Impact of chronic fructose infusion on hepatic metabolism during TPN administration

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Donmoyer, Christine M., D. Brooks Lacy, Yiqun Zhang, Sheng-Song Chen, and Owen P. McGuinness. Impact of chronic fructose infusion on hepatic metabolism during TPN administration. Am J Physiol Endocrinol Metab 283: E1151–E1158, 2002; 10.1152/ajpendo.00223.2001.—During chronic total parenteral nutrition (TPN), net hepatic glucose uptake (NHGU) is markedly elevated. However, NHGU is reduced by the presence of an infection. We recently demonstrated that a small, acute (3-h) intraportal fructose infusion can correct the infection-induced impairment in NHGU. The aim of this study was to determine whether the addition of fructose to the TPN persistently enhances NHGU in the presence of an infection. TPN was infused continuously into the inferior vena cava of chronically catheterized dogs for 5 days. On day 3, a bacterial clot was implanted in the peritoneal cavity, and either saline (CON, n = 5) or fructose (+FRUC, 1.0 mg·kg⁻¹·min⁻¹, n = 6) infusion was included with the TPN. Forty-two hours after the infection was induced, hepatic glucose metabolism was assessed in conscious dogs with arteriovenous and tracer methods. Arterial plasma glucose concentration was lower with chronic fructose infusion (120 ± 4 vs. 131 ± 3 mg/dl, +FRUC vs. CON, P < 0.05); however, NHGU was not enhanced (2.2 ± 0.5 vs. 2.8 ± 0.4 mg·kg⁻¹·min⁻¹). Acute removal of the fructose infusion dramatically decreased NHGU (2.2 ± 0.5 to −0.2 ± 0.5 mg·kg⁻¹·min⁻¹), and net hepatic lactate release also fell (1.6 ± 0.3 to 0.5 ± 0.3 mg·kg⁻¹·min⁻¹). This led to an increase in the arterial plasma glucose (Δ13 ± 3 mg/dl, P < 0.05) and insulin (Δ5 ± 2 μU/ml) concentrations and to a decrease in glucagon (Δ−11 ± 3 pg/ml) concentration. In conclusion, the addition of chronic fructose infusion to TPN during infection does not lead to a persistent augmentation of NHGU.

METHODS

Animal Preparation

Eleven mongrel dogs weighing 19–25 kg were fed a standard meat (Pedigree; Kalkan, Vernon, CA) and chow (Purina Lab Canine no. 5006; Purina Mills, St. Louis, MO) diet once a day and had free access to water. The composition of the diet, based on dry weight, was 52% carbohydrate, 31% protein, 11% fat, and 6% fiber. Dogs were housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines. The protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. Health of the animals was determined during the week before surgery and the week before TPN administration as a good appetite, i.e., consumption of at least three-fourths of the daily ration, normal stools, hematocrit >0.35, and leukocyte count >18,000 mm⁻³.

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**Experimental Preparation**

A laparotomy was performed on healthy dogs under general anesthesia, as described previously (8). During the laparotomy, Silastic (Dow Corning, Midland, MI) catheters (0.04 in. ID) for blood sampling were positioned in the right external iliac artery, portal vein, left common hepatic vein, and left common iliac vein. Two infusion catheters (0.03 in. ID) for TPN and fructose infusion were placed in the inferior vena cava (IVC). Flow probes (Transonic Systems, Ithaca, NY) were positioned about the portal vein, hepatic artery, and right external iliac artery. The free ends of the catheters and flow probes were placed in subcutaneous pockets. The dogs received 600,000 U of penicillin G (Procaine; Anthony Products, Irwindale, CA) in saline intravenously during surgery and 500 mg of ampicillin (Principen; Bristol-Meyers Squibb, Princeton, NJ) orally twice per day for 3 days after surgery.

