A High-Fat, High-Fructose Diet Accelerates Nutrient Absorption and Impairs Net Hepatic Glucose Uptake in Response to a Mixed Meal in Partially Pancreatectomized Dogs\textsuperscript{1,2}

Katie Colbert Coate,\textsuperscript{3,4} Guillaume Kraft,\textsuperscript{3} Margaret Lautz,\textsuperscript{3} Marta Smith,\textsuperscript{3} Doss W. Neal,\textsuperscript{3,4} and Alan D. Cherrington\textsuperscript{3,4}

\textsuperscript{3}Department of Molecular Physiology and Biophysics, \textsuperscript{4}Diabetes Research and Training Center, Vanderbilt University School of Medicine, Nashville, TN

Abstract

The aim of this study was to elucidate the impact of a high-fat, high-fructose diet (HFFD; fat, 52%; fructose, 17%), in the presence of a partial (~65%) pancreatectomy (PPx), on the response of the liver and extrahepatic tissues to an orally administered, liquid mixed meal. Adult male dogs were fed either a nonpurified, canine control diet (CTR; fat, 26%; no fructose; \(n = 5\)) or a HFFD (\(n = 5\)) for 8 wk. Diets were provided in a quantity to maintain neutral or positive energy balance in CTR or HFFD, respectively. Dogs underwent a sham operation or PPx at wk 0, portal and hepatic vein catheterization at wk 6, and a mixed meal test at wk 8. Postprandial glucose concentrations were significantly greater in the HFFD group than in CTR or HFFD, respectively. Dogs underwent a sham operation or PPx at wk 0, portal and hepatic vein catheterization at wk 6, and a mixed meal test at wk 8. Postprandial glucose concentrations were significantly greater in the HFFD group than in CTR or HFFD, respectively. Impaired glucose tolerance in HFFD was due in part to accelerated gastric emptying and glucose absorption, as indicated by a more rapid rise in arterial plasma acetaminophen and the rate of glucose output by the gut, respectively, in HFFD than in CTR. It was also attributable to lower net hepatic glucose uptake (NHGU) in the HFFD group (\(5.5 \pm 0.5\) mmol/L) than in the CTR group (\(9.2 \pm 0.5\) mmol/L). Impaired glucose tolerance in HFFD was due in part to accelerated gastric emptying and glucose absorption, as indicated by a more rapid rise in arterial plasma acetaminophen and the rate of glucose output by the gut, respectively, in HFFD than in CTR. It was also attributable to lower net hepatic glucose uptake (NHGU) in the HFFD group (\(5.5 \pm 0.5\) mmol/L) compared to the CTR group (\(26.6 \pm 7.0\) mmol/L), resulting in lower hepatic glycogen synthesis (GSYN) in the HFFD group (\(10.8 \pm 5.4\) mmol/kg/min) than in the CTR group (\(30.4 \pm 7.0\) mmol/kg/min). HFFD also displayed aberrant suppression of lipolysis by insulin. In conclusion, HFFD feeding accelerates gastric emptying and diminishes NHGU and GSYN, thereby impairing glucose tolerance following a mixed meal challenge. These data reveal a constellation of deleterious metabolic consequences associated with consumption of a HFFD for 8 wk. J. Nutr. 141: 1643–1651, 2011.

Introduction

Maintenance of glucose homeostasis during the fasting-to–fed transition is largely dictated by the ability of the liver to switch from glucose production to glucose consumption. Several factors have been reported to influence this dynamic process, including the load of glucose delivered to the liver, the concentration of insulin in the hepatic sinusoids, and the presence of a portal glucose-feeding signal (a negative arterial-portal glucose gradient generated by the delivery of glucose into the portal vein) \((1–4)\). Collectively, these factors augment glucose uptake and GSYN\textsuperscript{3} by the liver. Thus, under normal conditions, the liver serves as one of the principal buffers of perturbations in postprandial glycemia. However, individuals with diabetes display a marked impairment not only in the ability of hyperinsulinemia and hyperglycemia to suppress hepatic glucose production, but also in the ability of those postprandial stimuli to activate hepatic glucose uptake and GSYN \((5–8)\). As a result, diabetic individuals experience frequent bouts of postprandial hyperglycemia, which contributes to the elevation of their hemoglobin A\textsubscript{1C} and predisposes them to many of the complications associated with the disease.

