (35 \pm 3\% for fructose-fed and 33 \pm 2\% for control).

**Effect of Fructose Feeding on Myeloperoxidase Activity.** To assess neutrophil accumulation within the ischemic-reperfused myocardium, MPO activity was measured in the left ventricular free wall. Similar to our findings with infract size, MPO activity was significantly reduced in hearts from the fructose-fed rats compared with those from control rats (Table 3). Ischemic preconditioning decreased the MPO activity to a similar degree in hearts from both control and fructose-fed rats (Table 3). 5-HD pretreatment of fructose-fed rats caused a significant increase in MPO compared with hearts from fructose-fed rats not receiving treatment. MPO activity was not affected by 5-HD pretreatment in control rats. Administration of 5-HD prior to ischemic preconditioning increased the MPO activity to a similar degree in both groups compared with MPO values after ischemic preconditioning alone.

**Findings in the Zucker Model of Insulin Resistance.** To determine whether this protective effect afforded by fructose feeding was consistent among models of insulin resistance, we determined the effect of MI/R injury in the Zucker obese model of insulin resistance. Both the Zucker lean and obese groups of rats had similar areas at risk compared with the above results (38 \pm 3\% for lean and 41 \pm 3\% for obese). The infarct size is shown in Fig. 3. In contrast to the fructose-fed rat model of insulin resistance, the area of necrosis in the Zucker obese rats was significantly larger compared with its lean control (57 \pm 4\% for obese and 37 \pm 4\% for lean, p < 0.05). These findings demonstrate that the protection afforded by fructose feeding is not consistent with other models of insulin resistance.

**Effect of Acute Fructose Feeding.** To determine whether the aforementioned protective effect by fructose feeding is due to fructose itself and not insulin resistance, we determined the effect of MI/R injury in rats fed the high-fructose diet for 3 days, a time period prior to the development of metabolic effects. Again, there were no differences in the area at risk compared with the previous data (37 \pm 4\%). Similar to the findings in rats fed fructose for 4 weeks, the
infarct size was also significantly reduced (20 ± 5%, p < 0.01) compared with control saline rats (Fig. 3). These findings suggest that fructose feeding itself may be responsible for the observed protection.

Discussion

Previous studies have shown that following coronary ischemia/reperfusion injury, animals or humans possessing diabetes mellitus suffer greater myocardial damage compared with their nondiabetic counterparts (Nadeau et al., 1986; Cho et al., 2002; Donnan et al., 2002; Marfella et al., 2002). However, the effect of the prediabetic state of insulin resistance during ischemia/reperfusion has not been addressed. Since patients (and animal models) with insulin resistance possess some of the same qualities as type II diabetes, such as glucose intolerance, hypertriglyceridemia, increased free fatty acids, and coronary vascular impairment, we hypothesized that insulin-resistant rats would also suffer greater myocardial damage after ischemia/reperfusion injury than rats with normal glucose tolerance. In this study, we have shown that insulin resistance induced by fructose feeding is protective in the setting of ischemia/reperfusion injury. However, our findings suggest that this protective phenomenon is specifically related to this particular model (fructose-rich diet) and not to the state of insulin resistance.

It is currently unclear why feeding rats a high-fructose diet might afford protection during ischemia/reperfusion injury; however, we hypothesized that it might due to a "chemical preconditioning" response. Therefore, we designed further experiments to address this possibility. In both fructose-fed and control rats, IPC decreased ischemia/reperfusion injury as measured by infarct size; however, this protection was only significant in control animals. It should be noted that MPO was significantly reduced in fructose-fed rats subjected to IPC, suggesting that IPC likely adds some additional protection against myocardial ischemia/reperfusion injury. In contrast, pretreatment of fructose-fed rats with 5-HD, a mitochondrial K\textsubscript{ATP} channel antagonist and known inhibitor of ischemic preconditioning, completely abrogated the protection afforded by fructose feeding, whereas it had no effect on the control rats. These data suggest that the protection provided via fructose feeding is related to a "preconditioning" response and may be directly or indirectly linked to the mitochondrial K\textsubscript{ATP} channel. It should be noted that recent data have questioned the specificity of 5-HD as a mitochondrial K\textsubscript{ATP} channel antagonist (Hanley et al., 2002), thus, this channel may or may not be involved. However, 5-HD is a well accepted inhibitor of ischemic and pharmacologic preconditioning (Fryer et al., 2000; Riess et al., 2002), and thus we feel confident that the preconditioning phenomenon is a viable mechanism for the myocardial protection we observed.

