Hepatospecific effects of fructose on c-jun NH2-terminal kinase: implications for hepatic insulin resistance

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Wei, Yuren, and Michael J. Pagliassotti. Hepatospecific effects of fructose on c-jun NH2-terminal kinase: implications for hepatic insulin resistance. Am J Physiol Endocrinol Metab 287: E926–E933, 2004.—Sucrose- and fructose-enriched diets produce hepatic insulin resistance in rats independently of obesity. In humans, fructose infusion results in impaired insulin regulation of glucose production. The aim of the present study was to identify intracellular mediators of sucrose- and fructose-induced hepatic insulin resistance. In study 1, male rats were fed a control diet (STD, 68% of energy from corn starch, 12% from corn oil) or a sucrose-enriched diet (HSD, 68% sucrose, 12% corn oil) for 1, 2, or 5 wk. HSD produced hepatic insulin resistance at all time points. Hepatic protein tyrosine phosphatase 1B protein levels and activity were increased at 5 wk only, whereas c-jun NH2-terminal kinase (JNK) activity was increased at all time points. Normalization of JNK activity in hepatocytes isolated from HSD rats improved insulin-stimulated tyrosine phosphorylation of insulin receptor substrate (IRS) proteins and insulin suppression of glucose release. In study 2, male rats were provided STD for 1 wk and then were either fasted or fasted and refeed either STD or HSD for 3 or 6 h. Rats refeed HSD were characterized by increased hepatic JNK activity and phosphorylation of IRS1 on Ser307 after 6 h only. In study 3, hyperglycemic, hyperinsulinemic pancreatic clampings were performed for 3 or 6 h in the presence or absence of low or high intraportal fructose infusions. High intraportal fructose infusions, which increased portal vein fructose concentration to ~1 mM, increased hepatic JNK activity and phosphorylation of IRS1 on Ser307 at 6 h only. These data suggest that sucrose- and fructose-induced hepatic insulin resistance are mediated, in part, via activation of JNK activity. Thus high rates of fructose metabolism in the liver appear to acutely activate stress pathways.

METHODS

Animals. Male Wistar Crl:(WI)BR rats (Charles River Laboratories, Wilmington, MA) weighing ~180 g on arrival were provided free access to a high-starch diet (STD, 68% of energy from corn starch, 12% from corn oil, and 20% from casein (25)) and water. Rats were housed individually in a temperature- and humidity-controlled environment with a 12:12-h light-dark cycle. Protocols described below were performed after 1 wk of acclimatization. All procedures were reviewed and approved by the University of Colorado Health Sciences Center and/or Colorado State University institutional animal care committees.

Chronic dietary protocol. Rats were provided either STD or a high-sucrose diet (HSD, 68% sucrose, 12% corn oil, and 20% casein (25)) for 1, 2, or 5 wk. After an overnight fast, animals were anesthetized with pentobarbital sodium (70 mg/kg) and placed on a hyperglycemic and high rates of glucose flux in tissues contribute to the development of oxidative stress, insulin resistance, and protein glycosylation (16, 29, 32). Recent studies have demonstrated that acute hyperglycemia induced by a glucose infusion elicits a hepatic stress response and systemic inflammation (16). Sucrose (a disaccharide containing glucose and fructose) and fructose are unique nutrients with respect to the liver, because of the exceptionally high rate of hepatic fructose extraction and the ability of fructose to stimulate liver glucose uptake (17, 30, 37). In fact, chronic exposure to a diet enriched in fructose appears to elicit a hepatic stress response characterized by increased activator protein-1 activity (13). Therefore, our second aim was to determine whether sucrose ingestion or selective elevations in portal vein fructose acutely regulate the activity of PTP1B and/or JNK in the liver. Such data would support the notion that the liver was particularly susceptible to changes in sugar delivery, in particular sugars that undergo extensive hepatic metabolism.
heating pad. The abdomen was opened, the portal vein was exposed, and saline \( (n = 10/\text{diet/time}) \) or insulin \( (n = 7/\text{diet/time}, 2 \text{ mU/kg}) \) was injected. After injection (2 min), a blood sample was drawn, and portions of the liver were removed and processed for subsequent analyses.

