nature publishing group

# Hepatic Effects of a Fructose Diet in the Stroke-prone Spontaneously Hypertensive Rat

M. Julia Brosnan[1,2] and Richard D. Carkner[1]

**BACKGROUND**

Feeding stroke-prone spontaneously hypertensive rats (SHRSP) a diet rich in fructose results in a profound glucose intolerance not observed in the normotensive Wistar Kyoto (WKY) strain. The aim of this study was to investigate the role of the liver in the underlying mechanisms in the SHRSP.

**METHODS**

SHRSP and WKY rats were fed either 60% fructose or regular chow for 2 weeks with blood pressure being measured using tail-cuff plethysmography and radiotelemetry. Intraperitoneal glucose tolerance tests were performed and livers harvested for analysis of expression of inflammatory mediators and antioxidant proteins by western blotting and quantitative reverse transcriptase–PCR. The serum triglyceride content and fatty acid profiles were also measured.

**RESULTS**

Feeding SHRSP and WKY on 60% fructose for 2 weeks resulted in glucose intolerance with no increases in levels of blood pressure. Serum triglycerides were increased in both strains of fructose-fed rats with the highest levels being observed in the SHRSP. The serum fatty acid profiles were changed with large increases in the amounts of oleic acid (18.1) and reductions in linoleic acid (18.2). Levels of expression of c-jun N-terminal kinase/stress activated protein kinase (JNK/SAPK), and nuclear factor κB (NF-κB) were shown to be unchanged between the livers of the chow and fructose-fed groups. In contrast, protein levels of the three isoforms of superoxide dismutase (SOD) were upregulated in liver of SHRSP fed on fructose while only manganese SOD (MnSOD) was upregulated in fructose-fed WKY rats.

**CONCLUSIONS**

These results demonstrate that the major contribution of the liver in the early pathogenesis of metabolic syndrome may be an increased secretion of triglyceride containing altered proportions of fatty acid pools. Feeding rats a diet rich in fructose does not affect hepatic expression of inflammatory pathways and the increased hepatic SOD expression may constitute an early protective mechanism.

*Am J Hypertens* 2008; **21**:708-714 © 2008 American Journal of Hypertension, Ltd.

Metabolic syndrome, characterized by insulin resistance, obesity, hypertriglyceridemia, and hypertension, is a strong predictor of type 2 diabetes. The mechanisms underlying development of the metabolic syndrome remain to be resolved, although genetics and lifestyle factors are clearly involved. The spontaneously hypertensive rat (SHR), which presents with a predisposition to insulin resistance, has been used to identify genes contributing to metabolic syndrome.[1] Feeding SHR fructose provides a model in which the molecular events leading to metabolic syndrome can be determined.[2] The effects of fructose on blood pressure levels have been contradictory with some groups showing no effects and others demonstrating either hypertension or hypotension.[2–6]

At least three tissues, liver, adipose, and skeletal muscle are involved in the development of insulin resistance in the SHR[1,7–9] but the relative importance of each in the response to fructose is unknown. The key role of the liver in fructose and lipid metabolism suggests that early hepatic changes may be pivotal to the development of full-blown disease. Pathways of oxidative stress and inflammation have been implicated but the relative role of each is unknown.[10] A recent study in insulin resistant adipocytes showed that alterations in the expression of antioxidant enzymes were amongst the first changes and that subsequent treatment with antioxidants was sufficient to restore insulin sensitivity.[11] Recent studies also suggested that alterations in not only the amounts of circulating lipids but also the classes of fatty acids may contribute to the development of obesity and diabetes.[12,13]

In this study we examined metabolic changes, occurring within 2 weeks of fructose feeding, in the liver of stroke-prone SHRs (SHRSP), a sub-strain of SHR, and genetically normotensive Wistar Kyoto (WKY) fed with a 60% fructose diet.

## METHODS

All animal experiments were performed in conformity with the NIH Guidelines on the care and use of animals and protocols were approved by the Institutional Animal Care and Use Committee at Albany Medical College. Male SHRSP and WKY were purchased at 10 weeks of age from Charles River Laboratories (Wilmington, MA) and maintained in a

[1]Center for Metabolic Disease, Ordway Research Institute, Albany, New York, USA; [2]Center for Cardiovascular Sciences, Albany Medical College, Albany, New York, USA. Correspondence: M. Julia Brosnan (jbrosnan@ordwayresearch.org)

Received 12 December 2007; first decision 7 January 2008; accepted 27 February 2008; advance online publication 10 April 2008. doi:10.1038/ajh.2008.41

temperature-controlled room at the Animal Research Facility under 12-h light–dark cycles. On arrival all rats were fed on the standard chow, in which the carbohydrate content is made up of starch and contains only 0.2% fructose and 1.0% sucrose (Labdiet, Richmond, IN). 60, 26, and 14% of the calories in this diet were derived from carbohydrates, protein, and fat respectively.