Recently, our laboratory demonstrated that consumption of a HFFD has adverse effects on the regulation of whole-body glucose metabolism in vivo. For example, 8 wk of HFFD feeding significantly impaired glucose tolerance in response to an oral glucose challenge, as indicated by a 123% higher \(\Delta\text{AUC}\) for plasma glucose compared to baseline studies \((9)\). This was due in part to a defect in \(\beta\) cell function, given that glucose-stimulated insulin secretion (as indicated by the \(\Delta\text{AUC}\) for c-peptide) was not enhanced relative to that in baseline studies despite a 2-fold greater increase in the plasma glucose level. Furthermore, 13 wk...
of HFFD feeding was associated with an inability of the liver to switch from net glucose output to net glucose uptake despite hyperinsulinemia, hyperglycemia, and portal glucose delivery. Thus, the functional consequences of a HFFD on hepatic glucose metabolism were similar to those observed in diabetic individuals (7,8).

It is important to note that the metabolic consequences associated with HFFD feeding were detected in response to a glucose challenge (9), which lacked other meal-associated factors that can influence the gastric emptying rate, insulin and glucagon secretion, and NHGU (10–19). Administration of a standard mixed meal has been proposed as a more physiological challenge to the system. In view of these considerations, we conducted experiments in conscious dogs to determine if 8 wk of HFFD feeding: 1) impairs NHGU in the context of a mixed meal; 2) impairs insulin secretion in the presence of other insulin secretagogues (e.g. amino and fatty acids); 3) affects the acute protein and lipid responses to a mixed meal; and 4) alters gastrointestinal function and/or meal macronutrient absorption.

Research Design and Methods

Animals, diets, and surgical procedures. The protocol was approved by the Vanderbilt University Animal Care and Use Committee and all facilities met the standards published by the American Association for the Accreditation of Laboratory Animal Care. Ten adult male mongrel dogs were randomly assigned to either a meat (Kal Kan) and CTR (n = 5; Laboratory Canine Diet 5006, chunk form, PMI Nutrition LabDiet) (20) or to a HFFD (n = 5; High Fructose and Fat Canine Diet 5A4J, short cut pellet form, PMI Nutrition TestDiet) for 8 wk (Table 1). Diets were provided once daily in a quantity (CTR: 500 g + 1 can of meat/d; HFFD: 800 g/d) intended to keep the dogs’ weight stable (CTR body weight; initial: 25 ± 2 kg, final: 25 ± 2 kg) or in positive energy balance (HFFD body weight; initial: 26 ± 1 kg, final: 29 ± 1 kg) over time. Dogs had free access to the food that was provided over the course of 24 h. Mean energy intake was greater in HFFD (12.6 ± 0.75 MJ/d) than in CTR (8.34 ± 0.14 MJ/d) (P < 0.05). Our goal in the present study was to investigate the adverse effects of a HFFD on the response of the liver and extrahepatic tissues to a mixed meal challenge regardless of whether the defect was attributable to excess energy consumption or the macro- and micronutrient content of the diet. We are currently conducting studies to elucidate the relative contributions of fat, fructose, and excess energy to impaired hepatic glucose flux in vivo.

Dogs in the CTR or HFFD group underwent a sham or PPx (≤65% resection), respectively, at wk 0, as described previously (9). It was not our primary aim to isolate the effect of a PPx per se on metabolism; rather, we wanted to use it as a surgical tool to compromise endocrine pancreatic function in the hope that, when coupled with a dietary insult, a diabetic phenotype would emerge. We have now shown, however, that dogs fed the HFFD, with or without PPx (65%), displayed equivalent glucose intolerance, whole-body insulin resistance, and defective hepatic glucose metabolism, but did not become diabetic (9). Although we think, therefore, that the data presented herein represent an effect of the HFFD per se, the possibility cannot be excluded that factors secondary to the PPx influenced the results.