**Acute refeeding protocol.** After 1 wk on STD, rats were fasted for 48 h and then either continued fasting or were provided free access to either STD or HSD for 3 or 6 h \( (n = 8/\text{group/time}) \). Rats were anesthetized with pentobarbital sodium \( (70 \text{ mg/kg}) \) and placed on a heating pad. The abdomen was opened, a portal vein blood sample was drawn, and portions of the liver were removed and processed for subsequent analyses.

In vivo clamp protocols. Rats were deprived of food overnight and then were anesthetized using ketamine \( (50 \text{ mg/kg}) \), xylazine \( (10 \text{ mg/kg}) \), and acepromazine \( (5 \text{ mg/kg}) \). Catheters were then implanted in a jejunal vein (Helix Medical), carotid artery (Intramedic Clay-Adams, Bectin-Dickinson, Sparks, MD), and jugular vein (Intramedic Clay-Adams) as previously described \((25, 28)\). All catheters were filled with heparinized saline and were exteriorized on the dorsal side of the neck. Rats were used in studies after 7 days of recovery, during which they were provided STD. Rats used in studies were characterized by normal energy intake (equivalent to presurgery values) during recovery days 4–7 and body weights in excess of presurgery values. On the day of study, extensions were added to catheters of 6- to 8-h-fasted rats. Rats were allowed to rest for 30 min, and then a baseline blood sample was taken. To investigate the effects of the portal vein fructose concentration, rats underwent one of four experimental protocols. In all protocols, somatostatin \( (1.2 \text{ µg/kg} \cdot \text{min}^{-1}) \) was infused in the jugular vein to inhibit endogenous insulin secretion. In protocol 1, a pancreatic clamp was performed in which insulin was infused in the jugular vein at rates \((0.7 \pm 0.03 \text{ mU/kg} \cdot \text{min}^{-1})\) designed to maintain glucose levels at baseline values. In the remaining protocols \((2–4)\), the insulin infusion rate was \(3 \text{ mU/kg} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}\). In protocol 2 \( (G) \), a hyperglycemic, hyperinsulinemic clamp was performed in which glucose was infused at a constant rate in the jejunal vein \((4.5 \text{ mg/kg} \cdot \text{min}^{-1})\) and at a variable rate in the jugular vein to establish moderate hyperglycemia. This protocol was used to mimic the postprandial environment of hyperinsulinemia, hyperglycemia, and portal vein glucose levels in excess of arterial levels. Protocols 3 and 4 were identical to G with the addition of a fructose infusion in the jejunal vein that was designed to elevate portal vein fructose concentrations to \(\leq0.3 \text{ mmol/l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \) or \(>0.1 \text{ mmol/l} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). In F1 and F2, arterial glucose levels were adjusted in an attempt to equate the total hepatic sugar load (glucose + fructose) with that of G. Thus F1 and F2 were designed to examine the effects of low- vs. high-fructose concentrations on hepatic insulin signaling. The duration of these experiments was either 3 h \((n = 6/\text{group})\) or 6 h \((n = 6/\text{group})\).

At the end of the experimental periods, rats were anesthetized with pentobarbital sodium \( (70 \text{ mg/kg}) \) and placed on a heating pad. The abdomen was opened, simultaneous arterial and portal vein blood samples were obtained (terminal samples), and portions of the liver, kidney, and gastrocnemius muscle were removed and processed for subsequent analyses. Glucose concentrations in the final arterial sample taken before the administration of anesthesia were not significantly different from glucose concentrations measured after anesthesia.