**Nutritional Support**

After allowance of ≥14 days for recovery from surgery, the IVC catheters were exteriorized with animals under local anesthesia (2% Lidocaine; Abbott, North Chicago, IL). Dogs wore a jacket (Alice King Chatham, Los Angeles, CA) with two large pockets for 5 days of continuous TPN infusion by means of an ambulatory infusion pump (Dakmed, Buffalo, NY). The TPN was designed to be isocaloric on the basis of predicted resting energy expenditure, calculated as 144 + 62.2 × body weight (in kg) (26). The composition of the TPN included glucose, lipids, amino acids, saline, potassium phosphate (American Pharmaceutical Partners, Los Angeles, CA), and a multivitamin supplement (MV1-12; Astra, Westborough, MA). Glucose (50% dextrose; Abbott, -10 mg·kg⁻¹·min⁻¹) made up 75% of the nonprotein calories; Intralipid 20% (Baxter, Deerfield, IL) supplied the remaining 25% of the energy requirements. Travalosol 10% (Baxter) was infused to supply basal nitrogen requirements (1.5 × body wt⁻⁰.⁶⁷·g of protein⁻¹·day⁻¹). All enteral nutrients were discontinued upon initiation of TPN.

**Induction of Infection**

A 1% fibrinogen (Sigma, St. Louis, MO) solution was filtered (0.45 μm) under sterile conditions. A nonlethal dose (2 × 10⁸ organisms/kg body wt) of Escherichia coli, determined by serial dilution followed by plating, was added to the solution. Bacteria (American Type Tissue Culture Collection no. 25922) were prepared by inoculation of 1 liter of Trypticase soy broth (Becton Dickinson, Cockeysville, MD) and incubation overnight at 37°C. Bacteria were pelleted by centrifugation on the next day and were washed with and reconstituted in sterile saline before addition to the filtrate. To initiate clot formation, thrombin (1,000 U; Gentrac, Middleton, WI) was added to the filtrate.

On the 3rd day of TPN administration, ~10 ml of venous blood were withdrawn, centrifuged, and analyzed for serum chemistries. A second laparotomy was performed with animals under anesthesia. An abdominal midline incision was made at a point below that made during the previous surgery, and the bacterial clot was implanted. After clot implantation, a fructose (∼FRUC, n = 6) or saline (CON, n = 5) infusion was begun into the IVC catheter in addition to the TPN infusion. n(−)Fructose (Sigma; 25% wt/vol in saline) was infused at a rate of 1.0 mg·kg⁻¹·min⁻¹ (5.6 μmol·kg⁻¹·min⁻¹). All animals received additional saline during the laparotomy (500 ml) and on the next day (1 liter).

Infected animals were typically hyperthermic and mildly tachycardic but not hypotensive.

**Experimental Protocol**

On the 5th day of TPN and 42 h after clot implantation, studies were performed. Free ends of all catheters were exteriorized under local anesthesia with Lidocaine. Their contents were aspirated and flushed with saline. Leads from the flow probes were also exteriorized and connected to an ultrasonic flowmeter. The dog was placed in a Pavlov harness for the duration of the study. Angiocaths (18 gauge, Abbott) were inserted into a cephalic vein for infusion of radioactive tracer. Blood pressure, heart rate (Micro-Med, Louisville, KY), and rectal temperature (Yellow Springs Instruments, Yellow Springs, OH) were assessed during the study.

A primed (38 μCi, constant (0.4 μCi/min) infusion of [3-¹⁴C]glucose (New England Nuclear, Wilmington, DE) was begun with a Harvard Apparatus syringe pump (Holliston, MA) and continued for the duration of the study. TPN and fructose or saline infusions were also continued at constant rates. After a 120-min tracer-equilibration period and a 60-min basal sampling period, the chronic fructose infusion was discontinued for 90 min. Blood samples were taken from the iliac artery, portal vein, hepatic vein, and iliac vein every 20 min during the basal sampling period and during the last 60 min of the experimental period. Blood flows and hemocrit were recorded at each sampling point. Saline was infused to replace blood volume withdrawn by sampling. At the end of blood sampling, animals were killed with an overdose of pentobarbital sodium (Veterinary Lab, Lenexa, KS). Tissue samples from each liver lobe were freeze-clamped with Wallenberg clamps precooled in liquid nitrogen, and the entire liver was removed and weighed. Tissues were stored at -70°C until analyzed.

One dog in ∼FRUC was studied on three consecutive days (before clot implantation and 18 and 42 h after infection). After sampling on each day, catheters and flow probe ends were tucked into subcutaneous pockets, and additional saline was infused to replace blood withdrawn. Only data from day 2 of infection are included.

