Myocardial heat shock protein 60 expression in insulin-resistant and diabetic rats

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Abstract

Heat shock protein 60 (HSPD1) plays a critical role in myocardial protection. Its reduced expression may lower myocardial protection against ischemic injury in the diabetic state. This study was conducted to investigate the natural course of fructose-fed insulin-resistant rats, define changes in myocardial HSPD1 expression, and determine the effects of thiazolidinedione or anti-hypertensive treatment. Results showed that insulin resistance with hyperinsulinemia and hypertension developed after 6 weeks of fructose feeding. This time-course study also showed that myocardial HSPD1 expression was mildly increased in week 6 (P=0.05) and significantly increased in week 8. Rosiglitazone-treated rats had restored systolic blood pressure (BP) and normalized plasma insulin level during oral glucose tolerance tests, whereas amlodipine-treated rats restored only systolic BP. Both amlodipine and rosiglitazone treatments normalized the abundance of myocardial HSPD1 expression in fructose-fed rats. When these rats received streptozotocin injection and diabetes developed, myocardial HSPD1 expression decreased despite persistent hypertension. In conclusion, this is the first study to report that myocardial HSPD1 expression is increased in high-fructose-fed rats, which may be due to increased BP. Once the high-fructose-fed rats developed diabetes with insulin deficiency, the myocardial HSPD1 expression decreased in spite of persistent hypertension. Journal of Endocrinology (2009) 200, 151–157

Introduction


Increased HSPD1 expression during ischemic and reperfusion injuries (Latchman 2001, Kirchhoff et al. 2002) and in ischemic and hypertrophic cardiomyopathies (Li et al. 1998, Sakai et al. 2003) probably represent a myocardial self-defense mechanism (Schafler et al. 2002). However, HSPD1 expression has been found to be reduced in diabetic myocardium (Chen et al. 2005). Thus, reduced expression of HSPD1 may lower myocardial protection against ischemic injuries in the diabetic state. Insulin resistance and impaired insulin secretion are the main pathophysiologic defects responsible for the development of type 2 diabetes (Saad 1991, Nathan 2002). This study proposed the hypothesis that myocardial HSPD1 expression may be increased in the prediabetic state with insulin resistance and hyperinsulinemia, but is decreased with progressive loss of β-cell function.

To address this issue, fructose-fed rats were used to investigate the effects of insulin resistance on cardiac HSPD1 expression. A previous study has shown that high-fructose supplementation can induce insulin resistance, hyperinsulinemia, and hypertension (Hwang et al. 1987). To further determine the independent effects of insulin resistance and hypertension on changes in myocardial HSPD1, the study used rosiglitazone and amlodipine to treat fructose-fed rats. Rosiglitazone is an agonist for peroxisome proliferator-activated receptor γ, which is a ligand-activated nuclear transcription factor that lowers blood glucose primarily by increasing insulin sensitivity in peripheral tissues (Yki-Jarvinen 2004). Amlodipine is a...
Research design and methods

Animals and diets

Male Sprague–Dawley rats weighing 200–250 g were housed in a cage in an air-conditioned room (22 °C ± 2 °C) on a 12-h light cycle (1800 h to 0600 h). These animals were maintained according to the guidelines established in the ‘Taiwan Government Guide for the Care and Use of Laboratory Animals’. The rats were divided into two groups: the control group, which was given deionized distilled water to drink and fed standard rat chow (60% vegetable starch, 12% fat, and 28% protein, Purina, St. Louis, MO, USA) and the fructose group, which was given deionized distilled water and fed a diet of 66% fructose, 12% fat, and 22% protein (Teklad, Madison, WI, USA).

Diabetes was induced by injecting low-dose streptozotocin (STZ, 40 mg/kg body weight, i.p.) into the high-fructose-fed rats. BP was measured every 2 weeks after randomization of diet supplementation. Oral glucose tolerance tests (OGTT) were performed in these rats at the age of 4, 6, 8, 10, and 12 weeks to define insulin resistance and β-cell function. Amlodipine (15 mg/kg) and rosiglitazone (3 mg/kg) was administered via oral gavage for 8 weeks accompanied with fructose feeding. At the end of the experiment, the rats were killed after overnight fasting, and heart and blood samples were collected in heparinized tubes.