**Hepatocyte cell suspensions.** Hepatocytes were obtained from rats after 1 wk on either STD \((n = 8)\) or HSD \((n = 8)\) by collagenase perfusion \((3)\). Viability was determined by trypsin blue exclusion, which was \(\geq92\%\). Isolated cells were suspended \((30 \text{ mg/ml})\) in oxygenated Krebs-Henseleit buffer containing \(1\%\) gelatin and incubated at \(37^\circ\text{C} \). SP-600125 \((10 \mu\text{M})\), a selective reversible inhibitor of JNK-JUN, was used where noted \((Biomol, Plymouth Meeting, PA)\).

**Processing and analysis of blood and media samples.** All blood samples were immediately centrifuged, and the plasma was used for analysis of glucose \((Beckman Glucose Analyzer, Fullerton, CA\) or Sigma, St. Louis, MO\), fructose \((Sigma)\), free fatty acids \((FFA; Wako Chemicals, Richmond, VA)\), triglycerides \((Sigma)\), insulin \((Linco Research, St. Charles, MO)\), or glucagon \((Linco)\). In the clamp protocols, insulin, FFA, and fructose concentrations were measured on the baseline sample, two samples taken during the final 30-min steady-state period, and after anesthesia. Glucagon concentrations were only measured on the baseline sample and one sample taken during the final 30-min steady-state period.

**Preparation of cell lysates from liver and hepatocytes.** Freshly removed liver or hepatocytes were homogenized and/or sonicated on ice in a buffer containing \(20 \text{ mM HEPES, pH 7.4, 1% Triton X-100, 10% glycerol, 2 mM EGTA, 1 mM sodium vanadate, 2 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 50 mM β-glycerophosphate, 3 mM benzamidine, 10 µM leupeptin, 5 µM pepstatin, and 10 µg/ml aprotinin. Samples were rotated for 30 min at 4°C and centrifuged at 14,500 rpm for 1 h. Total protein was determined by the detergent-compatible modified Lowry assay (Bio-Rad).**

**Liver and hepatocyte membrane preparations.** Homogenates were made using the protocol described above and were centrifuged at \(100,000 \text{ g} \cdot \text{h}^{-1} \cdot 4°C \). Supernatants were retained as the cytosolic fraction. The pellet was resuspended, incubated on ice, and centrifuged at \(100,000 \text{ g} \cdot \text{h}^{-1} \cdot 4°C \). Supernatants were retained as the solubilized membrane fraction. Purity was determined using glucose-6-phosphate dehydrogenase and 5'-nucleotidase activity \((Sigma)\). Preparations with \(>90\%\) purity were used for subsequent analyses.

**Immunoprecipitation and Western blotting.** For immunoprecipitation, equivalent amounts of protein were incubated with antibodies against insulin receptor substrate-1 \((IRS1; Upstate Biotechnology, Lake Placid, NY)\) or IRS2 \((Upstate)\). Protein A- or G-agarose \((Upstate)\) was then provided, and incubations were continued for \(2 \text{ h} \cdot 4°C\). Immunoprecipitated proteins were resolved by SDS-PAGE. After electrotransfer of proteins from the gels to Hybond-P membranes \((Amersham Pharmacia Biotech, Piscataway, NJ)\), membranes were incubated with antibodies against phosphotyrosine \((BD Transduction Laboratories)\). Detection was performed using enhanced chemiluminescence reagents, and band intensity was analyzed by optical densitometry \((UVP, Upland, CA)\).

In some cases, protein amounts were determined without immunoprecipitation. Equivalent amounts of protein \((50–100 \mu\text{g})\) were subjected to SDS-PAGE as described above and incubated with antibodies against phospho-Akt \((Ser^{473})\), Cell Signaling Technology, Beverly, MA\), phospho-IRS1 \((Ser^{302}; \text{Upstate})\), protein kinase C-α and -δ \((PKC, Santa Cruz Biotechnology, Santa Cruz, CA)\), and PTP1B \((Oncogene Research Products, San Diego, CA)\). Membranes were incubated and analyzed as described above.