Approximately 2 wk before the study (wk 6 of feeding), each dog underwent a laparotomy under general anesthesia to implant sampling catheters into the femoral artery, the hepatic portal vein, and the left common hepatic vein and to place infusion catheters into a splenic and jejunal vein (2). At the same time, ultrasonic flow probes (Transonic Systems) were positioned around the hepatic artery and the portal vein for the assessment of hepatic blood flow, as previously described (2). After 8 wk of feeding, dogs were challenged with a mixed meal (see next paragraph) and net gut/hepatic substrate balance (see “Calculations”) was measured. All dogs were healthy, as indicated by leukocyte count <18000/mm³, hematocrit >0.35, and good appetite and normal stools.

Experimental design and mixed meal composition. Oral mixed meal tests were carried out in dogs that had been feed deprived for 24 h. On the morning of the study, a liquid mixed meal was drawn up into two 60-mL syringes. Following the control period, it was delivered directly into the dog’s mouth over the course of 2 min to activate the cephalic response to a meal. Experiments consisted of consecutive 60-min equilibration (0–60 min) and control (60–120 min) periods, followed by

### TABLE 1 Composition of the experimental diets

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>High-fat, high-fructose diet composition</th>
<th>Nonpurified Diet + meat composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/kg diet</td>
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<tr>
<td>Protein</td>
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<td>289</td>
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<tr>
<td>Fat</td>
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<tr>
<td>Saturated fat</td>
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<td>Polyunsaturated fat</td>
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<tr>
<td>Total carbohydrate</td>
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<tr>
<td>Starch</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Fructose</td>
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<td>Sucrose</td>
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<td>Lactose</td>
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<td>Crude fiber</td>
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<td>Protein, % energy</td>
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<tr>
<td>Fat, % energy</td>
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<td>Total carbohydrate, % energy</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Fructose</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>Lactose</td>
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<td>0.6</td>
</tr>
<tr>
<td>Energy density, kJ/g</td>
<td>18.9</td>
<td>17.3</td>
</tr>
</tbody>
</table>

1 Protein sources include porcine meat meal, dehulled soybean meal, corn gluten meal, wheat middlings, and dried whey.
2 Fat sources include lard, porcine animal fat, vegetable shortening, and/or unsalted butter.
3 Corresponds to saturated, monounsaturated, and polyunsaturated fat content of nonpurified diet only; these data are not provided by the manufacturer for the can of meat.
4 Starch sources include wheat germ and middlings.
5 Fiber sources include wheat, beat pulp, and corn.
6 Vitamin mix, mg/kg prepared diet: provitamin A carotenoids, 1.0; retinol, 12.0; cholecalciferol, 0.11; α-tocopherol, 29.4; menadione, 0.3; thiamin hydrochloride, 8.9; riboflavin, 4.5; niacin, 78; pantothenic acid, 20; folic acid, 2.8; pyridoxine, 13; biotin, 0.2; vitamin B-12, 27; choline chloride, 1492.
7 Mineral mix, g/kg (unless otherwise specified) prepared diet: calcium, 19.2; sodium, 4.5; chloride, 7.1; fluorine, 48 mg/kg; iron, 380 mg/kg; zinc, 160 mg/kg; manganese, 55 mg/kg; copper, 14 mg/kg; cobalt, 0.5 mg/kg; iodine, 1.7 mg/kg; chromium, 2.3 mg/kg; selenium, 0.36 mg/kg.
oral administration of a defined liquid mixed meal and then a 270-min postprandial sampling period (120–390 min). The test meal consisted of 20% protein [26.9 g Beneprotein (96 kcal); Nestle Healthcare Nutrition], 56% carbohydrate [67.2 g Polycose (255 kcal); Abbott Nutrition], and 24% fat [25.3 mL Microlipid (114 kcal); Nestle Healthcare Nutrition]. Beneprotein and Polycose were dissolved in 60 mL of water along with Microlipid. Each meal was spiked with acetaminophen (500 mg) to measure the gastric emptying rate during mixed meal testing (21). Blood was drawn every 10 min during the early postprandial period (120–150 min) and every 30 min thereafter (180–390 min).

Processing and analysis of samples. Hematocrit, plasma glucose, glucagon, insulin, c-peptide, FFA, TG, and active GLP-1 concentrations, and blood lactate, alanine, and glycerol concentrations were measured using standard procedures as previously described (9,22–25). Arterial plasma acetaminophen levels were determined using a modified protocol designed for HPLC, as previously described (26–28).