Analytical methods

BP was measured using a tail-cuff method and a Narco Bio-System Physiograph (Houston, TX, USA; Bunag 1973). The rats were transported to a quiet environment and kept in cages with free access to water. The small animal unit had a rat holder base with a built-in warming element that allowed an increase of ambient temperature to 37 °C, thus maintaining adequate circulation in the rats’ tail for reliable measurements. Amlodipine (15 mg/kg) and rosiglitazone (3 mg/kg) was administered via oral gavage for 8 weeks accompanied with fructose feeding. At the end of the experiment, the rats were killed after overnight fasting, and heart and blood samples were collected in heparinized tubes.

Statistical analysis

The data were expressed as mean ± S.E.M. based on data derived from two to six independent experiments. Areas under the glucose and insulin curves (AUC) during the OGTT were calculated by the trapezoid rule. The band intensity from western blots was scanned with densitometry and digitally analyzed. Statistical significance as tested by Student’s t-test or by the post hoc test made after a two-way ANOVA resulted in a significant F test among groups for multiple measurements. A P value < 0.05 was considered statistically significant.

Results

Characteristics of fructose-fed rats

There was no weight difference between the two groups over the 8-week period although the weight of fructose-fed rats increased after 10 weeks of feeding (Fig. 1A). The systolic BP in the fructose-fed group began to increase after 2 weeks of randomization and there were significant differences between the two groups from week 4 to the end of the experiment (Fig. 1B).

The OGTT was performed every two weeks from weeks 4 to 12. During the 2 h following glucose ingestion, blood glucose levels showed no significant difference at any time point between the two groups from weeks 4 to 8 (Supplementary Fig. 1, see
Supplementary data in the online version of the Journal of Endocrinology at http://joe.endocrinology-journals.org/content/vol200/issue2/). However, the plasma glucose levels in the fructose-fed group were significantly higher than those in the control group at 30, 60, and 90 min in week 10, and at 30, 60, 90, and 120 min in week 12 (Supplementary Fig. 1). Plasma insulin levels had no significant differences at any time point between the two groups in weeks 4, 10, and 12 (Supplementary Fig. 2, see Supplementary data in the online version of the Journal of Endocrinology at http://joe.endocrinology-journals.org/content/vol200/issue2/). However, plasma insulin levels in the fructose-fed group increased in all time points in weeks 6 and 8 (Supplementary Fig. 2).

The total area under the curve (AUC) for glucose during the 2-h OGTT increased in the fructose-fed rats in weeks 10 and 12 (Fig. 1C). The AUC for plasma insulin of fructose-fed rats also increased in weeks 6 and 8 (Fig. 1D). Based on Fig. 1C and D, hyperinsulinemia occurs at weeks 6 and 8. Thereafter, insulin levels go back to control levels and glucose levels are increased. These data suggested that insulin resistance and hyperinsulinemia developed after 6 weeks of fructose feeding, and that impaired insulin secretion due to β-cells failure after 10 weeks of fructose feeding.

Myocardial HSPD1 expression increased in high-fructose-fed rats

To investigate whether the insulin resistance affected myocardial HSPD1 expression, we used 24 animals (n = 2 in each group) to test the myocardial HSPD1 expression in the different time, and the rat hearts were collected every 2 weeks from weeks 4 to 12. The time-course study showed that the expression of myocardial HSPD1 mildly increased in week 6 and significantly increased in week 8 (data not shown). The over-expression of myocardial HSPD1 disappeared after 10 weeks of fructose feeding (data not shown). The increased myocardial HSPD1 expressions after 6 and 8 weeks of high-fructose feeding were confirmed with more experimental animals (Fig. 2). These data suggested that fructose-fed rats could increase the expression of the myocardial HSPD1.

Hypertension increased myocardial HSPD1 expression

The animal model here not only presented with insulin resistance and hyperinsulinemia, but also with elevated systolic BP. The next experiment was designed to determine the independent effects of hyperinsulinemia and hypertension on changes in myocardial HSPD1 expression. Fructose-fed rats were treated with amlodipine (5 mg/kg), rosiglitazone (3 mg/kg), or placebo for 8 weeks accompanied with fructose feeding. BPs were taken every 2 weeks and OGTT was performed in week 7. The rats were then killed at the end of 8 weeks and samples were collected for further evaluation.

Systolic BP (Fig. 3A), blood glucose AUC, and plasma insulin AUC during OGTT in high-fructose-fed rats were the same as the previous experiments (Table 1). Rosiglitzone-treated rats had restored systolic BP and normalized.