Since we used a dietary model (fructose feeding) to assess the effect of insulin resistance on myocardial ischemia/reperfusion injury, it was unclear whether the myocardial protection that we noted was due to the metabolic state of insulin resistance or was more specifically related to ingesting large amounts of fructose. In an attempt to clarify this issue, we performed a second set of experiments in a different model of insulin resistance, the Zucker obese rat. This model is similar to the fructose-fed rat in that it has comparable metabolic abnormalities (such as glucose intolerance, hyperinsulinemia, and dyslipidemias); however, the mechanism of these disturbances is due to a genetic alteration in leptin signaling (Jiang et al., 1999). These rats also differ from the fructose-fed model by being markedly obese and mildly hyperglycemic (Table 1). In Zucker obese rats, application of the same ischemia/reperfusion protocol resulted in greater myocardial injury compared with the control Zucker lean rats. Based on these findings and those in diabetic animals, we believe that the myocardial protection seen in the fructose-fed rat is more likely due to the high-fructose diet as opposed to the state of insulin resistance. Moreover, these findings suggest that MI/R in a genetically induced model of insulin resistance and glucose intolerance results in greater myocardial damage, which is consistent with our original hypothesis concerning the prediabetic state. However, there are obvious metabolic differences between the Zucker obese and fructose-fed models; thus, we can not state conclusively that the metabolic syndrome induced by fructose feeding is not responsible for the observed myocardial protection.

Thus, to clarify this issue further, we performed a third set of experiments where we fed age-matched (10-week) control rats a high-fructose diet for only 3 days and followed this by the same myocardial ischemia/reperfusion protocol. As evidenced by normal fasting glucose and insulin levels seen at the end of 3 days (Table 1), this time period of high fructose ingestion is not long enough to induce the metabolic changes seen after longer periods of fructose feeding. In this set of experiments, we demonstrate that the same protection is afforded after 3 days of fructose feeding as that of 4 weeks of fructose feeding, suggesting that this diet-induced myocardial protection is a direct effect of fructose and not due to the metabolic changes resulting from the diet.

The mechanism by which fructose feeding is protective during myocardial ischemia/reperfusion is not known. However, we theorize that alterations in glycogen storage and/or glycolytic efficiency may be responsible. During hypoxia and ischemia, myocardial energy production switches from the use of fatty acids to carbohydrates, thereby allowing maintenance of adequate ATP synthesis when oxygen availability is limited. Although somewhat controversial depending on the model employed (Depre et al., 1999), there is an abundance of
data that suggest that increased preischemic glycogen stores may provide protection against a myocardial ischemic insult (Cross et al., 1996; Eynan et al., 2002). Moreover, it has been shown that small amounts of dietary fructose increase glycogen storage in the liver (Conlee et al., 1987; Watford, 2002). Therefore, it is possible that ingesting large amounts of fructose may increase myocardial glycogen stores. Additionally, previous in vitro studies in liver have shown fructose to be protective during both hypoxia and anoxia (Okabe et al., 1991; Gasbarrini et al., 1992; Hatenaka et al., 1994). The mechanism for this protection is purportedly due to an increased production in ATP during anaerobic metabolism (Okabe et al., 1991). Thus, it is possible, though untested, that fructose feeding increases glycogen storage that in turn provides energy to the myocardium during ischemia. Obviously, this mechanistic proposal is speculative; however, it is clear from the current data that fructose feeding alters the myocardial metabolism in some way such that it is protected from ischemia.

**Summary.** The intent of this study was to determine the effect of insulin resistance on myocardial ischemia/reperfusion injury; however, during our pursuit of this question, we serendipitously discovered that fructose feeding of rats is protective against myocardial ischemia/reperfusion injury. This protection is specific to the dietary ingestion of fructose and not secondary to the metabolic abnormalities associated with it. Moreover, fructose feeding appears to provide protection via a preconditioning phenomenon. The mechanism of this protection is unclear. However, it may have the potential to be harnessed into a useful clinical tool.

**References**


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