**c-Jun terminal kinase activity.** Liver and hepatocyte cell lysates were incubated with an NH₂-terminal c-Jun-(1–98) fusion protein bound to glutathione Sepharose beads \((Cell Signaling)\). The kinase reaction was initiated, after several washing steps, in the presence of \(100 \mu\text{M ATP}\). Western blot analysis was then used to detect c-Jun phosphorylation using an antibody specific for Ser^{63} \((Cell Signaling)\).

**PTP1B activity.** Lysates were precleared with protein A-Sepharose for \(15 \text{ min} \cdot 4°C\). Equal quantities of protein were subjected to immunoprecipitation with anti-PTP1B antibody \((Oncogene Research Products)\) at \(4°C\) overnight. PTP1B immunocomplexes were precipitated with protein A-Sepharose at \(4°C\) for an additional \(2 \text{ h}\). Immunoprecipitates were washed, and the pp60src-COOH-terminal phospho-regulatory peptide \((TSTEPQpYQPGEN)\) was added to a final concentration of \(200 \mu\text{M}\). The reaction was terminated after 1 h, aliquots were treated with Biolumin Green reagent \((Biomol)\), and absorbance was measured at \(630 \text{ nm}\).

**Data analysis and statistics.** Data are presented as means ± SE. Two-way ANOVA was used to analyze the effects of diet and time. Post hoc analyses included linear contrasts and Student-Newman-Keuls’s test. Where appropriate, group means were compared using...
unpaired t-tests or ANOVA. An α-level of $P < 0.05$ was used for statistical significance.

RESULTS

Chronic dietary feeding protocol. Fasting plasma insulin levels were increased significantly after 5 wk on the HSD, whereas plasma triglyceride concentrations were increased significantly at 1, 2, and 5 wk (Table 1). Similar to our previous observations (23, 25), HSD-induced hepatic insulin resistance was evident at 1, 2, and 5 wk [Fig. 1, similar results were observed for tyrosine phosphorylation of IRS2 (data not shown)]. Increased PTP1B protein expression and activity were observed in HSD at the 5-wk time point only (Fig. 2). Hepatic JNK activity and serine phosphorylation of IRS1 were not significantly different between saline-injected muscle or kidney JNK activity and serine phosphorylation of IRS1 were not significantly different between saline-injected dietary groups (data not shown).

To examine the role of JNK in HSD-induced insulin resistance, hepatocytes isolated from rats fed either STD or HSD for 1 wk were used. The increase in JNK activity observed in HSD was retained when measured in freshly isolated hepatocytes (Fig. 4). Treatment of hepatocytes with SP-600125 restored JNK activity to levels observed in hepatocytes isolated from STD-control rats (Fig. 4). Insulin stimulation of tyrosine phosphorylation of IRS1 and insulin suppression of glucose release (Fig. 5) were reduced in hepatocytes isolated from HSD rats. SP-600125 improved insulin action on both IRS1 and suppression of glucose release in hepatocytes isolated from HSD rats. However, insulin stimulation of tyrosine phosphorylation of IRS1 and suppression of glucose release were not restored to values observed in the STD group (Fig. 5).

Acute refeeding studies. Chronic exposure to high-sucrose diets (≥1 wk) produces a hepatic phenotype characterized by insulin resistance, enhanced gluconeogenesis, elevated hepatic and plasma triglycerides, and increased saturated fatty acids in sinusoidal membranes (7, 25, 27). The next series of studies was performed to determined whether acute exposure to either HSD or fructose infusion could elicit selective changes in hepatic JNK activity. Energy intake during the 3-h (35 ± 4 kcal in STD, 31 ± 3 kcal in HSD) or 6-h (50 ± 6 kcal in STD, 54 ± 5 kcal in HSD) refeeding period was not significantly different between refed groups. Portal vein insulin and glucose levels were increased significantly in refed vs. fasted rats but were not significantly different between refed groups (data not shown). Liver phosphate was significantly lower, and xylulose 5-phosphate (a pentose phosphate pathway intermediate) significantly higher in rats refed HSD compared with those refed