**Sample Processing and Analysis**

Venous blood samples were withdrawn before induction of infection and on the study day for serum chemistry analysis, which was performed within 24 h by an outside veterinary laboratory certified by the American Animal Hospital Association. On the study day, all other blood samples were placed in chilled tubes containing EDTA and processed as described (18). For analysis of epinephrine and norepinephrine, whole blood treated with EGTA and glutathione was analyzed by HPLC [coefficients of variation (CVs) were 11 and 6%, respectively] (14). Plasma treated with aprotinin (500 kallikrein inhibitor units/ml; Miles, Kankakee, IL) was analyzed for glucagon and C-peptide content (CVs 8 and 8%) (22). Samples were measured for plasma insulin (CV 9%) (22) and cortisol (CV 11%) (13) concentrations and glucose specific activity (SA) (25).

The method of Beutler (3) was adapted for use with a Technicon Autoanalyzer II (Bran Luebbe, Buffalo Grove, IL) to measure blood fructose content (lower detection limit = 15 μM). To remove the majority of glucose, samples were deproteined with 4% perchloric acid (PCA), neutralized with 10% KOH, and incubated for 60 min with an equal volume of 0.1 M phosphate buffer (pH 7.4) containing glucose oxidase (10 U/ml) and catalase (600 U/ml).
Plasma glucose concentration was measured with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) by use of the glucose oxidase method. Metabolite (lactate, alanine, glycerol, fructose, and NEFA) load entering the liver was calculated as the sum of the loads in the hepatic artery and portal vein (A_s × HABF) + (P_s × PBF), where A_s and P_s represent the substrate concentrations in the artery and portal vein, and HABF and PBF represent blood flow in the hepatic artery and portal vein. Similarly, the substrate load leaving the liver equaled H_le × THBF, in which H_le and THBF represent the hepatic vein substrate concentration and total hepatic blood flow (HABF + PBF). Net hepatic substrate balance was calculated as the difference between the entering and exiting substrate loads, always denoted as uptake or output to give a positive value. Similarly, net hindlimb (or gut) glucose balance was calculated using the formula (A_le − V_le) × ABF, where A_le and V_le are the glucose concentrations in the artery and iliac (or portal vein) vein, and ABF is the iliac artery (or portal vein) blood flow. Net hepatic fractional extraction (HFE) of substrate was calculated as net hepatic substrate balance divided by substrate load entering the liver. Plasma flow was used for NEFA calculations and was calculated by multiplying blood flow by (1 − hematocrit).

The rate of total glucose appearance (R_a) was calculated with a one-compartmental model as described by Wall et al. (29) and modified by DeBodo et al. (7). Whole body glucose clearance rate was the ratio of total glucose disappearance (R_e) and arterial plasma glucose concentration. Unidirectional hepatic glucose uptake (HGU) was calculated as the ratio of [3H]glucose uptake by the liver and inflowing [3H]glucose SA. Hepatic glucose production (HGP) was the difference between HGU and NHGU.

**Statistics**

Results are expressed as means ± SE of four samples in both the basal (120- to 180-min) and experimental (210- to 270-min) periods. Animals were randomized into groups denoted as control (CON, n = 5) and chronic fructose (+FRUC, n = 6), unless otherwise indicated. Student’s t-test was used for group comparisons in the basal period, and the paired t-test was used for comparisons of serum chemistries between days 0 and 2. Statistical comparisons of the effect of fructose removal were made with a two-way ANOVA followed by an F-test (SYSTAT, Evanston, IL) for comparisons within the group. Statistical significance was designated as a P value <0.05.

**RESULTS**

**Serum Chemistries and Hemodynamics**

Before induction of infection, all serum chemistry variables were in the normal range. Liver enzyme (alkaline phosphatase, alanine transaminase, and aspartate transaminase) activities and albumin and triglyceride concentrations before and after infection are shown in Table 1. Alkaline phosphatase activity was elevated on day 2 of infection in CON and +FRUC (P < 0.05), but alanine transaminase and aspartate transaminase activities remained within the normal range. Infection reduced the albumin concentration to a similar extent, to 1.9 ± 0.4 and 2.2 ± 0.2 g/dl in CON and +FRUC, whereas triglyceride concentrations were in

Table 1. Plasma enzyme activities, albumin, total protein, and triglyceride concentrations before and 2 days after infection in saline- and chronic fructose-infused groups receiving TPN