*Glucose tolerance test.* Groups of SHRSP and WKY rats ($n$ = 4–5) were switched to a 60% fructose diet (TD89247; Harlan Teklad, Madison, WI) or remained on the standard chow for 2 weeks. At the end of the fructose-feeding protocol rats were fasted overnight (12 h). The next morning, rats were anesthetized with isoflurane and blood samples (200 µl) taken for the measurement of blood glucose, insulin and triglycerides. The rats were weighed and an IP injection of sterile glucose solution (20 mg/kg) administered. Blood samples (10–20 µl) were taken from the tail vein at 10, 20, 30, 60, and 90 min from the anesthetized rats.

*Hemodynamic measurements.* Hemodynamic measurements were made in groups of unanesthetized SHRSP and WKY using the CODA-2 tail-cuff plethysmography system (Kent Scientific, Torrington, CT). After a training period, accomplished by subjecting the animals to plethysmography at least twice prior to the start of the experimental period, systolic blood pressure values were obtained before and at the end of the 2-week fructose-feeding protocol.[14] Rats were allowed to acclimatize to the restrainer (supplied by the manufacturer) for at least 15 min prior to data collection, the body temperature was monitored throughout the protocol and the mean value of five recordings was taken ($n$ = 5 per group).

Systolic blood pressures were also measured using radiotelemetry in a third set of rats. Probes were implanted into the abdominal aorta ($n$ = 10 for each strain), under standard conditions, and blood pressure and heart rate measurements collected using the Data Quest ART Gold Radiotelemetry system (Data Sciences International, St Paul, MN) as described previously.[15] Data were recorded for 10 s every 5 min and then averaged into 12-h means according to the day and nighttime scheduling in the holding room. Following a postoperative recovery period of 5 days the rats were arbitrarily split into groups and baseline measurements collected for the next week. Fourteen days after surgery, the rats were fed either the 60% fructose or maintained on the standard diet. Water was freely available.

Before killing, blood was withdrawn by cardiac puncture from rats anesthetized with isoflurane. Tissues were harvested and weighed. Blood was collected into either heparin or EDTA tubes and spun at 2000 *g* for 20 min, and tissues were immediately frozen at −80 °C.

*Metabolic phenotyping.* Glucose was measured with a glucose oxidase assay using the AccuChek Active system (Roche Diagnostics, Indianapolis, IN). Plasma insulin levels were measured using the rat-specific enzyme-linked immunosorbent assay kit (Crystal Chem, Downers Grove, IL). Enzymatic assays were used for the measurement of plasma triglycerides (Wako Chemicals, Neuss, Germany).

*Western blotting.* Proteins were extracted from frozen tissues in ice-cold cell lysis buffer containing 20 mmol/l HEPES, 1 mmol/l EGTA, pH 7.5 (Cell Signaling Technology, Danvers, MA), in the presence of complete, EDTA free, mini protease tablets (Roche Diagnostics, Indianapolis, IN) and phosphatase inhibitors cocktail (Calbiochem, San Diego, CA) using a Polytron PT3100 homogenizer (Brinkman, Westbury, NY) at 28,000 r.p.m. The homogenates were spun at 20,000 *g* for 5 min at 4 °C and protein content of the supernatant measured using the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA). Western blotting was performed as previously described.[15] Antibodies against MnSOD (manganese superoxide dismutase) were obtained from Prof. Taniguchi (Osaka University, Japan), anti-ECSOD (extracellular SOD) was from Stressgen (Ann Arbor, MI), antibodies against JNK (c-jun N-terminal kinase), jun and phospho-c-jun were from Cell Signaling Technology (Danvers, MA), and antibodies against proteins on the NF-κB (nuclear factor κB) pathway were from Santa Cruz. Proteins were separated by polyacrylamide gel electrophoresis and electroblotted onto a Hybond-P membrane (Amersham). Membranes were incubated with the primary antibody before washing and application of a horseradish peroxidase–conjugated secondary antibody. Bands were detected by chemiluminescence (ECL kit, Amersham) and quantified using a Bio-Rad Image Analyzer densitometry system.

*Quantitative reverse transcriptase–PCR.* RNA was extracted from livers using RNAzol and 1 µg converted into cDNA using a commercial reverse transcriptase kit (Promega, Madison, WI).[16] Rat-specific primers for TNF-α tumor necrosis factor-α) IL-1α (interleukin-1α), IκB-α (Inhibitor of κB) and β-actin were used with previously described PCR conditions[17] in an iCycler (Bio-Rad) and crossover points used to calculate relative expression levels.

β-Actin: 5′AAGTCCCTCA CCCTCCCAAAAG3′ and 5′AAGCAATGCT GTCACCTTCCC3′; IL-1α: 5′AAGACA AGCC TGTGTTGCTAAGG3′ and 5′TCCCAGAAGAA AATGAGGTCGGTC3′; TNF-α: 5′AAATGGGCTC CCTCTC ATCAGTTC3′ and 5′TCTGCTTGGT GGTTTGCTACGAC 3′; IκB-α: 5′TGAGTACCTG GACTTGCAGA ACG3′ and 5′TGTAGATGCC TCTCCAAGGATGG3′.