Table 1. Animal characteristics from chronic feeding protocol

<table>
<thead>
<tr>
<th>Group</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 5</th>
</tr>
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<tbody>
<tr>
<td><strong>Body weight, g</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>256 ± 11</td>
<td>297 ± 12</td>
<td>381 ± 13</td>
</tr>
<tr>
<td>HSD</td>
<td>250 ± 10</td>
<td>295 ± 14</td>
<td>387 ± 12</td>
</tr>
<tr>
<td><strong>Basal insulin, μU/ml</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>34 ± 5</td>
<td>37 ± 4</td>
<td>30 ± 6</td>
</tr>
<tr>
<td>HSD</td>
<td>33 ± 4</td>
<td>39 ± 5</td>
<td>45 ± 5†</td>
</tr>
<tr>
<td><strong>Basal glucose, mg/dl</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>135 ± 9</td>
<td>130 ± 11</td>
<td>137 ± 12</td>
</tr>
<tr>
<td>HSD</td>
<td>130 ± 10</td>
<td>128 ± 9</td>
<td>133 ± 11</td>
</tr>
<tr>
<td><strong>Basal FFA, μM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>485 ± 28</td>
<td>447 ± 30</td>
<td>501 ± 48</td>
</tr>
<tr>
<td>HSD</td>
<td>502 ± 30</td>
<td>486 ± 38</td>
<td>492 ± 29</td>
</tr>
<tr>
<td><strong>Basal TG, mM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STD</td>
<td>0.45 ± 0.07</td>
<td>0.55 ± 0.04</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>HSD</td>
<td>0.77 ± 0.05*</td>
<td>1.24 ± 0.11*</td>
<td>1.15 ± 0.12*</td>
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</tbody>
</table>

Values are means ± SE for 10 saline-injected rats/group/time. STD, starch-enriched control diet; HSD, sucrose-enriched diet; FFA, free fatty acids; TG, triglycerides. $P < 0.05$, significant diet effect (*) and significant time effect (†).
STD (Table 2). The significant changes in these two intermediates likely reflect the greater hepatic sugar metabolism after exposure to HSD. Hepatic JNK activity and serine phosphorylation of IRS1 were increased in HSD after 6 h but not 3 h, whereas no changes in PTP1B activity were observed (Fig. 6).

Fructose infusion studies. In the 3-h protocol, the total sugar load to the liver (fructose + glucose) averaged 188, 193, and 194 mg/dl in G, F1, and F2, respectively (Table 3). In the 6-h protocol, the total sugar load to the liver averaged 192, 191, and 198 mg/dl in G, F1, and F2, respectively. This estimate assumes a distribution of blood flow to the liver of 20% in the artery and 80% in the portal vein (21). Glucagon concentrations were not significantly different among groups (data not shown). A selective increase in the portal vein fructose concentration (to levels achieved after ingestion of sucrose- or fructose-enriched meals) increased hepatic JNK activity at 6 h only (Fig. 7). No effects were observed on hepatic PTP1B activity, kidney JNK activity, or muscle JNK activity (data not shown).

Lipid intermediates, such as diacylglycerol, can activate some isoforms of PKC, and recent evidence has suggested that FFA-induced hepatic insulin resistance may be mediated, in part, through altered regulation of PKC-δ (15). High-sucrose and -fructose diets increase hepatic lipids and lipid intermediates, and it is likely that lipid intermediates are also increased in the fructose infusion studies performed here. Thus we measured the amount of PKC-α and -δ in cytosolic and membrane fractions prepared from liver samples taken after fructose infusion studies. No differences were observed in the amount of PKC-α or -δ in either fraction among groups (data not shown).