Calculations. Net hepatic substrate balances were calculated using the arteriovenous difference method according to the following formula: net hepatic substrate balances = load_{out} – load_{in}, where load_{out} = [H] × HF and load_{in} = [A] × AF + [P] × PF. [A], [P], and [H] represent substrate concentrations in femoral artery, portal vein, and hepatic vein blood or plasma, respectively, and AF, PF, and HF represent blood or plasma flow (as measured using ultrasonic flow probes) through the hepatic artery, the portal vein, and total liver, respectively. With this calculation, positive values reflect net hepatic production and negative values reflect net hepatic uptake. Net gut balance was determined by multiplying the [A] – [P] substrate difference by PF. Net hepatic fractional substrate extraction, hepatic sinusoidal hormone concentrations, and non-HGU were calculated as previously described (22,29). Net hepatic carbon retention, a reflection of hepatic GSYN, was calculated as the sum of the hepatic balances of glucose, lactate, alanine × 2 (to account for the contribution of amino acids other than alanine), and glycerol, with all factors in glucose equivalents as previously described and validated (30–34).

Statistical analyses. All data are presented as means ± SEM. Statistical comparisons between groups and over time were carried out using 2-way, repeated-measures ANOVA (SigmaPlot Statistical Software). When the F test was significant, the Student-Newman-Keuls test was conducted. Changes relative to the basal period only are reported. Differences were considered significant at P < 0.05 vs. basal period and CTR for 2.5 h postmeal delivery (Fig. 1A). Glucose output by the gut also increased more rapidly from basal in HFFD, albeit for a shorter duration, peaking 1.5 h postmeal administration (P < 0.05 vs. basal period and CTR) and then falling such that during the last hour of the experiment, it was significantly lower in the HFFD group than in the CTR group (Fig. 1B). In agreement with their gut glucose absorption profile, arterial plasma acetaminophen levels were significantly greater in the HFFD group than in the CTR group during the early postprandial period (Fig. 1C).

In response to the meal, there were 4-fold and 5-fold increases from basal in arterial plasma c-peptide (P < 0.05) and insulin concentrations, respectively, in the CTR group as well as a significant elevation from basal in arterial and portal vein plasma GLP-1 (active form) concentrations 3 h postmeal administration (Fig. 2A–C; Table 2). In agreement with a sustained rate of gastric emptying and delivery of nutrients to the gut, plasma GLP-1 levels tended to remain elevated from basal throughout the experiment in the CTR group (Fig. 2G; Table 2). In contrast, there were 5-fold and 7-fold increases from basal (P < 0.05) in arterial plasma c-peptide and insulin concentrations, respectively, in the HFFD group, which were significantly greater than those in the CTR group during the experiment (Fig. 2A,B). Likewise, arterial and portal vein plasma GLP-1 levels
During the basal period, net hepatic glucose output was comparable between the CTR and HFFD groups (Fig. 3A). In response to meal consumption, the livers of dogs in the CTR group rapidly switched from net glucose output to net glucose uptake ($P < 0.05$ vs. basal period and HFFD) and remained in an uptake mode for the duration of the study (Fig. 3A). In contrast, NHGU was nearly absent in the HFFD group, as evidenced by the lack of a significant change from basal in the net hepatic glucose balance following meal consumption (Fig. 3A). As a result, NHGU was significantly lower in the HFFD group than in the CTR group during the last 2 h of the experiment (Fig. 3A). On the other hand, nonhepatic glucose uptake (non-HGU) in response to meal consumption was markedly higher in the HFFD group than in the CTR group ($P < 0.05$ vs. basal period and CTR) (Fig. 3C). Given that the ratios of non-HGU:arterial insulin (CTR: 0.15 ± 0.04, HFFD: 0.10 ± 0.01) and nonhepatic glucose clearance:arterial insulin (CTR: 0.15 ± 0.04, HFFD: 0.09 ± 0.01) did not differ between the groups, these data indicate that the muscle was able to compensate for impaired NHGU in the HFFD group but at the expense of increased postprandial plasma insulin and glucose.