*Serum fatty acids.* The total lipids in 100 µl of serum were isolated using standard methods.[18] Briefly, 100 µg of 17.0 heptadecanoic acid was added to the serum to monitor recovery of fatty acids and a 1:2 mix of chloroform/methanol added. After centrifugation, the lower phase was dried under nitrogen and the lipids resuspended in chloroform. For fatty acid examination, lipids were hydrolyzed with acidified methanol and the fatty acid methyl esters analyzed by gas chromatography/mass spectrometry using an Agilent 6800 series gas chromatograph equipped with a 5873 mass-selective laser.[18]

*Statistics.* All results are expressed as mean ± s.e. Area under the curve was calculated for the glucose tolerance curves and analyzed by analysis of variance. Repeated measures analysis of variance was performed for telemetry data. A $P$ value <0.05 was considered significant throughout.

## RESULTS

### Glucose intolerance

We fed SHRSP and WKY rats a fructose-rich diet and performed intraperitoneal glucose tolerance tests as shown in Figure 1. Even on the regular chow diet, the SHRSP showed a higher glucose peak in response to the glucose load than did the chow-fed WKY (312 ± 30 vs. 267 ± 15 mg/dl, respectively). Two weeks of fructose feeding further increased the peak glucose in the SHRSP group with the glucose levels being 540 ± 13 mg/dl ($P < 0.0001$, $F = 74$ compared to chow-fed SHRSP). In contrast, peak glucose levels were 307 ± 12 mg/dl in the fructose-fed WKY group which was not significantly different from the chow-fed WKY group. Thus, as previously reported, SHRSP is predisposed to development of features of the metabolic syndrome including dietary induced glucose intolerance and represents an excellent model in which to investigate the inter-relationship between physiology and molecular events.[2]

### Physiological parameters

Feeding SHRSP a fructose diet resulted in elevations in circulating triglycerides and a small increase in the weight of the epididymal fat pads (Table 1), which we used as a measure of adiposity. Serum triglyceride levels were statistically significant between the groups with the highest levels (164.0 ± 24.3 mg/dl) observed in the fructose-fed SHRSP group. We did not find an increase in body weight gain between the fructose and chow-fed groups of either strain. Consistent with previous results, no significant changes were detected in either fed or fasting glucose, but there was an increase in the fasting insulin in the fructose-fed SHRSP group compared to the groups fed on chow ($P = 0.003$ vs. chow-fed WKY; $P = 0.005$ vs. chow-fed SHRSP; $P = 0.002$ vs. chow-fed WKY).

Having demonstrated increased serum triglycerides in fructose-fed rats, we wanted to know whether there were changes in the patterns of fatty acids and whether they were altered between the strains. We found that high fructose resulted in similar changes in the serum fatty acid profiles with both groups showing reduced proportions of linoleic acid (18.2) and increased monounsaturated oleic acid (18.1) compared to their chow control groups (Figure 2).

We used both radiotelemetry and tail-cuff plethysmography to measure blood pressure in chow and fructose-fed rats (Table 2).

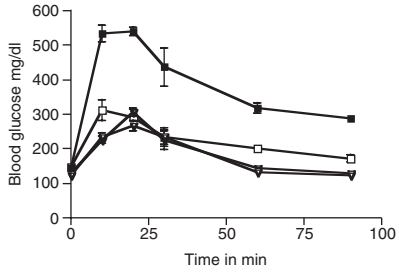


**Figure 1 |** Glucose intolerance in SHRSP and WKY rats. After the 2-week feeding protocol rats were fasted overnight and injected IP with glucose (20 mg/kg body weight). Blood was with drawn and glucose levels determined. Filled and open squares represent fructose- and chow-fed SHRSP, respectively ($n = 4$–5). Filled and open triangles represent fructose and chow-fed WKY, respectively ($P < 0.0001$, $F = 74$).

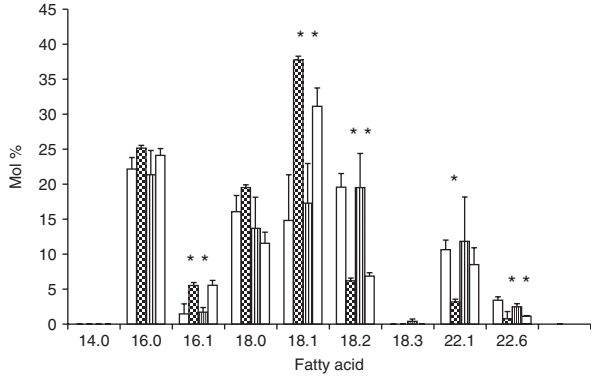


**Figure 2 |** Profile of major fatty acids detected in serum. The identity of fatty acids extracted from the serum at the end of the 2-week feeding protocol was determined using gas chromatography/mass spectrometry and shown here as a proportion of the total fatty acid pool (Mol %). Open and closed bars represent WKY and SHRSP fed regular chow, whereas the checkered and striped bars represent WKY and SHRSP fed fructose ($n = 5$ per group). There were no significant differences between the profiles from the two strains of rat fed fructose, although there was a clear diet effect observed when fructose-fed rats were compared to chow-fed rats ($P = 0.0001$ chow vs. fructose diet and *$P < 0.05$ vs. fatty acid in respective control groups).