Fig. 2. Protein tyrosine phosphatase 1B protein (PTP1B; A) and activity (B) in the liver after 1, 2, or 5 wk on either the STD (n = 10/wk) or HSD (n = 10/wk) diet. Data are from saline-injected rats, as described in the legend of Fig. 1. STD at 1 wk is set to 1. *Significantly different from STD at same time point (P < 0.05).

Fig. 3. Hepatic c-Jun terminal kinase activity (JNK) and phosphorylation of IRS1 on Ser307 (Pser-IRS1) after 1 wk on the STD (n = 10) or HSD (n = 10) diet. Data are from saline-injected rats, as described in the legend to Fig. 1. STD is set to 1. Representative gels are provided at top. *Significantly different from STD (P < 0.05).

Fig. 4. JNK activity in hepatocytes isolated from rats after 1 wk on either the STD (n = 8) or HSD (n = 8) diet. JNK activity was measured after exposure of hepatocytes to vehicle or SP-600125. *Significantly different from STD (P < 0.05).
The present study provides data directly implicating JNK in sucrose- and fructose-induced hepatic insulin resistance. This conclusion is based on data that demonstrated 1) a similar time course between sucrose-induced hepatic insulin resistance and elevated hepatic JNK activity and 2) that normalization of elevated hepatic JNK activity improved insulin activation of tyrosine phosphorylation of IRS proteins and insulin suppression of glucose release in hepatocytes isolated from rats fed a sucrose-enriched diet. In addition, this study has demonstrated that administration of a single sucrose-enriched meal or the selective elevation of portal vein fructose concentrations increased hepatic JNK activity and Ser307 phosphorylation of IRS1. Therefore, these data complement and add to a previous study in which it was proposed that fructose can activate stress-induced pathways in the liver (13).

In the present study, hepatic insulin resistance was observed after 1, 2, and 5 wk of exposure to a sucrose-enriched diet. Although hepatic JNK activity was increased at all time points, increased PTP1B was only observed at the 5-wk time point. These data suggest that PTP1B does not play a significant role in the initial induction of hepatic insulin resistance in this model. This is consistent with a previous study (31) in which elevated hepatic JNK activity and 2) that normalization of elevated hepatic JNK activity improved insulin activation of tyrosine phosphorylation of IRS proteins and insulin suppression of glucose release in hepatocytes isolated from rats fed a sucrose-enriched diet. In addition, this study has demonstrated that administration of a single sucrose-enriched meal or the selective elevation of portal vein fructose concentrations increased hepatic JNK activity and Ser307 phosphorylation of IRS1. Therefore, these data complement and add to a previous study in which it was proposed that fructose can activate stress-induced pathways in the liver (13).

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Table 3. Metabolites and hormones in fructose infusion studies

<table>
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<tr>
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<th>C</th>
<th>G</th>
<th>F1</th>
<th>F2</th>
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<tbody>
<tr>
<td>3-h Protocol</td>
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<tr>
<td>Arterial glucose, mg/dl</td>
<td>125±8</td>
<td>165±12*</td>
<td>160±11*</td>
<td>156±10*</td>
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<tr>
<td>Arterial fructose, mg/dl</td>
<td>ND</td>
<td>ND</td>
<td>2.3±0.2</td>
<td>6.1±0.5†</td>
</tr>
<tr>
<td>Portal vein glucose, mg/dl</td>
<td>120±7</td>
<td>194±15*</td>
<td>191±14*</td>
<td>184±14*</td>
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<tr>
<td>Portal vein fructose, mg/dl</td>
<td>ND</td>
<td>ND</td>
<td>6.4±0.4</td>
<td>19.1±1.4†</td>
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<tr>
<td>Arterial insulin, μU/ml</td>
<td>30±3</td>
<td>133±9*</td>
<td>140±11*</td>
<td>129±10*</td>
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<td>Portal vein insulin, μU/ml</td>
<td>87±5</td>
<td>415±29*</td>
<td>389±31*</td>
<td>393±27*</td>
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<td>Arterial glucose, mg/dl</td>
<td>135±7</td>
<td>171±10*</td>
<td>161±13*</td>
<td>154±11*</td>
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<tr>
<td>Arterial fructose, mg/dl</td>
<td>ND</td>
<td>ND</td>
<td>2.1±0.2</td>
<td>6.3±0.4†</td>
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<tr>
<td>Portal vein glucose, mg/dl</td>
<td>132±6</td>
<td>198±17*</td>
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<tr>
<td>Portal vein fructose, mg/dl</td>
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<td>ND</td>
<td>7.1±0.3</td>
<td>21.2±1.8†</td>
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<td>Arterial insulin, μU/ml</td>
<td>37±5</td>
<td>128±6*</td>
<td>134±12*</td>
<td>126±11*</td>
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<tr>
<td>Portal vein insulin, μU/ml</td>
<td>91±8</td>
<td>378±33*</td>
<td>380±27*</td>
<td>371±31*</td>
</tr>
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</table>