**Lactate metabolism.** In both the CTR and HFFD groups, arterial blood lactate levels increased from basal following meal consumption (Fig. 4A). In the CTR group, this was consistent with a robust switch from net hepatic lactate uptake to output (Fig. 4B) and a significant increase from basal in NHCR (Fig. 3B), an index of net GSYN. Net hepatic lactate output (Fig. 4B) and GSYN (Fig. 3B) continued for the remainder of the study in the CTR group. In the HFFD group, on the other hand, there was only a transient switch from net hepatic lactate uptake to output following meal consumption and the rate was significantly lower in the HFFD group than in the CTR group during the experiment (Fig. 4B). This was consistent with a significantly lower rate of NHCR in the HFFD group compared to the CTR group (Fig. 3B). These data suggest that in the absence of meal-associated glucose uptake, the livers of dogs in the HFFD group produced significantly less lactate and synthesized significantly less glycogen.

**Glycerol, nonesterified fatty acid, and TG metabolism.** During the basal period, arterial blood glycerol levels were significantly higher in the HFFD group than in the CTR group. In the CTR group, this was consistent with a significantly lower arterial blood glycerol and glycogen concentrations during the basal period or differences between groups in arterial or hepatic sinusoidal plasma glucagon concentrations following meal consumption (Table 2).

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>Basal period, min</th>
<th>Experimental period, min</th>
<th>$P$ value</th>
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<tbody>
<tr>
<td></td>
<td>60–120</td>
<td>180</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Diet</td>
</tr>
<tr>
<td>Portal vein plasma GLP-1, pmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>3.9 ± 0.5</td>
<td>5.6 ± 0.7</td>
<td>12.7±1.8</td>
</tr>
<tr>
<td>HFFD</td>
<td>3.1 ± 0.3</td>
<td>14.4 ± 4.7**</td>
<td>9.8±1.4</td>
</tr>
<tr>
<td>Arterial plasma glucagon, ng/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>47.4 ± 6.4</td>
<td>40.6 ± 4.3</td>
<td>40.9±4.2</td>
</tr>
<tr>
<td>HFFD</td>
<td>31.9 ± 6.0</td>
<td>34.4 ± 4.1</td>
<td>36.2±6.0</td>
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<tr>
<td>Hepatic sinusoidal plasma glucagon, ng/L</td>
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<td></td>
</tr>
<tr>
<td>CTR</td>
<td>51.0 ± 7.7</td>
<td>43.5 ± 6.8</td>
<td>45.2±4.6</td>
</tr>
<tr>
<td>HFFD</td>
<td>40.1 ± 6.1</td>
<td>43.4 ± 4.0</td>
<td>40.6±3.3</td>
</tr>
</tbody>
</table>

1 Data are mean ± SEM; n = 5. # Different from basal, $P < 0.05$; * different from CTR, $P < 0.05$.  
2 NS, Nonsignificant, $P > 0.05$.
levels fell rapidly in both groups following meal consumption, glycerol concentrations remained significantly higher in the HFFD group than in the CTR group for the duration of the study (Table 3). Likewise, arterial plasma FFA concentrations began to rise in the HFFD group 3 h postmeal delivery such that by the end of the study, they were similar to basal values and significantly greater than those in the CTR group (Table 3). Changes in net hepatic glycerol uptake paralleled those in arterial blood glycerol; by the end of the study, net hepatic glycerol uptake was significantly greater in the HFFD group compared to the CTR group (Table 3). Arterial plasma TG concentrations declined in both groups over the first 3 postprandial hours, after which they remained low in the CTR group but returned to basal levels in the HFFD group (Table 3).

Alanine metabolism. Arterial blood alanine levels rose to a significantly greater extent in the HFFD group than in the CTR group 2 h postmeal administration (Table 4). This was partly attributable to a 5-fold compared to 3-fold increase from basal in alanine output from the gut in HFFD vs. CTR, respectively, 1 h postmeal delivery, coupled with a lower hepatic fractional extraction of alanine during the mid- to late-postprandial period in HFFD compared to CTR (Table 4).

Discussion

The objective of the present study was to investigate whether chronic consumption of a HFFD, in combination with PPx, alters the response of the liver and extrahepatic tissues to an orally delivered, liquid mixed meal under nonclamped experimental conditions. A HFFD was utilized, because it reflects the macronutrient composition of a Western diet, which contains foods that are replete with fat and fructose and, when consumed in increasing quantities, has been associated with a heightened risk for the development of type 2 diabetes (35,36). We report herein that 8 wk of HFFD feeding elicited excessive postprandial hyperglycemia due to accelerated gastric emptying and glucose absorption as well as diminished NHGU and a reduction in the ability of insulin to suppress lipolysis.