**Table 1 | Characteristics of fructose-fed SHRSP and WKY**

| | Body weight gain | Fed glucose (mg/dl) | Fasted glucose (mg/dl) | Fasted insulin (ng/ml) | Fasted triglyceride (mg/dl) | Adiposity (g/kg) |
|---|---|---|---|---|---|---|
| WKY | | | | | | |
| Regular chow | 23 ± 2 | ND | 154 ± 12 | 1.59 ± 0.24 | 61.2 ± 5.0 | 3.83 ± 0.74 |
| 60% Fructose chow | 21 ± 3 | ND | 156 ± 5 | 1.88 ± 0.28 | 89.7 ± 7.7 | 4.73 ± 0.51 |
| SHRSP | | | | | | |
| Regular chow | 28 ± 3 | 157 ± 1 | 148 ± 4 | 2.11 ± 0.37 | 103.5 ± 8.1 | 4.92 ± 1.57 |
| 60% Fructose chow | 29 ± 2 | 176 ± 23 | 153 ± 6 | 3.70 ± 0.10*,** | 164.0 ± 24.3*,** | 6.96 ± 0.69*** |

*$P < 0.003$ vs. WKY-chow; **$P < 0.005$ vs. SHRSP-chow; ***$P < 0.05$ vs. WKY chow.

**Table 2 | Final systolic blood pressures in fructose and chow-fed SHRSP and WKY**

| | SBP (mm Hg) | | |
|---|---|---|---|
| | Plethysmography | Radiotelemetry | |
| | Daytime | Daytime | Nighttime |
| WKY | | | |
| Regular chow | 125 ± 4 | 119 ± 1 | 122 ± 2 |
| 60% Fructose chow | 130 ± 5 | 118 ± 2 | 120 ± 4 |
| SHRSP | | | |
| Regular chow | 164 ± 5 | 141 ± 3* | 147 ± 7 |
| 60% Fructose chow | 168 ± 5 | 119 ± 3* | 131 ± 4 |

Final average systolic blood pressures (SBP), as measured by radiotelemetry (daytime and nighttime) or plethysmography (daytime only) in rats fed either fructose or chow for 2 weeks. Repeated measures analysis of variance of the nighttime telemetry data showed that the blood pressure response to fructose feeding was different between the two strains ($P = 0.0001$, $F = 21.49$). No statistically significant differences were detected in the blood pressure responses to fructose of either the SHRSP or WKY when blood pressure was measured using plethysmography. The measurement of daytime SBP in chow and fructose fed SHRSP was different using the two techniques *$P < 0.05$ vs. daytime SBP in SHRSP measured using plethysmography by Student's *t*-test.

No differences in the baseline mean systolic pressures between the SHRSP-fructose and SHRSP-chow or between the WKY-fructose and WKY-chow groups were detected using telemetry. At the end of 2 weeks' feeding, the telemetrically determined nighttime SBP of the fructose-fed SHRSP was 131 ± 4, whereas that of the chow-fed group rose to 147 ± 7 mm Hg. No changes were detected between the final nighttime SBP of the WKY chow and fructose groups. Statistical analyses of these data revealed that when blood pressure was measured using radiotelemetry the two strains responded differently. (Repeated measures analysis of variance of nighttime SBP, $P = 0.0001$, $F = 21.49$).

When blood pressure was measured using plethysmography, we found baseline values to be higher in the SHRSP, than those measured using plethysmography, with pre-diet systolic blood pressures being 168 ± 5 and 164 ± 6 in the fructose- and chow-fed SHRSP groups. These values were not significantly changed by the fructose diet (Table 2). Measured blood pressure values in WKY rats did not significantly differ between the two techniques with the post-diet values being 130 ± 5 and 128 ± 5 for the WKY-fructose and chow groups, respectively.

No changes were detected in either mean heart rate or physical activity measurements, as determined by telemetry, between any of the groups (not shown).

### Oxidative stress

The levels of expression of the three SODs were measured by western blotting as shown in Figure 3a. We found that the levels of ECSOD, MnSOD, and CuZnSOD were increased to 231 ± 50%, 231 ± 19%, and 135 ± 2%, respectively, of those observed in the chow-fed SHRSP ($P < 0.05$ for each). Fructose feeding also resulted in a significant increase in MnSOD expression in WKY rats ($P < 0.05$).