Data are means ± SE for n = 6/group/time. C, control pancreatic clamp experiments in which glucose and insulin levels were maintained at basal values; G, pancreatic clamp experiments in which glucose and insulin levels were increased in the presence of a low portal vein fructose infusion; F1, pancreatic clamp experiments in which glucose and insulin levels were increased in the presence of a high portal vein fructose infusion; ND, not detected. Arterial glucose, fructose, and insulin concentrations represent the mean of 2 values taken during the final 30 min of the clamp. Portal vein concentrations are based on a single sample taken from anesthetized rats at the termination of the clamp.

*Significantly different from control, and †significantly different from F1 (P < 0.05).

It was hypothesized that upregulation of PTP1B after fructose feeding was likely the result of hyperinsulinemia, which in our model occurs between 2 and 5 wk (25). Normalization of hepatic JNK activity in hepatocytes isolated from sucrose-fed rats improved but did not completely restore insulin action, based on measurements of tyrosine phosphorylation of IRS proteins and glucose release. Thus JNK contributes to, but may not be solely responsible for, sucrose-induced hepatic insulin resistance. Sucrose- and fructose-enriched diets produce a hepatic phenotype that is characterized by insulin resistance, elevated lipids, and increased gluconeogenesis (7, 25, 33). Thus it is difficult, using chronic feeding models, to distinguish between direct or acute effects of fructose metabolism and indirect effects mediated via chronic changes to the intrahepatic lipid environment (4, 27). In the present study, administration of a single sucrose-enriched meal was used to determine whether sucrose-induced activation of JNK in the liver required chronic changes to the intrahepatic lipid environment. Hepatic JNK activity and serine phosphorylation of IRS1 were increased in response to a single exposure to a sucrose-enriched meal. Moreover, selective elevation of portal vein fructose concentrations, to levels observed when rats ingest a sucrose-enriched meal, increased hepatic JNK activity. Thus a chronic change to the intrahepatic lipid environment does not appear to be required for activation of hepatic JNK.

Activation of hepatic JNK in response to a single sucrose-enriched meal or fructose infusion required a significant period of time (3–6 h), suggesting that the intrahepatic signal(s) involved may not include typical carbohydrate intermediates (e.g., phosphorylated sugars, xylulose 5-phosphate, lactate). However, the contribution of these important signaling metabolites cannot be excluded based on the present data (8, 20). Moreover, fructose infusion did not result in selective translocation of PKC-α or -δ, suggesting that activation of JNK is not mediated via classical lipid intermediates (14). Sucrose- and fructose-induced accumulation of precursors that generate advanced glycation end products provides an alternative hypothesis (13) that may be consistent with the delayed time course observed in the present study. Consistent with this notion are studies that have demonstrated that metformin (9) not only reduces sucrose-induced insulin resistance but also systemic methylglyoxal (formed by β-elimination of the phosphate group of triose phosphates) levels in type 2 diabetic subjects (2).

