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

Harn-Shen Chen^{1,2}, Tzu-En Wu², Chi-Chang Juan³ and Hong-Da Lin^{1,2}

¹Division of Endocrinology and Metabolism, Department of Medicine, Taipei Veterans General Hospital, No. 201, Shih-Pai Road, Sec 2, Taipei 11217, Taiwan, ROC

²Department of Medicine and ³Institute of Physiology, National Yang-Ming University School of Medicine, Taipei 11227, Taiwan, ROC

(Correspondence should be addressed to H-S Chen; Email: chenhs@vghtpe.gov.tw)

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

Abnormalities of the cardiovascular systems are common in diabetic patients and the consequences are devastating (Fein 1990, Grossman & Messerli 1996, Sowers *et al.* 2001, Fang *et al.* 2004). Heat shock proteins play cardio-protective roles during myocardial injury and their expression typically increases upon myocardial stress (Latchman 2001, Lepore *et al.* 2001, Delogu *et al.* 2002, Cornelussen *et al.* 2003). Several studies have shown the potentially important function of heat shock protein 60 (HSPD1) in the heart (Lau *et al.* 1997, Latchman 2001, Snoeckx *et al.* 2001, Kirchhoff *et al.* 2002, Schaffer *et al.* 2002, Shamaei-Tousi *et al.* 2007). Its expression is increased under ischemic distress (Snoeckx *et al.* 2001), while its over-expression leads to the inhibition of apoptosis in cardiomyocytes that have undergone ischemic and reperfusion injuries (Izaki *et al.* 2001). These findings emphasize the protective effects of HSPD1 in the cardiac muscle.

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 (Schaffer *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

comparator because it effectively lowers blood pressure (BP) but has no specific effect on insulin sensitivity (Kjeldsen *et al.* 2006).

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 ($22\text{ }^{\circ}\text{C} \pm 2\text{ }^{\circ}\text{C}$) 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 $37\text{ }^{\circ}\text{C}$, thus maintaining adequate circulation in the rats’ tail for reliable measurements of systolic BP. The mean of five consecutive readings was calculated for each BP measurement.

OGTT was performed after overnight fasting. A 0-min blood sample (0.5 ml) was taken by cutting the tail tip and then a glucose solution (2 g/ml per kg body weight) was immediately administered by a gavage. Four more blood samples were taken from the tail vein at 30, 60, 90, and 120 min after glucose administration. All of the blood samples were collected in Eppendorf tubes pre-rinsed with heparin solution (10 IU/ml) and were kept on ice until centrifugation (3500 *g* at $4\text{ }^{\circ}\text{C}$ for 30 min) to separate the plasma.

The concentration of blood glucose was measured using a glucometer (SureStep, Lifescan, Milpitas, CA, USA). Plasma specimens were frozen at $-20\text{ }^{\circ}\text{C}$ until insulin assay via RIA

(INSIK-5, DiaSorin, Saluggia, Italy) to determine the relationship between glucose homeostasis and myocardial HSPD1 expression.

Western blot

Mouse anti-HSPD1 monoclonal IgG3 antibody was purchased from StressGen Biotechnologies (Victoria, British Columbia, Canada). The tissues and cells were lysed with lysis buffer (137 mmol/l NaCl, 20 mmol/l Tris–HCl (pH 7.5), 10% glycerol, 1% Triton X-100, 0.5% Nonidet P-40, 2 mmol/l EDTA (pH 8.0), 3 $\mu\text{g}/\text{ml}$ aprotinin, 3 $\mu\text{g}/\text{ml}$ leupeptin, 2 mmol/l phenylmethylsulfonyl fluoride, 20 mmol/l NaF, 10 mmol/l NaPP, and 2 mmol/l Na_3VO_4). Equal amounts of proteins from each sample were separated by SDS-PAGE and then transferred to polyvinylidene difluoride membrane and incubated with a blocking buffer (3% BSA in 20 mmol/l Tris–HCl (pH 7.5), 137 mmol/l NaCl, and 0.1% Tween 20) for 1 h at room temperature. The membranes were incubated sequentially with primary antibodies overnight at $4\text{ }^{\circ}\text{C}$, washed twice (20 mmol/l Tris–HCl (pH 7.5), 137 mmol/l NaCl, and 0.1% Tween 20), incubated with HRP-conjugated secondary antibodies (1:7500 to 1:13 000 dilution) for 1 h at room temperature, washed thrice, and then detected with ECL (Amersham Biosciences).