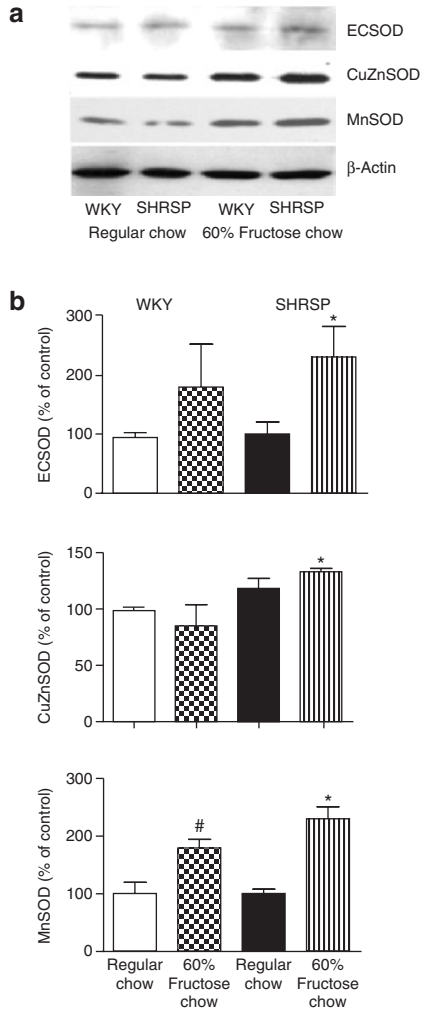


**Figure 3 |** SOD protein expression in livers of fructose- and chow-fed rats. (**a**) Representative western blots of MnSOD, CuZnSOD, and ECSOD expression in liver homogenates, $n = 4$ in each group. (**b**) Densitometric analysis of SOD expression in chow- and fructose-fed rat livers: open bars represent chow-fed WKY; and black bars represent chow-fed SHRSP, checkered and striped bars represent fructose-fed WKY and SHRSP, respectively. (*$P < 0.05$ vs. chow-fed SHRSP #$P < 0.05$ vs. chow-fed WKY).

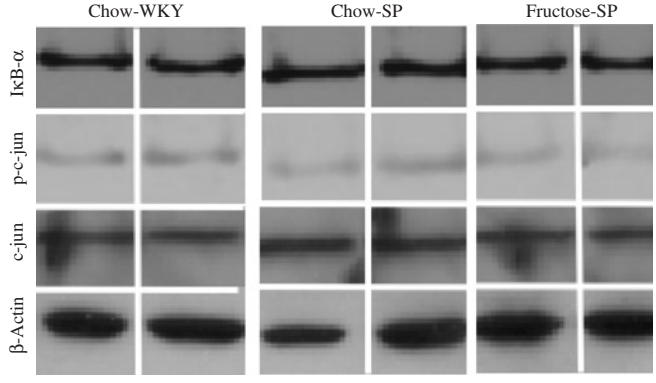


**Figure 4 |** Expression of inflammatory mediators. Representative western blots of the IκB-α subunit of the NF-κB complex and native and phosphorylated c-jun.

### Inflammatory pathways

Modulation of inflammatory pathways, including those mediated by JNK/SAPK (c-jun N-terminal kinase/stress activated protein kinase) and NF-κB, has been proposed to contribute to the pathogenesis of metabolic syndrome.[19] We measured the levels of JNK protein in livers from chow- and fructose-fed SHRSP and found no difference (100 ± 15% vs. 98 ± 12% chow vs. fructose, respectively, not significant). We also determined JNK/SAPK activity by measuring the proportion of c-jun phosphorylation and found that the ratio of c-jun to phosphorylated c-jun was not altered by the fructose feeding (**Figure 4**). The activity of the NF-κB transcription factor was determined by western blotting of the IκB-α subunit. We were unable to detect any differences in the amounts of IκB-α between the chow- and fructose-fed groups suggesting that, at least in this early stage, this is not an important pathway in this model. Finally, we quantified the hepatic expression of TNF-α, IL-1α and IκB-α in livers of fructose-fed SHRSP by performing Quantitative-PCR and found no differences with the relative expression levels being 1.05 ± 0.2, 1.11 ± 0.2 and 0.95 ± 0.3, respectively, of those found in chow-fed SHRSP.