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 5)</th>
<th>+FRUC (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 2</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>61 ± 11</td>
<td>224 ± 34*</td>
</tr>
<tr>
<td>(1-70 U/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine transaminase</td>
<td>33 ± 4</td>
<td>48 ± 10</td>
</tr>
<tr>
<td>(10-100 U/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10-100 U/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate transaminase</td>
<td>21 ± 4</td>
<td>24 ± 8</td>
</tr>
<tr>
<td>(10-100 U/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (2.3-4.3 g/dl)</td>
<td>2.0 ± 0.3</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>Total protein (5.4-7.8 g/dl)</td>
<td>6.5 ± 0.2</td>
<td>5.5 ± 0.4*</td>
</tr>
<tr>
<td>Triglyceride (25-160 mg/dl)</td>
<td>66 ± 9</td>
<td>70 ± 20</td>
</tr>
</tbody>
</table>

Values are means ± SE; units in parentheses indicate normal range. Day 0, before infection; day 2, day 2 postinfection. CON group had saline infusion; +FRUC group had chronic fructose infusion. TPN, total parenteral nutrition. *Significantly different from day 0.
the normal range and were not altered during infection.

General and basal hemodynamic variables (Table 2) were not different between groups. On day 2 of infection, body temperature and heart rate were elevated in CON and +FRUC, a reaction similar to that of previous infected animals in our laboratory (10). Portal vein blood flow (27 ± 2 and 26 ± 2 ml·kg⁻¹·min⁻¹) was similar in CON and +FRUC. Hepatic arterial blood flow (18 ± 2 and 23 ± 1 ml·kg⁻¹·min⁻¹) was elevated with infection (normal = 6 ± 1 ml·kg⁻¹·min⁻¹) (19).

**Fructose**

Blood fructose concentrations in +FRUC (n = 5) in the artery, portal vein, and hepatic vein were 340 ± 44, 319 ± 41, and 222 ± 34 μM, respectively, and were 10-fold higher than those in animals not receiving fructose infusion (arterial concentration, 32 ± 7 μM). Net hepatic fructose uptake in +FRUC was 5.3 ± 0.8 μmol·kg⁻¹·min⁻¹ (94 ± 15% of the fructose infusion rate), and net hepatic fractional extraction of fructose was 0.32 ± 0.03 μmol·kg⁻¹·min⁻¹.

**Hormones**

Table 3 shows basal arterial hormone (insulin, C-peptide, glucagon, cortisol, and catecholamine) concentrations on day 2 of infection. Hormones were not significantly different in +FRUC compared with CON.

**Glucose**

In the basal period, arterial plasma glucose concentrations in CON and +FRUC were 131 ± 3 and 120 ± 4 mg/dl (P = 0.05). Whole body glucose Rₐ (9.7 ± 0.5 and 10.2 ± 0.7 mg·kg⁻¹·min⁻¹) and clearance rates (7.6 ± 0.5 and 8.4 ± 0.8 ml·kg⁻¹·min⁻¹) were similar in CON and +FRUC [n = 5; not significant (NS)].

NHGU rates were 2.8 ± 0.4 and 2.2 ± 0.5 mg·kg⁻¹·min⁻¹ in CON and +FRUC, respectively, and glucose HFE was also similar (0.07 ± 0.01 and 0.06 ± 0.02 mg·kg⁻¹·min⁻¹). Unidirectional HGU (3.1 ± 0.7 and 2.6 ± 0.6 mg·kg⁻¹·min⁻¹) and HGP (0.3 ± 0.6 and 0.6 ± 0.7 mg·kg⁻¹·min⁻¹) rates were comparable.

Net hindlimb glucose uptake rates were similar (13 ± 2 and 12 ± 2 mg/min, CON and +FRUC) in the basal period. Net glucose uptake rates by the gut were also similar (1.3 ± 0.1 and 1.1 ± 0.1 mg·kg⁻¹·min⁻¹).