![Figure 1](image-url) 

**Figure 1** (A) Body weight and (B) systolic blood pressure in rats during 12 weeks on different diets. Body weight and blood pressure were measured every 2 weeks after randomization of diet supplementation. Oral glucose tolerance tests (OGTT) was performed at the 4, 6, 8, 10, and 12 weeks after randomization of diet supplementation. (C) Blood glucose area under curve and (D) plasma insulin area under curve during OGTT in rats during 12 weeks on different diets. *P<0.05 compared with the controls.
plasma insulin AUC during the OGTT, while amlodipine-treated rats only had restored systolic BP. Both amlodipine and rosiglitazone treatment normalized the abundance of myocardial HSPD1 expression in high-fructose-fed rats (Fig. 3B). These data provided in vivo evidence that myocardial HSPD1 expression was regulated by BP but not by insulin level in this insulin-resistant animal model.

**Myocardial HSPD1 expression decreased when diabetes occurred**

The previous study indicated that insulin deficiency down-regulated myocardial HSPD1 expression in diabetic rats (Chen et al. 2005). This study hypothesized that decreased insulin action, which may be due to insulin resistance or insulin deficiency, could decrease myocardial HSPD1 expression. However, the time-course study revealed that hypertension and hyperinsulinemia occurred after 6–8 weeks of high-fructose feeding, which increased myocardial HSPD1 expression. The following experiment was designed to determine how diabetes altered HSPD1 expression.

To investigate whether the occurrence of plasma insulin and blood glucose levels led to perturbation of myocardial HSPD1 expression, diabetes was induced by STZ (40 mg/kg) in 12 high-fructose-fed S–D rats at week 6. OGTT was performed on week 7 and the rats were killed at week 8. Systolic BP in the fructose-fed group began to increase after 2 weeks of randomization and STZ treatment did not alter BP changes (Fig. 4A). The 12 diabetic rats revealed heterogeneous blood glucose homeostasis, which were separated into severely high and moderately high blood glucose groups (Table 2). Figure 4B–E shows the results of OGTT in these four experimental groups of S–D rats. The pure high-fructose-fed rats revealed hyperinsulinemia without significant hyperglycemia compared with the control groups. The moderately high blood glucose rats revealed significant hyperglycemia and normal insulin levels compared with the control, while the severely high blood glucose rats showed hyperglycemia and insulin deficiency. Figure 4F revealed that myocardial HSPD1 expression increased in the high-fructose-fed rats. It decreased in the severely high glucose rats and decreased, but not significantly, in the moderately high blood glucose rats.

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**Figure 2** Myocardial HSPD1 expressions in the control and high-fructose-fed rats at weeks 6 and 8. Myocardium was harvested and myocardial proteins were immunoblotted for HSPD1. Immunoblotting with β-actin served as controls. Each bar represents mean ± S.E.M. summarized from multiple experiments (n=6, in each group). The abundance of heat shock protein normalized to the abundance of β-actin in each sample. *P<0.05 compared with the controls.

**Figure 3** (A) Systolic blood pressure changes in the four experimental groups. *P<0.05 compared with the control group and †P<0.05 compared with the high-fructose-fed group. (B) Blood glucose AUC and (C) plasma insulin AUC during OGTT. *P<0.05 compared with the control group and †P<0.05 compared with high-fructose-fed rats without further treatment. (D) Myocardial HSPD1 expression in the four experimental groups with different treatments. Each bar represents mean ± S.E.M. summarized from multiple experiments (n=6 in each group). The abundance of heat shock protein normalized to the abundance of β-actin in each sample. *P<0.05 compared with the control group and †P<0.05 compared with high-fructose-fed rats without further treatment.
In this study, S–D rats fed with a high-fructose diet developed hypertension, hyperinsulinemia, and increased myocardial HSPD1 expression. The data also revealed that hypertension was a novel mechanism that up-regulated myocardial HSPD1 expression in insulin-resistant rats. When these rats received STZ injection, the myocardial HSPD1 expression decreased after diabetes occurred. These results provide in vivo evidence that myocardial HSPD1 expression was up-regulated by elevated BP but not by hyperinsulinemia in the insulin-resistant animal model. Once the β-cells could not provide enough insulin, diabetes occurred and myocardial HSPD1 decreased.