## DISCUSSION

In 1988 Reaven coined the term metabolic syndrome to describe a clustering of risk factors associated with carbohydrate consumption that predispose to the development of coronary events. Fructose is readily absorbed and metabolized by the liver, where it bypasses the main regulatory step of glycolysis and stimulates lipogenesis and secretion of triglyceride-containing very low–density lipoprotein. Furthermore, fructose consumption does not trigger the release of insulin, or insulin-stimulated leptin, and is believed to contribute, in the long term, to obesity.[20,21]

Many studies have used fructose diets to induce features of metabolic syndrome in people and in rodent models.[2,3,20,22] We confirmed that feeding SHRSP a diet rich in fructose results in glucose intolerance and several other features of the metabolic syndrome, as defined for a clinical population, by the International Diabetes Foundation.[23] Melancon *et al.* have also previously shown that feeding SHR (from which SHRSP was derived) a diet rich in fructose leads to a reduction in insulin sensitivity.[9] Thus, we confirmed that feeding fructose to SHRSP generates a valuable model, in which the early changes underlying the development of metabolic syndrome can be determined.[2] One of the most striking differences between SHRSP and WKY rats fed fructose is the levels of circulating triglyceride measured, with the SHRSP having almost twice as much as the control strain. We demonstrated that both strains show changes in the profile of serum fatty acids with large increases in the proportion of the fatty acid pool detected as oleic acid (18.1) and reductions in the proportion of linoleic acid (18.2) and other long chain fatty acids. Fructose has been shown to alter the expression of key lipogenic enzymes including that of stearoyl-CoA desaturase (Scd1), which converts saturated fatty acids into monounsaturated fatty acids such as oleic acid.[24] Despite the fact that oleic acid is an unsaturated fatty acid, it is becoming increasingly implicated in the development of cardiovascular disease.[13,25]

It is difficult to understand why the hypertriglyceridemia was not associated with alterations in blood pressure as demonstrated in some other studies. Again, it may be that the changed fatty acids profile is important. The modified fatty acid TTA, (tetradecylthioacetic acid), reduced blood pressure in hypertensive rats, concomitant with reductions in serum linoleic acid and increases in oleic acid, as observed here.[26]

### Effects on blood pressure

Although fructose has been used to induce insulin resistance and hypertension in rodents, the effects of fructose feeding on blood pressure in rats have been contradictory with different groups showing increases, decreases, or no changes.[4–7,9] In this study we showed that, despite the development of hypertriglyceridemia, fructose did not increase blood pressure in the SHRSP or WKY over a 2-week period. In fact although our plethysmography data showed no changes in blood pressure, our telemetric data, in agreement with several earlier reports, showed a significant and sustained decrease in blood pressure in SHRSP fed fructose over this time course.[4,2] D'Angelo *et al.* fed Sprague-Dawley rats high fructose for 8 weeks and showed that when blood pressure levels were measured using telemetry there were no detectable differences between chow and fructose-fed rats.[4] The blood pressure values that we measured are relatively low, particularly those obtained using telemetry, but concur with those measured by others using young SHR strains from this supplier.[15] Melancon fed SHR high sucrose for 4 weeks and found mean arterial pressures to be 135 and 133 mm Hg in the chow and sucrose groups, respectively. When blood pressure measurements were made in the same rats using plethysmography, a difference of 23 mm Hg between the groups was found indicating that, similar to this study, the results are technique sensitive.

### Hepatic gene expression

The relatively short time course of our experiments presents a valuable window for investigating the early events contributing to metabolic syndrome. In this study, we focused on changes in hepatic expression of genes associated with inflammation and oxidative stress. We investigated the expression levels of inflammatory cytokines and were unable to detect significant dietary-induced changes in liver at this stage. The transcriptional regulator, NF-κB, has been linked with insulin resistance,[19] but we did not detect any changes in the amount of the inhibitor component, IκB-α suggesting no changes in activation in the liver. We were also unable to detect any changes in the levels of JNK proteins or their activities. In good agreement with Wei *et al.* we showed that fructose has no effect on the level of phosphorylation of the nuclear target of JNK, c-jun,[27] suggesting that other mediators must be changing in the liver. This contrasts with the findings of others, who demonstrated increased levels of p-c-jun and may be explained by the fact that those studies were performed in different models.[27–29] It is possible that inflammatory pathways are altered in other tissues, such as adipose, or at a later stage in this model.

We found altered expression of the SOD family of enzymes and propose that these changes reflect changes in ROS metabolism, as suggested by others.[22,30,31] SOD catalyzes the dismutation reaction of superoxide to hydrogen peroxide. MnSOD has been shown to be upregulated by, and protect against, oxidative damage.[32,33] Several studies have suggested a role for antioxidants in blood pressure regulation, particularly with respect to nitric oxide levels.[34] In the experiments here, increased expression of SOD in liver, and potentially other tissues, may protect the levels of nitric oxide and contribute to the minimal effects on blood pressure. It is interesting to note that glutathione peroxidase is regulated differently in SHR than in WKY and may contribute to changes in blood pressure,[35] whereas Girard *et al.* have shown that feeding a high fructose diet significantly increased the activity of glutathione peroxidase in adipose and brain.[7]

Ideally, we would have measured superoxide but this is made difficult because of its high reactivity, and the fact that hepatic cytochrome p450 enzymes preclude the use of lucigenin as a substrate[36] while the hydroethidine-based assays[37] were not sufficiently sensitive.