### Metabolites

Arterial lactate concentrations in the basal period were 7.3 ± 1.4 and 7.6 ± 1.4 mg/dl in CON and +FRUC (n = 5). Net hepatic lactate release (NHLR) was 1.3 ± 0.2 and 1.5 ± 0.3 mg·kg⁻¹·min⁻¹. The percentages of NHGU released as lactate were 49 ± 6 and 57 ± 6% in CON and +FRUC. Arterial alanine concentrations (305 ± 32 and 294 ± 32 μM), net hepatic alanine uptake rates (2.6 ± 0.5 and 2.7 ± 0.3 μmol·kg⁻¹·min⁻¹), and net HFE of alanine (0.19 ± 0.05 and 0.19 ± 0.03) in CON and +FRUC were similar.

Arterial NEFA concentrations in the basal period were 261 ± 31 and 219 ± 14 μM, CON and +FRUC, and net hepatic NEFA uptakes were 0.7 ± 0.2 and 0.6 ± 0.2 μmol·kg⁻¹·min⁻¹ (NS). Likewise, arterial glyceral concentrations (63 ± 2 and 62 ± 2 μM) and net hepatic glycerol uptake rates (1.8 ± 0.2 and 1.9 ± 0.1 μmol·kg⁻¹·min⁻¹) were similar.

### Experimental Period

**Hemodynamics.** Both hepatic arterial (HA) and portal venous (PV) blood flow fell slightly over time in CON and +FRUC (HA: Δ−2 ± 0 and Δ−2 ± 1 ml·kg⁻¹·min⁻¹; PV: Δ−2 ± 1 and Δ−1 ± 1 ml·kg⁻¹·min⁻¹). These changes resulted in a significant decline (Δ−4 ± 2 and Δ−4 ± 2 ml·kg⁻¹·min⁻¹) in total hepatic blood flow.

**Fructose.** When the fructose infusion was discontinued, fructose concentrations in +FRUC decreased by −70% to 96 ± 23, 96 ± 27, and 75 ± 17 μM in the artery, portal vein, and hepatic vein, respectively. Net hepatic uptake of fructose fell dramatically to 0.6 ± 0.4 μmol·kg⁻¹·min⁻¹ (n = 4), which was not significantly different from zero.

**Hormones.** As shown in Fig. 1, arterial plasma insulin increased to 23 ± 6 μU/ml (Δ5 ± 2 μU/ml; P < 0.05) during acute fructose removal. Arterial C-peptide values increased to 0.95 ± 0.19 ng/ml (Δ0.19 ± 0.13 ng/ml; P < 0.05). Parallel to the rise in insulin, glucagon fell to 56 ± 3 pg/ml (Δ−11 ± 3 pg/ml; P < 0.05). There were no hormone changes over time in CON. Cortisol and catecholamine concentrations were not altered by acute removal of fructose (data not shown).

**Glucose.** Arterial plasma glucose rose to 133 ± 6 mg/dl (Δ13 ± 3 mg/dl; P < 0.05) when fructose was removed but did not change in CON (Δ2 ± 2 mg/dl; Fig. 2). Whole body glucose Rₐ (Δ0.2 ± 0.1 and Δ0.5 ± 0.3 mg·kg⁻¹·min⁻¹) was also similar.

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>CON (n = 5)</th>
<th>+FRUC (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin, μU/ml</td>
<td>14 ± 2</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>C-peptide, ng/ml</td>
<td>0.78 ± 0.13</td>
<td>0.76 ± 0.16 (n = 5)</td>
</tr>
<tr>
<td>Glucagon, pg/ml</td>
<td>54 ± 9</td>
<td>67 ± 5</td>
</tr>
<tr>
<td>Cortisol, μg/dl</td>
<td>3.9 ± 0.7</td>
<td>4.8 ± 0.3</td>
</tr>
<tr>
<td>Epinephrine, pg/ml</td>
<td>100 ± 40</td>
<td>142 ± 26</td>
</tr>
<tr>
<td>Norepinephrine, pg/ml</td>
<td>224 ± 78</td>
<td>270 ± 58</td>
</tr>
</tbody>
</table>

Values are means ± SE. All differences between groups are NS.
mg·kg⁻¹·min⁻¹) and clearance (Δ0.2 ± 0.1 vs. Δ−0.3 ± 0.2 ml·kg⁻¹·min⁻¹) in CON and +FRUC did not significantly increase in the experimental period.