Several factors probably contributed to postprandial hyperglycemia in HFFD-fed animals. For example, the rates of gastric emptying and glucose absorption can influence the timing and magnitude of postprandial glucose excursions in healthy and diabetic individuals (37,38). Previously, Davis et al. (39) demonstrated that glucose absorption (and presumably, gastric emptying) occurs very slowly in overnight feed-deprived dogs when fed a test meal of the same composition as their normal diet. In the present study, a sustained rate of glucose output by the gut occurred in CTR following meal consumption, consistent with the observations of Davis et al. (39). In contrast, the rates of gastric emptying and glucose output by the gut were significantly higher in the HFFD group than in the CTR group.
TABLE 3  Arterial blood glycerol, net hepatic glycerol uptake, and arterial plasma FFA and TG concentrations during the basal (60 to 120 min) and experimental (120 to 390 min) periods following oral administration of a liquid mixed meal to 24-h feed-deprived dogs that had been fed a nonpurified, canine control diet (CTR) or a high-fat, high-fructose diet (HFFD) for 8 wk.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Basal period min</th>
<th>Experimental period min</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60–120</td>
<td>180</td>
<td>240</td>
</tr>
<tr>
<td></td>
<td>Diet</td>
<td>Time</td>
<td>Diet x time</td>
</tr>
<tr>
<td>Arterial blood glycerol, (\mu)mol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>0.218 ± 0.019</td>
<td>0.188 ± 0.021</td>
<td>0.165 ± 0.013</td>
</tr>
<tr>
<td>HFFD</td>
<td>0.226 ± 0.025</td>
<td>0.183 ± 0.026</td>
<td>0.146 ± 0.019</td>
</tr>
</tbody>
</table>

Net hepatic glycerol uptake, \(\mu\)mol kg\(^{-1}\) min\(^{-1}\)

|                |       |       |       |       |       |       |
| CTR | 926 ± 122 | 144 ± 7 | 123 ± 5 | 104 ± 12 | 81 ± 4 | 92 ± 8 |
| HFFD | 914 ± 43 | 137 ± 34 | 92 ± 21 | 137 ± 59 | 280 ± 104 | 402 ± 110 |

Arterial plasma FFA, \(\mu\)mol/L

|                |       |       |       |       |       |       |
| CTR | 0.218 ± 0.019 | 0.188 ± 0.021 | 0.165 ± 0.013 | 0.153 ± 0.013 | 0.193 ± 0.014 | 0.194 ± 0.033 |
| HFFD | 0.226 ± 0.025 | 0.183 ± 0.026 | 0.146 ± 0.019 | 0.177 ± 0.018 | 0.157 ± 0.026 |             |

Arterial plasma TG, mmol/L

|                |       |       |       |       |       |       |
| CTR | 286 ± 46 | 350 ± 20 | 321 ± 9 | 313 ± 17 | 307 ± 20 | 311 ± 19 |
| HFFD | 318 ± 35 | 442 ± 29 | 452 ± 29 | 452 ± 48 | 409 ± 46 | 374 ± 63 |

\(^1\) Data are mean ± SEM, \(n = 5\). \# Different from baseline, \(P < 0.05\); * different from CTR, \(P < 0.05\); NS, not significant, \(P > 0.05\).

\(^2\) NS, Nonsignificant, \(P = 0.05\).

during the early postprandial period, consistent with excessive postprandial hyperglycemia in the former. Furthermore, there was a tendency for alanine production by the gut to be greater in the HFFD group compared to the CTR group 1 h postmeal delivery, suggesting that accelerated absorption in the HFFD group was not exclusive to glucose. By the end of the study, however, temporal differences in gastric emptying and nutrient absorption between groups were reversed such that net glucose and alanine output by the gut were significantly greater in the CTR group than in the HFFD group, indicative of accelerated meal macronutrient absorption in the latter.