Compared with the general population, patients with type 2 diabetes have higher cardiovascular morbidity and mortality due to many contributory risk factors, such as dyslipidemia, insulin resistance, and hypertension (Panzram 1987). Previous studies have shown that high-fructose supplementation can induce insulin resistance, hyperinsulinemia, hypertension, and hypertriglyceridemia in rats (Hwang et al. 1987). The present study of Sprague–Dawley rats fed a diet in which fructose-replaced carbohydrates produced insulin resistance, hyperinsulinemia, and elevated BP. After 6–8 weeks of high-fructose feeding, these rats presented with hypertension and hyperinsulinemia without hyperglycemia. Further feeding with high-fructose diet revealed hypertension and hyperglycemia without significant hyperinsulinemia. These data indicated that due to the progressive

Table 1 Characteristics of experimental animals. Fructose-fed rats were treated with amlodipine (15 mg/kg), rosiglitazone (3 mg/kg), or placebo for 8 weeks after fructose feeding. OGTT was performed in week 7.

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Body weight (g)</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Fasting plasma insulin (µU/ml)</th>
<th>Glucose AUC (mg/dl per min)</th>
<th>Insulin AUC (µU/ml per min)</th>
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<tr>
<td>Control</td>
<td>6</td>
<td>401.1 ± 31.4</td>
<td>89.7 ± 9.6</td>
<td>13.91 ± 8.78</td>
<td>16.302 ± 1080</td>
<td>3783 ± 376</td>
</tr>
<tr>
<td>High-fructose diet</td>
<td>6</td>
<td>430.2 ± 28.3</td>
<td>93.5 ± 14.2</td>
<td>23.18 ± 3.17*</td>
<td>16.590 ± 1819</td>
<td>5153 ± 403*</td>
</tr>
<tr>
<td>High-fructose diet +</td>
<td>6</td>
<td>405.4 ± 26.6</td>
<td>87.2 ± 6.3</td>
<td>24.22 ± 3.94*</td>
<td>15.977 ± 1109</td>
<td>5055 ± 239*</td>
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<tr>
<td>amlodipine Tx</td>
<td>6</td>
<td>444.3 ± 28.9*</td>
<td>82.0 ± 9.3</td>
<td>18.46 ± 5.45</td>
<td>16.507 ± 1140</td>
<td>4112 ± 397</td>
</tr>
<tr>
<td>rosiglitazone Tx</td>
<td>6</td>
<td>430.2 ± 28.3</td>
<td>93.5 ± 14.2</td>
<td>23.18 ± 3.17*</td>
<td>16.590 ± 1819</td>
<td>5153 ± 403*</td>
</tr>
</tbody>
</table>

*P<0.05 compared with control group.

Discussion

In this study, S–D rats fed with a high-fructose diet developed hypertension, hyperinsulinemia, and increased myocardial HSPD1 expression. The data also revealed that hypertension was a novel mechanism that up-regulated myocardial HSPD1 expression in insulin-resistant rats. When these rats received STZ injection, the myocardial HSPD1 expression decreased after diabetes occurred. These results provide in vivo evidence that myocardial HSPD1 expression was up-regulated by elevated BP but not by hyperinsulinemia in the insulin-resistant animal model. Once the β-cells could not provide enough insulin, diabetes occurred and myocardial HSPD1 decreased.

Figure 4  (A) Systolic blood pressure changes in the three experimental groups. Blood pressure was taken every 2 weeks after randomization of diet supplementation. Values are expressed as means ± s.d. *P<0.05 compared with the controls. Further analysis using rats divided into moderately high and severely high blood glucose showed (B) the response curve of blood glucose during OGTT without STZ injection; (C) response curve of blood glucose during OGTT with STZ injection; (D) response curve of plasma insulin during OGTT without STZ injection; and (E) response curve of plasma insulin during OGTT with STZ injection. *P<0.05 compared with the control group. (F) Myocardial HSPD1 expressions increased in high-fructose-fed rats and decreased after STZ treatment. Each bar represents mean ± S.E.M. summarized from multiple experiments (n = 6 in each group). The abundance of heat shock protein normalized to the abundance of β-actin in each sample. *P<0.05 compared with the controls.
Table 2  Characteristics of the experimental animals. Diabetes was induced by streptozotocin (STZ, 40 mg/kg) injection in 12 high-fructose-fed S-D rats at week 6 and OGTT was performed at week 7.