During acute fructose removal, NHGU fell rapidly and significantly from 2.2 ± 0.5 to −0.2 ± 0.5 mg·kg⁻¹·min⁻¹ (Fig. 2; P < 0.05). The change in NHGU was due to a Δ−1.1 ± 0.7 fall in unidirectional HGU (2.6 ± 0.6 to 1.5 ± 0.5 mg·kg⁻¹·min⁻¹) as well as a Δ1.2 ± 0.5 mg·kg⁻¹·min⁻¹ increase in HGP (0.6 ± 0.7 to 1.9 ± 0.8 mg·kg⁻¹·min⁻¹). In CON, however, the change in NHGU was only Δ−0.3 ± 0.2 mg·kg⁻¹·min⁻¹, because neither HGU nor HGP changed significantly (Δ−0.7 ± 0.6 and Δ−0.5 ± 0.6 mg·kg⁻¹·min⁻¹, respectively).

Hindlimb glucose uptake rates increased when fructose was removed (Δ5 ± 2 mg/min; P < 0.05) but did not change in CON (Δ2 ± 1 mg/min). There was no change in gut glucose uptake in the experimental period (data not shown).

Tissue analysis of terminal liver biopsies is presented in Table 4. Hepatic glycogen content in CON and +FRUC (n = 5) was 44 ± 10 and 35 ± 7 mg/g liver (NS). GK and G-6-Pase activities were similar in the groups; liver glucose and G-6-P levels were not significantly different. However, the ratio of glucose to G-6-P fell, whereas the ratio of F-6-P to G-6-P was not altered in +FRUC. Moreover F-6-P levels were increased in +FRUC. Total glycogen phosphorylase and glycogen synthase activities and SA ratios were not different.

Metabolites. As shown in Fig. 3, acute fructose removal in +FRUC (n = 5) reduced arterial lactate concentration (Δ−20 ± 7 mg/dl) and net hepatic lactate release (NHLR; Δ−1.1 ± 0.2 mg·kg⁻¹·min⁻¹). In contrast, neither lactate concentration nor NHLR changed significantly in CON (Δ−8 ± 3 mg/dl and Δ0.0 ± 0.1 mg·kg⁻¹·min⁻¹, respectively). The percentage of NHGU released as lactate did not change in CON (Δ0.13 ± 0.10). However, in +FRUC it rose markedly.

Table 4. Tissue analysis of liver biopsies obtained at end of study

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 5)</th>
<th>+FRUC (n = 6)</th>
</tr>
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<tbody>
<tr>
<td>Glycogen, mg/g</td>
<td>44 ± 10</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>Glucokinase, nU/mg protein</td>
<td>6.8 ± 1.0</td>
<td>6.7 ± 0.9</td>
</tr>
<tr>
<td>G-6-Pase, h/d/mg protein</td>
<td>38 ± 7</td>
<td>41 ± 4</td>
</tr>
<tr>
<td>Glycogen phosphorylase</td>
<td>36 ± 2</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>U/mg protein activity ratio</td>
<td>0.48 ± 0.05</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>Glycogen synthase</td>
<td>5.6 ± 0.8</td>
<td>6.3 ± 1.4</td>
</tr>
<tr>
<td>U/mg protein activity ratio</td>
<td>0.15 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>Glucose, µmol/g</td>
<td>5.7 ± 0.3</td>
<td>5.1 ± 0.4</td>
</tr>
<tr>
<td>G-6-P, µmol/g</td>
<td>0.24 ± 0.01</td>
<td>0.32 ± 0.03</td>
</tr>
<tr>
<td>Glucose/G-6-P</td>
<td>23 ± 2</td>
<td>16 ± 1⁰</td>
</tr>
<tr>
<td>F-6-P, µmol/g</td>
<td>47 ± 7</td>
<td>65 ± 6⁰</td>
</tr>
<tr>
<td>F-6-P/G-6-P</td>
<td>0.16 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>F-2,6-P₂, µmol/g</td>
<td>13 ± 1</td>
<td>16 ± 1⁰</td>
</tr>
</tbody>
</table>

Values are means ± SE. G-6-P, glucose 6-phosphate; G-6-Pase, glucose-6-phosphatase; F-6-P, fructose 6-phosphate; F 2,6-P₂, fructose 2,6-bisphosphate. *P < 0.05, significantly different from CON.
because NHGU fell to a greater extent than NHLR. The percentage of NHGU released as lactate could not be calculated, because the liver was no longer a significant consumer of glucose. Arterial alanine concentration (Δ−6 ± 10 and −25 ± 7 μM, CON and +FRUC) and net hepatic alanine uptake (Δ0.0 ± 0.2 and Δ−0.3 ± 0.5 μmol·kg⁻¹·min⁻¹) were similar. There was no change in net HPE of alanine (Δ−0.02 ± 0.02 and Δ−0.04 ± 0.02).