While these studies were in progress, Kelley et al. (13) demonstrated that chronic fructose feeding resulted in increased phosphorylation of JNK and DNA binding activity of activator protein-1 in the liver. In their study, lipoxygenase inhibitors reversed plasma hypertriglyceridemia and reduced activation of activator protein-1, but data on JNK activity were not provided. The present studies add significantly to the
notion that high rates of sugar metabolism in the liver activate stress/inflammatory pathways. First, we have provided data directly linking elevated hepatic JNK activity to sucrose- and fructose-induced insulin resistance. Second, we have demonstrated acute activation of JNK activity and downstream serine phosphorylation of IRS1 in response to a single, sucrose-enriched meal or infused fructose. Third, we have demonstrated that sucrose- and fructose-mediated effects are tissue specific (i.e., liver and not kidney or muscle) and potentially independent of classical carbohydrate and lipid intermediates.

Fructose stimulates glucose phosphorylation and uptake and glycogen synthesis in the liver (30, 37). This stimulatory effect occurs at fructose concentrations of \( \leq 0.2\) mM; thus, low “catalytic” concentrations of fructose can improve glucose tolerance (19). Consistent with the positive effects of low-fructose concentrations on glucose tolerance, we did not observe induction of hepatic JNK activity when portal vein fructose concentrations were clamped at \( \geq 0.3\) mM. In contrast, chronic exposure to sucrose- or fructose-enriched diets produces a number of negative effects in the liver, most notably impaired insulin action, hepatic steatosis, and accelerated gluconeogenesis (7, 25). Importantly, diets containing physiological doses of sucrose (i.e., 18% of energy as sucrose) elicit similar hepatic adaptations but over a more prolonged time course (24). Portal vein fructose concentrations after ingestion of these diets range from 0.8 to 1.4 mM, and portal vein fructose concentrations between 0.5 and 1.5 mM have been observed in humans, primates, and rats in the postprandial setting (17, 26). These concentrations of fructose have interesting and potent effects on the liver that appear to depend on the duration of exposure. Thus, under hyperglycemic, hyperinsulinemic conditions, a 2-h exposure to portal vein fructose concentrations \( \geq 1\) mM leads to the selective upregulation of hepatic glucose-6-phosphatase gene expression (38), whereas more prolonged exposure (3–6 h) is required to provoke activation of JNK.

It has recently been demonstrated that SP-600125 can activate p38 MAPK in MIN6 cells (35). In the present studies, we did not observe any significant activation of either p38 MAPK or ERK in response to chronic sucrose ingestion, acute sucrose ingestion, or portal vein fructose infusion (data not shown). In addition, we were unable to detect significant activation of p38 MAPK in response to SP-600125 in isolated hepatocytes (data not shown). Thus it is possible that the degree of selectivity of SP-600125 may be cell specific.

Aguirre et al. (1) demonstrated that phosphorylation of Ser\(^{307}\) was critical for the inhibitory effect of anisomycin and tumor necrosis factor-\(\alpha\) in Chinese hamster ovary cells, and suggested that this might be mediated via the association of activated JNK with IRS1. More recent evidence demonstrated that JNK activity was abnormally elevated in muscle and activated JNK with IRS1. More recent evidence demonstrated that this might be mediated via the association of JNK and IRS-1. It has recently been demonstrated that SP-600125 can act as a specific inhibitor of p38 MAPK in MIN6 cells (35). In the present studies, we did not observe any significant activation of either p38 MAPK or ERK in response to chronic sucrose ingestion, acute sucrose ingestion, or portal vein fructose infusion (data not shown). In addition, we were unable to detect significant activation of p38 MAPK in response to SP-600125 in isolated hepatocytes (data not shown). Thus it is possible that the degree of selectivity of SP-600125 may be cell specific.

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