In conclusion, these studies confirm that feeding SHRSP high fructose generates a model in which the mechanisms underlying metabolic syndrome can be studied. The inability to clear a glucose load does not require an upregulation of hepatic inflammatory pathways although ROS metabolism was altered. We showed that fructose increased levels of serum triglycerides and alterations in the fatty acid profiles. Together, these results suggest that the major contribution of the liver in the fructose-fed SHRSP, in the development of metabolic syndrome, is an alteration in the amount and/or composition of secreted triglyceride.

Acknowledgments: We thank Dr Doyle for help with the analyses of telemetry data, Drs Anfinogenova and Tang for help with the tail-cuff plethysmography, the FATTT lab, in particular, Drs DiRusso and Black for teaching us how to analyze fatty acids and, the Charitable Leadership Foundation for funding this work.

Disclosure: The authors declared no conflict of interest.

1. Aitman TJ, Glazier AM, Wallace CA, Cooper LD, Norsworthy PJ, Wahid FN, Al-Majali KM, Trembling PM, Mann CJ, Shoulders CC, Graf D, St Lezin E, Kurtz TW, Kren V, Pravenec M, Ibrahimi A, Abumrad NA, Stanton LW, Scott J. Identification of Cd36 (Fat) as an insulin-resistance gene causing defective fatty acid and glucose metabolism in hypertensive rats. *Nat Genet* 1999; 21:76–83.
2. Strahorn P, Graham D, Charchar FJ, Sattar N, McBride MW, Dominiczak AF. Genetic determinants of metabolic syndrome components in the stroke-prone spontaneously hypertensive rat. *J Hypertens* 2005; 23:2179–2186.
3. Bezerra RM, Ueno M, Silva MS, Tavares DQ, Carvalho CR, Saad MJ, Gontijo JA. A high-fructose diet induces insulin resistance but not blood pressure changes in normotensive rats. *Braz J Med Biol Res* 2001; 34:1155–1160.
4. D'Angelo G, Elmarakby AA, Pollock DM, Stepp DW. Fructose feeding increases insulin resistance but not blood pressure in Sprague-Dawley rats. *Hypertension* 2005; 46:806–811.
5. Hsieh PS. Reversal of fructose-induced hypertension and insulin resistance by chronic losartan treatment is independent of AT2 receptor activation in rats. *J Hypertens* 2005; 23:2209–2217.
6. Vasudevan H, Nagareddy PR, McNeill JH. Gonadectomy prevents endothelial dysfunction in fructose-fed male rats, a factor contributing to the development of hypertension. *Am J Physiol Heart Circ Physiol* 2006; 291:H3058–H3064.
7. Girard A, Madani S, Boukortt F, Cherkaoui-Malki M, Belleville J, Prost J. Fructose-enriched diet modifies antioxidant status and lipid metabolism in spontaneously hypertensive rats. *Nutrition* 2006; 22:758–766.
8. James DJ, Cairns F, Salt IP, Murphy GJ, Dominiczak AF, Connell JM, Gould GW. Skeletal muscle of stroke-prone spontaneously hypertensive rats exhibits reduced insulin-stimulated glucose transport and elevated levels of caveolin and flotillin. *Diabetes* 2001; 50:2148–2156.
9. Melancon S, Bachelard H, Badeau M, Bourgoin F, Pitre M, Lariviere R, Nadeau A. Effects of high-sucrose feeding on insulin resistance and hemodynamic responses to insulin in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 2006; 290:H2571–H2581.
10. Lin Y, Berg AH, Iyengar P, Lam TK, Giacca A, Combs TP, Rajala MW, Du X, Rollman B, Li W, Hawkins M, Barzilai N, Rhodes CJ, Fantus IG, Brownlee M, Scherer PE. The hyperglycemia-induced inflammatory response in adipocytes: The role of reactive oxygen species. *J Biol Chem* 2004; 280:4617–4626.
11. Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 2006; 440:944–948.
12. Flowers MT, Miyazaki M, Liu X, Ntambi JM. Probing the role of stearoyl-CoA desaturase-1 in hepatic insulin resistance. *J Clin Invest* 2006; 116:1478–1481.
13. Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci USA* 2002; 99:11482–11486.
14. Brosnan MJ, Hamilton CA, Graham D, Lygate CA, Jardine E, Dominiczak AF. Irbesartan lowers superoxide levels and increases nitric oxide bioavailability in blood vessels from spontaneously hypertensive stroke-prone rats. *J Hypertens* 2002; 20:281–286.
15. Miller WH, Brosnan MJ, Graham D, Nicol CG, Morecroft I, Channon KM, Danilov SM, Reynolds PN, Baker AH, Dominiczak AF. Targeting endothelial cells with adenovirus expressing nitric oxide synthase prevents elevation of blood pressure in stroke-prone spontaneously hypertensive rats. *Mol Ther* 2005; 12:321–327.
16. Hamilton CA, Brosnan MJ, Al-Benna S, Berg G, Dominiczak AF. NAD(P)H oxidase inhibition improves endothelial function in rat and human blood vessels. *Hypertension* 2002; 40:755–762.
17. Peinnequin A, Mouret C, Birot O, Alonso A, Mathieu J, Clarencon D, Agay D, Chancerelle Y, Multon E. Rat pro-inflammatory cytokine and cytokine related mRNA quantification by real-time polymerase chain reaction using SYBR green. *BMC Immunol* 2004; 5:3.
18. Weng Y, DiRusso CC, Reilly AA, Black PN, Ding X. Hepatic gene expression changes in mouse models with liver-specific deletion or global suppression of the NADPH-cytochrome P450 reductase gene. Mechanistic implications for the regulation of microsomal cytochrome P450 and the fatty liver phenotype. *J Biol Chem* 2005; 280:31686–31698.
19. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, Wynshaw-Boris A, Poli G, Olefsky J, Karin M. IκK-β links inflammation to obesity-induced insulin resistance. *Nat Med* 2005; 11:191–198.
20. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond)* 2005; 2:5.
21. Gross LS, Li L, Ford ES, Liu S. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. *Am J Clin Nutr* 2004; 79:774–779.
22. Delbosc S, Paizanis E, Magous R, Araiz C, Dimo T, Cristol JP, Cros G, Azay J. Involvement of oxidative stress and NADPH oxidase activation in the development of cardiovascular complications in a model of insulin resistance, the fructose-fed rat. *Atherosclerosis* 2005; 179:43–49.
23. Zimmet P, Magliano D, Matsuzawa Y, Alberti G, Shaw J. The metabolic syndrome: a global public health problem and a new definition. *J Atheroscler Thromb* 2005; 12:295–300.
24. Miyazaki M, Dobrzyn A, Man WC, Chu K, Sampath H, Kim HJ, Ntambi JM. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms. *J Biol Chem* 2004; 279:25164–25171.
25. Sampath H, Miyazaki M, Dobrzyn A, Ntambi JM. Stearoyl CoA desaturase-1 mediates the pro-lipogenic effects of dietary saturated fat. *J Biol Chem* 2007; 282:2483–2493.
26. Gudbrandsen OA, Hultstrom M, Leh S, Monica Bivol L, Vagnes O, Berge RK, Iversen BM. Prevention of hypertension and organ damage in 2-kidney, 1-clip rats by tetradecylthioacetic acid. *Hypertension* 2006; 48:460–466.
27. Wei Y, Wang D, Pagliassotti MJ. Fructose selectively modulates c-jun N-terminal kinase activity and insulin signaling in rat primary hepatocytes. *J Nutr* 2005; 135:1642–1646.
28. Kelley GL, Allan G, Azhar S. High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. *Endocrinology* 2004; 145:548–555.