Arterial NEFA concentration (Δ−5 ± 10 and −14 ± 11 μM, CON and +FRUC) and net hepatic NEFA uptake (Δ0.1 ± 0.2 and Δ0.1 ± 0.3 μmol·kg⁻¹·min⁻¹) were not altered. Arterial glycerol and net hepatic glycerol uptake were also unchanged in the experimental period (data not shown).

**DISCUSSION**

Previously, we demonstrated that acute (3-h) fructose infusion could correct the infection-induced impairment in NHGU in TPN-adapted dogs (9). In the present study, we evaluated whether the acute effect of fructose persisted chronically in the presence of an infection. Inclusion of fructose (42 h) with TPN did not enhance NHGU above rates observed during infection. However, acute discontinuation of the chronic fructose infusion resulted in a dramatic reduction in NHGU, suggesting that there are chronic adaptations that limit the effectiveness of fructose. Thus chronic fructose infusion was unable to correct the impairment in NHGU seen during infection, despite fructose having a persistent effect on hepatic glucose metabolism.

Fructose was combined with the TPN infusion and infused into a peripheral vein. Peripheral infusion was chosen because if fructose were effective at enhancing NHGU, the optimal clinical therapy would be to include fructose within the TPN infusate. We chose a 40% greater rate than that in our previous study (1.0 vs. 0.7 mg·kg⁻¹·min⁻¹ (9)), in which fructose was infused into the portal vein. This allowed us to achieve a comparable portal vein fructose concentration (367 ± 36 vs. 319 ± 41 μM; chronic vs. acute). As observed previously with portal fructose infusion, >90% of the infused fructose was removed by the liver, indicating that the effect of fructose was mediated primarily on the liver.

Despite the marked stimulation of NHGU in response to acute fructose infusion (9), NHGU was not improved with chronic fructose infusion. The reason for the different responses in the acute and chronic infusion studies is unclear; we hypothesize that subtle adjustment in the prevailing hormone levels or intrahepatic adaptations may be involved. The differing route of fructose delivery cannot explain the lack of a chronic effect of fructose. In a pilot study of a noninfected dog receiving chronic TPN, a peripheral fructose infusion (1.0 mg·kg⁻¹·min⁻¹) increased NHGU over a period of 90 min. The increase was not as dramatic as the rise in the intraportal study (4. vs. 2.5 mg·kg⁻¹·min⁻¹), because it was opposed by declines in arterial glucose and insulin concentrations (Δ−10 mg/dl and Δ−4 μU/ml, respectively). In the acute intraportal fructose studies, insulin and glucagon levels were held constant, which unveiled the full effects of fructose in the absence of changes in insulin and glucagon. We did not detect significant changes in arterial insulin or glucagon with chronic fructose infusion. However, given the exceptional sensitivity of the liver to these hormones, subtle but physiologically significant changes at the liver cannot be excluded. Another explanation for the lack of a sustained stimulation of NHGU by chronic fructose infusion is a compensatory change in total enzyme activities that limits the effectiveness of fructose. We found that the activities of the enzymes thought to have an important role in HGU (total GK, phosphofructokinase, and G-6-Pase) activities were not altered by chronic fructose infusion. Thus it is likely that adaptations other than changes in total enzyme activity may have a more important role in limiting the sustained effects of fructose on NHGU.

The liver was clearly sensitive to the chronic infusion of fructose. Within 30 min of discontinuation of the chronic fructose infusion, NHGU decreased substantially. This resulted in corresponding rises in arterial plasma glucose and insulin levels (Δ11 ± 2 and Δ27 ± 6%, respectively) and a 15 ± 3% decrease in glucagon concentration. Because these hormonal changes would
normally stimulate HGU or reduce glucose production (5), they cannot explain the decrease in NHGU.