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Body weight (g)</th>
<th>Fasting blood glucose (mg/dl)</th>
<th>Fasting plasma insulin (µU/ml)</th>
<th>Glucose AUC (mg/dl per min)</th>
<th>Insulin AUC (µU/ml per min)</th>
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<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>430±28.3</td>
<td>90.8±8.6</td>
<td>18.68±2.86</td>
<td>13 119±937</td>
<td>3177±289</td>
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<tr>
<td>High-fructose diet (HFD)</td>
<td>6</td>
<td>443±28.3</td>
<td>95.8±8.1</td>
<td>28.64±5.74*</td>
<td>15 081±2067</td>
<td>4652±114*</td>
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<tr>
<td>HFD with STZ had moderate</td>
<td>6</td>
<td>414±6.17±7*</td>
<td>171±8.35±2*</td>
<td>21.72±5.19</td>
<td>34 665±2311</td>
<td>3148±479</td>
</tr>
<tr>
<td>high blood glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HFD with STZ had severe</td>
<td>6</td>
<td>391±33.6*</td>
<td>332±0.55±6*</td>
<td>13.60±2.73*</td>
<td>48 513±2497*</td>
<td>1762±433*</td>
</tr>
<tr>
<td>high blood glucose</td>
<td></td>
<td></td>
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</table>

*P<0.05 compared with control group.

insulin resistance in the rats, the β-cell could not provide enough insulin to overcome the insulin resistance. This result was comparable with some human data that insulin resistance was the primary abnormality and that β-cell dysfunction was a late event arising from prolonged, increased secretory demand caused by insulin resistance (DeFronzo & Ferrannini 1991, Kruszynska & Olefsky 1996).

Insulin resistance and hyperinsulinemia occur several years prior to the development of type 2 diabetes. Insulin resistance may decrease insulin action to down-regulate myocardial HSPD1 expression but may also increase insulin level to up-regulate myocardial HSPD1. Because of these complex pathophysiological effects of insulin resistance, it is difficult to explore its effects on the myocardial HSPD1 expression. This study examined the abundance of myocardial HSPD1 in high-fructose-fed rats, while the time-course study supported the concept that hyperinsulinemia contributes to an over-expression of myocardial HSPD1. Thus, plasma insulin level may play an important role in the regulation of myocardial HSPD1 expression. However, the animal model not only presented insulin resistance and hyperinsulinemia, but also elevated systolic BP. The next experiment aimed to determine the independent effects of hyperinsulinemia and hypertension on changes in myocardial HSPD1 expression using rosiglitazone and amiodipine. The rosiglitazone-treated rats had restored insulin level and normalized BP, whereas the amiodipine-treated rats had only restored BP. Both amiodipine and rosiglitazone treatment normalized the abundance of myocardial HSPD1 expression after BP was controlled despite persistent hyperinsulinemia. These data provided in vitro evidence that hypertension is the key factor leading to increased myocardial HSPD1 expression in insulin-resistant rats. These findings also supported the previous report that heat shock factor 1 is activated for the induction of heat shock protein in the arterial wall during acute hypertension (Xu 2002), a response that is likely to play an important role in protecting arteries during hemodynamic stress.

As discussed earlier, HSPD1 expression is increased in ischemic and hypertrophic cardiomyopathies (Li et al. 1998, Schaffer et al. 2002, Sakai et al. 2003), and decreased in the diabetic myocardium (Chen et al. 2005). The present study shows that in fructose-fed rats, administration of amiodipine normalizes both BP and HSPD1 levels despite elevated insulin levels, suggesting that only hyperinsulinemia does not up-regulate myocardial HSPD1 levels. In order to compare with a previous report, where insulin deficiency down-regulated myocardial HSPD1 expression (Chen et al. 2005), the rats were injected with STZ to induce diabetes. Myocardial HSPD1 expression also increased in rats with hypertension and hyperinsulinemia, but significantly decreased in rats with severe hyperglycemia. The data indicate that insulin resistance with hypertension can increase myocardial HSPD1 expression. Once these rats developed diabetes and insulinemia, myocardial HSPD1 expression decreased despite the hypertension and insulin resistance. Because HSPD1 has cardiac protective action, reducing it in heart muscle can potentially lead to decreased myocardial protection during myocardial ischemia. It may also play a fundamental role during the development of diabetic cardiomyopathy.

In conclusion, this is the first report that myocardial HSPD1 expression increases in high-fructose-fed rats. This effect may be due to increased BP. Once diabetes with insulin deficiency develops, myocardial HSPD1 expression decreases despite persistent hypertension. These data indicate that both insulin secretion and myocardial HSPD1 expression are increased to compensate and prevent hyperglycemia, as well as protect cardiac muscle against injury. Once the β-cells cannot provide enough insulin, diabetes occurs and myocardial HSPD1 decreases.

Declaration of interest

The authors declare no conflict of interest prejudicial to the impartiality of this scientific work.

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References


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