In addition, there was a doubling in arterial and portal vein plasma GLP-1 levels 10 min postmeal delivery in the HFFD group, whereas there was virtually no change 10 min postmeal in the CTR group. GLP-1 is an incretin hormone secreted by the L-cells of the distal small intestine primarily in response to nutrient ingestion (40,41). GLP-1 is thought to delay gastric emptying and potentiate glucose-dependent insulin secretion, thus limiting postprandial hyperglycemia (42–51). Previous studies conducted in our laboratory, however, demonstrated that a physiologic rise in endogenous GLP-1 is without significant effect on insulin secretion, gastric emptying, and glucose utilization in dogs (24,28,32). Thus, we think that the temporal changes in GLP-1 levels in the current study are a reflection of the differential gastric emptying rates in the HFFD compared to the CTR.

Previous studies conducted in individuals in the early stages of type 2 diabetes or those without autonomic neuropathy also reported an accelerated gastric emptying rate following consumption of a glucose solution or liquid mixed meal (53–60). Although the mechanisms that mediate differential gastric emptying rates in healthy and diabetic individuals remain poorly defined, one study (55) attributed augmented gastric emptying to increased phasic contractility of the proximal stomach in

TABLE 4  Arterial blood alanine, gut production of alanine, net hepatic alanine uptake, and hepatic fractional extraction of alanine during the basal (60 to 120 min) and experimental (120 to 390 min) periods following oral administration of a liquid mixed meal to 24-h feed-deprived dogs that had been fed a nonpurified, canine control diet (CTR) or a high-fat, high-fructose diet (HFFD) for 8 wk.\(^1\)

<table>
<thead>
<tr>
<th></th>
<th>Basal period min</th>
<th>Experimental period min</th>
<th>(P) value</th>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Diet</td>
<td>Time</td>
<td>Diet x time</td>
</tr>
<tr>
<td>Arterial blood alanine, (\mu)mol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTR</td>
<td>286 ± 46</td>
<td>350 ± 20</td>
<td>321 ± 9</td>
</tr>
<tr>
<td>HFFD</td>
<td>318 ± 35</td>
<td>442 ± 29</td>
<td>452 ± 29</td>
</tr>
</tbody>
</table>

Gut production of alanine, \(\mu\)mol kg\(^{-1}\) min\(^{-1}\)

|                |       |       |       |       |       |       |
| CTR | 1.1 ± 0.2 | 3.5 ± 0.4 | 3.6 ± 0.5 | 3.7 ± 0.3 | 3.9 ± 0.4 | 3.8 ± 0.6 |
| HFFD | 0.9 ± 0.1 | 4.4 ± 0.5 | 3.7 ± 0.4 | 2.8 ± 0.4 | 3.4 ± 1.0 | 1.1 ± 0.4 |

Net hepatic alanine uptake, \(\mu\)mol kg\(^{-1}\) min\(^{-1}\)

|                |       |       |       |       |       |       |
| CTR | 3.0 ± 0.1 | 5.8 ± 0.6 | 5.4 ± 0.6 | 6.5 ± 0.1 | 5.8 ± 0.6 | 5.3 ± 0.6 |
| HFFD | 1.9 ± 0.3 | 4.9 ± 0.7 | 4.5 ± 0.6 | 4.6 ± 0.5 | 3.9 ± 0.5 | 2.5 ± 0.5 |

Hepatic fractional extraction of alanine

|                |       |       |       |       |       |       |
| CTR | 0.29 ± 0.04 | 0.32 ± 0.02 | 0.33 ± 0.02 | 0.41 ± 0.02 | 0.37 ± 0.03 | 0.35 ± 0.03 |
| HFFD | 0.22 ± 0.02 | 0.25 ± 0.02 | 0.27 ± 0.03 | 0.32 ± 0.04 | 0.32 ± 0.04 | 0.25 ± 0.03 |

\(^1\) Data are mean ± SEM, \(n = 5\). \# Different from Basal, \(P < 0.05\); * different from CTR, \(P < 0.05\); NS, not significant, \(P > 0.05\).

\(^2\) NS, Nonsignificant, \(P = 0.05\).

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patients with asymptomatic (no autonomic neuropathy) type 2 diabetes. It is also possible that the HFFD induced perturbations in the gut microbiota, which might have elicited an increase in intestinal permeability as a consequence of endotoxemia, as previously reported (61–66). Future studies will need to be conducted to explore the mechanism(s) responsible for accelerated gastric emptying in HFFD-fed animals.