Statistical analysis

The data were expressed as mean $\pm$ 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

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.

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). Rosiglitazone-treated rats had restored systolic BP and normalized

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 $\pm$ 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.

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

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 $\pm$ 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.

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.

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.

	No.	Body weight (g)	Fasting blood glucose (mg/dl)	Fasting plasma insulin (μ U/ml)	Glucose AUC (mg/dl per min)	Insulin AUC (μ U/ml per min)
Control	6	401.1 $\pm$ 31.4	89.7 $\pm$ 9.6	13.91 $\pm$ 8.78	16 302 $\pm$ 1080	3783 $\pm$ 376
High-fructose diet (HFD)	6	430.2 $\pm$ 28.3	93.5 $\pm$ 14.2	23.18 $\pm$ 3.17*	16 590 $\pm$ 1819	5153 $\pm$ 403*
HFD with amlodipine Tx	6	405.4 $\pm$ 26.6	87.2 $\pm$ 6.3	24.22 $\pm$ 3.94*	15 977 $\pm$ 1109	5055 $\pm$ 239*
HFD with rosiglitazone Tx	6	444.3 $\pm$ 28.9*	82.0 $\pm$ 9.3	18.46 $\pm$ 5.45	16 507 $\pm$ 1140	4112 $\pm$ 397

* $P < 0.05$ 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.

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

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 $\pm$ 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 $\pm$ 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.

	No.	Body weight (g)	Fasting blood glucose (mg/dl)	Fasting plasma insulin (μU/ml)	Glucose AUC (mg/dl per min)	Insulin AUC (μU/ml per min)
Control	6	430.2±28.3	90.8±8.6	18.68±2.86	13 119±937	3177±289
High-fructose diet (HFD)	6	444.3±28.3	95.8±8.1	28.64±5.74*	15 081±2067	4652±114*
HFD with STZ had moderate high blood glucose	6	414.6±17.7*	171.8±35.2*	21.72±5.19	34 665±2311*	3148±479
HFD with STZ had severe high blood glucose	6	391.8±33.6*	332.0±55.6*	13.60±2.73*	48 513±2497*	1762±433*

*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 amlodipine. The rosiglitazone-treated rats had restored insulin level and normalized BP, whereas the amlodipine-treated rats had only restored BP. Both amlodipine and rosiglitazone treatment normalized the abundance of myocardial HSPD1 expression after BP was controlled despite persistent hyperinsulinemia. These data provided *in vivo* 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 amlodipine 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.

Funding

This work was supported by grants from the National Science Council (94-2314-B-075-050) and Taipei Veterans General Hospital (V94B-169).

References

Bunag RD 1973 Validation in awake rats of a tail-cuff method for measuring systolic blood pressure. *Journal of Applied Physiology* **34** 279–282.
Chen HS, Shan XY, Yang TL, Lin H-D, Chen J-W, Lin S-J & Wang PH 2005 Insulin deficiency down-regulated Hsp60 and IGF-1 receptor signaling in diabetic myocardium. *Diabetes* **54** 175–181.