29. Wei Y, Pagliassotti MJ. Hepatospecific effects of fructose on c-jun NH2-terminal kinase: implications for hepatic insulin resistance. *Am J Physiol Endocrinol Metab* 2004; 287:E926–E933.
30. Busserolles J, Rock E, Gueux E, Mazur A, Grolier P, Rayssiguier Y. Short-term consumption of a high-sucrose diet has a pro-oxidant effect in rats. *Br J Nutr* 2002; 87:337–342.
31. Busserolles J, Zimowska W, Rock E, Rayssiguier Y, Mazur A. Rats fed a high sucrose diet have altered heart antioxidant enzyme activity and gene expression. *Life Sci* 2002; 71:1303–1312.
32. Faraci FM and Didion SP. Vascular protection: superoxide dismutase isoforms in the vessel wall. *Arterioscler Thromb Vasc Biol* 2004; 24:1367–1373.
33. Macmillan-Crow LA, Cruthirds DL. Invited review: manganese superoxide dismutase in disease. *Free Radic Res* 2001; 34:325–336.
34. Fennell JP, Brosnan MJ, Frater AJ, Hamilton CA, Alexander MY, Nicklin SA, Heistad DD, Baker AH, Dominiczak AF. Adenovirus-mediated overexpression of extracellular superoxide dismutase improves endothelial dysfunction in a rat model of hypertension. *Gene Ther* 2002; 9:110–117.
35. Fortepiani LA, Reckelhoff JF. Increasing oxidative stress with molsidomine increases blood pressure in genetically hypertensive rats but not normotensive controls. *Am J Physiol Regul Integr Comp Physiol* 2005; 289:R763–R770.
36. Schepetkin IA. Lucigenin as a substrate of microsomal NAD(P)H-oxidoreductases. *Biochemistry (Mosc)* 1999; 64:25–32.
37. Zou AP, Li N, Cowley AW Jr. Production and actions of superoxide in the renal medulla. *Hypertension* 2001; 37:547–553.