Another factor that contributed to meal-associated glucose intolerance in the HFFD group was inadequate stimulation of NHGU. Under normal conditions, the liver is highly responsive to the route of glucose delivery (peripheral vs. enteral/intraperitoneal), the hepatic glucose load, and the hepatic sinusoidal insulin level (2,67,68). However, NHGU was markedly lower in the HFFD group than in the CTR group despite 1.6- and 2.4-fold greater increases in peak plasma glucose and insulin levels, respectively. These data suggest that HFFD feeding rendered the liver insensitive to the stimulatory effects of glucose, insulin, and portal glucose delivery on NHGU in the context of a physiologic mixed meal challenge, consistent with our previous findings with a glucose challenge (9).

In agreement with impaired NHGU, net hepatic lactate production, an index of net glycolytic flux, and NHCR, an index of net GSYN, were markedly lower in the HFFD group than in the CTR group following meal consumption. Previously, Basu et al. (7,8) reported that decreased hepatic UDP-glucose flux in type 2 diabetic individuals during hyperinsulinemia and hyperglycemia is entirely accounted for by a decrease in the contribution of extracellular glucose to the UDP-glucose pool, suggestive of reduced HGK in diabetes. Moreover, restoration of HGK expression in 20-wk-old Zucker diabetic fatty rats, a genetic model of obese type 2 diabetes, normalized their hepatic glucose flux and the incorporation of glucose into glycogen during a hyperglycemic clamp (69). Thus, it is likely that HFFD feeding impaired HGK activity, which resulted in diminished NHGU and GSYN in response to a mixed meal challenge. We are currently investigating the mechanism(s) associated with impaired hepatic glucose flux after HFFD feeding.

Interestingly, non-HGU, which is primarily reflective of glucose uptake in the skeletal muscle (70), was augmented in the HFFD group compared to the CTR group in response to meal ingestion. This was due in part to the fact that the skeletal muscle of dogs in the HFFD group was postprandially exposed to a much higher concentration of glucose and insulin. Thus, through a mass action effect of glucose as well as through the pleiotropic effects of insulin on muscle glucose uptake (70–73), the skeletal muscle responded accordingly by increasing its consumption of glucose. Indeed, when non-HGU or clearance was expressed relative to the arterial plasma insulin level in HFFD and CTR, the ratios were similar between groups, suggesting that augmented non-HGU in HFFD was secondary to elevated insulin and glucose. These data also underscore the predominance of the defect in hepatic glucose uptake.

Eight weeks of HFFD feeding was also associated with a remarkable resistance to insulin at the level of TG hydrolysis within the adipose tissue. This was evident from the fact that postprandial blood glycerol concentrations were significantly higher in the HFFD group than in the CTR group despite peak arterial plasma insulin concentrations that were 100% greater in the HFFD group. Conversely, plasma FFA concentrations were similar between groups, indicative of a selective impairment in the ability of insulin to suppress lipolysis, whereas the ability of hyperinsulinemia and hyperglycemia to stimulate reesterification of FFA into TG remains intact. The latter is exemplified by the fact that plasma FFA concentrations began to increase in the HFFD group toward the end of the study as plasma insulin and glucose concentrations waned.

We cannot ascertain from these data whether relative β cell failure contributed to meal-associated glucose intolerance in the HFFD group, because we do not know how much insulin would have been secreted in the CTR group had their plasma glucose concentrations been matched to those of the HFFD group. What we now know that was not evident at the time when we designed these experiments is that resection of 65% of the pancreas is insufficient to exacerbate the glucose intolerance induced by HFFD feeding (9). This is consistent with previous studies conducted in rodents in which removal of 85–95% of the pancreas was required before diabetes ensued, and even then, there was a heterogeneous hyperglycemic response that correlated with the extent of pancreatic resection (74,75). Nevertheless, the possibility cannot be excluded that in the context of a mixed meal, factors associated with a PPx might have influenced the results in the present study.

This study revealed novel metabolic consequences of a HFFD on the function of the gastrointestinal tract, liver, and adipose tissue in response to a mixed meal. These data highlight the need for additional studies aimed at elucidating the mechanism(s) by which a HFFD per se perturbs the coordinated response of the aforementioned tissues in the postprandial state.

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**Literature Cited**