- Cornelussen RN, Vanagt WY, Prinzen FW & Snoeckx LH 2003 Proteins involved in salvage of the myocardium. *Advances in Experimental Medicine and Biology* **543** 277–291.
- DeFronzo RA & Ferrannini E 1991 Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care* **14** 173–194.
- Delogu G, Signore M, Mechelli A & Famularo G 2002 Heat shock proteins and their role in heart injury. *Current Opinion in Critical Care* **8** 411–416.
- Fang ZY, Prins JB & Marwick TH 2004 Diabetic cardiomyopathy: evidence, mechanisms, and therapeutic implications. *Endocrine Reviews* **25** 543–567.
- Fein FS 1990 Diabetic cardiomyopathy. *Diabetes Care* **13** 1169–1179.
- Grossman E & Messerli FH 1996 Diabetic and hypertensive heart disease. *Annals of Internal Medicine* **125** 304–310.
- Hwang IS, Ho H, Hoffman BB & Reaven GM 1987 Fructose-induced insulin resistance and hypertension in rats. *Hypertension* **10** 512–516.
- Izaki K, Kinouchi H, Watanabe K, Owada Y, Okubo A, Itoh H, Kondo H, Tashima Y, Tamura S, Yoshimoto T *et al.* 2001 Induction of mitochondrial heat shock protein 60 and 10 mRNAs following transient focal cerebral ischemia in the rat. *Brain Research. Molecular Brain Research* **88** 14–25.
- Kirchhoff SR, Gupta S & Knowlton AA 2002 Cytosolic heat shock protein 60, apoptosis, and myocardial injury. *Circulation* **105** 2899–2904.
- Kjeldsen SE, Julius S, Mancia G, McInnes GT, Hua T, Weber MA, Coca A, Ekman S, Gierd X, Jamerson K *et al.* 2006 Effects of valsartan compared to amlodipine on preventing type 2 diabetes in high-risk hypertensive patients: the VALUE trial. *Journal of Hypertension* **24** 1405–14712.
- Kruszynska YT & Olefsky JM 1996 Cellular and molecular mechanisms of non-insulin dependent diabetes mellitus. *Journal of Investigative Medicine* **44** 413–428.
- Latchman DS 2001 Heat shock proteins and cardiac protection. *Cardiovascular Research* **51** 637–646.
- Lau S, Patnaik N, Sayen MR & Mestril R 1997 Simultaneous over-expression of two stress proteins in rat cardiomyocytes and myogenic cells confers protection against ischemia-induced injury. *Circulation* **96** 2287–2294.
- Lepore DA, Knight KR, Anderson RL & Morrison WA 2001 Role of priming stresses and Hsp70 in protection from ischemia-reperfusion injury in cardiac and skeletal muscle. *Cell Stress and Chaperones* **6** 93–96.
- Li G, Li RK, Mickle DA, Weisel RD, Merante F, Ball WT, Christakis GT, Cusimano RJ & Williams WG 1998 Elevated insulin-like growth factor-I and transforming growth factor-beta 1 and their receptors in patients with idiopathic hypertrophic obstructive cardiomyopathy. A possible mechanism. *Circulation* **98** II144–II149.
- Nathan DM 2002 Clinical practice. Initial management of glycemia in type 2 diabetes mellitus. *New England Journal of Medicine* **347** 1342–1349.
- Panzram G 1987 Mortality and survival in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **30** 123–131.
- Saad ME, Knowler WC, Pettitt DJ, Nelson RG, Charles MA & Bennett PH 1991 A two-step model for development of non-insulin-dependent diabetes. *American Journal of Medicine* **90** 229–235.
- Sakai J, Ishikawa H, Kojima S, Satoh H, Yamamoto S & Kanaoka M 2003 Proteomic analysis of rat heart in ischemia and ischemia-reperfusion using fluorescence two-dimensional difference gel electrophoresis. *Proteomics* **3** 1318–1324.
- Schaffler AE, Kirmanoglou K, Balbach J, Pecher P, Hannekum A & Schumacher B 2002 The expression of heat shock protein 60 in myocardium of patients with chronic atrial fibrillation. *Basic Research in Cardiology* **97** 258–261.
- Shamaei-Tousi A, Steptoe A, O'Donnell K, Palmen J, Stephens JW, Hurel SJ, Marmot M, Homer K, D'Aiuto F, Coates AR *et al.* 2007 Plasma heat shock protein 60 and cardiovascular disease risk: the role of psychosocial, genetic, and biological factors. *Cell Stress and Chaperones* **12** 384–392.
- Snoeckx LH, Cornelussen RN, Van Nieuwenhoven FA, Reneman RS & Van Der Vusse GJ 2001 Heat shock proteins and cardiovascular pathophysiology. *Physiological Reviews* **81** 1461–1497.
- Sowers JR, Epstein M & Frohlich ED 2001 Diabetes, hypertension, and cardiovascular disease: an update. *Hypertension* **37** 1053–1059.
- Xu Q 2002 Role of heat shock proteins in atherosclerosis. *Arteriosclerosis, Thrombosis, and Vascular Biology* **22** 1547–1559.
- Yki-Jarvinen H 2004 Drug therapy: thiazolidinediones. *New England Journal of Medicine* **351** 1106–1118.

Received in final form 17 October 2008

Accepted 20 October 2008

Made available online as an Accepted Preprint

22 October 2008