The fall in NHGU after acute fructose removal was due to reciprocal changes in unidirectional HGU and HGP; however, the relative contribution of these two processes is uncertain. If labeled glucose accumulated in hepatic glycogen during the 180-min tracer infusion and was then released from the liver upon fructose removal, this would result in an overestimation of the fall in HGU in the experimental period. In addition, release of labeled glucose from glycogen would underestimate the rise in HGP. The observed increase in HGP most likely reflects enhanced glycogen breakdown, because net hepatic gluconeogenic precursor (alanine and glycerol) uptake rate was unaltered. In addition, consistent with a stimulation of hepatic glycogenolysis, the fall in NHLR was less than that predicted by the fall in NHGU. NHLR fell by only 1.1 mg·kg\(^{-1}\)·min\(^{-1}\), which was one-half of the decline in NHGU (Δ2.3 ± 0.3 mg·kg\(^{-1}\)·min\(^{-1}\)). Although the primary source of carbon for hepatic lactate release is NHGU, it is clear that breakdown of glycogen stores also contributes carbon to glycolysis. Thus stimulation of glycogenolysis and subsequent conversion of glucose to lactate could account for the diminished fall in NHLR.

The abrupt fall in hepatic glucose entry after discontinuation of the fructose infusion is consistent with a fall in active GK. Fructose mediates its effect on the liver by translocation and activation of GK rather than by increasing total (active and inactive) GK activity (2). GK translocation is rapidly reversible in hepatocytes (1). Given its importance for hepatic glucose entry and subsequent metabolism (11), a decrease in GK translocation should decrease glucose phosphorylation and, hence, decrease the intracellular G-6-P concentration. We did not detect a fall in total G-6-P 90 min after discontinuation of the fructose infusion, maybe because the rise in glycogenolysis reversed the fall in G-6-P levels.

The mechanism for the increase in hepatic glycogenolysis and glucose production upon discontinuation of the chronic fructose infusion is unclear. The rapidity of the increase in hepatic glycogenolysis attests to the marked sensitivity of this process to fructose removal. Moreover, this increase occurred without corresponding changes in either the total activity or the activity ratio of glycogen synthase and glycogen phosphorylase. The fact that liver glucose uptake remained impaired for the entire 90 min despite the lack of a detectable change in enzyme activity suggests that other factors, such as enzyme localization, may also contribute to the activation of glycogenolysis upon fructose removal. Translocation of GK can allosterically modify the activities and localization of these enzymes in vivo without altering the activity of the enzymes measured in vitro (16). Given that fructose is metabolized to fructose 1-phosphate, which is known to inhibit G-6-Pase (12), removal of the inhibitory signal and subsequent stimulation of G-6-Pase may contribute to the decrement in NHGU after acute fructose removal. However, given that G-6-P was not decreased, a rise in G-6-Pase activity and a fall in GK activity alone cannot explain the accompanying changes in glycogenolysis.

Chronic fructose did not alter fatty acid metabolism; however, a modest rise in triglyceride was detected. Although there are no reported studies of TPN supplemented with small amounts of fructose, several long-term human studies with higher amounts of fructose (~10% of total calories) in the diet have been performed. Most studies show no effect of fructose on lipid metabolism, but dietary fructose studies lasting several weeks found detrimental effects in hyperinsulinemic and/or hypertriglyceridemic individuals (6). In the present study, less fructose (6.6% of the total calories) was infused peripherally over a shorter duration, which may explain the only modest rise in triglyceride.

In conclusion, acute enhancement of NHGU was not sustained with a small chronic fructose infusion during the course of an infection. Despite the failure to chronically stimulate NHGU, fructose played a considerable role in hepatic glucose metabolism. Acute removal of fructose markedly decreased NHGU despite hormone and glucose changes that would normally stimulate NHGU. Our results suggest that, although facilitation of hepatic glucose entry and subsequent glycogen deposition mediated by translocation of GK may be important sites for the acute regulation of hepatic glucose uptake, in the setting of continuous nutritional support there are as yet unexplained hepatic adaptations that limit its sustained effectiveness. Glycolysis is the primary metabolic fate during continuous nutritional support; fructose is likely ineffective chronically because it does not enhance glycolysis. Thus therapy that targets GK translocation alone may not be effective in leading to a sustained improvement in hepatic glucose disposal in infected states. The ability of fructose to reduce hyperglycemia could be useful as an adjunct therapy with TPN in severely hyperglycemic patients, although evidence to support this will require future work.

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