Gut and liver fat metabolism in depancreatized dogs: effects of exercise and acute insulin infusion

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The data show that transplanted from fat stores mobilized from splanchnic adipose and 75% of the FFA consumed by the liver is effectively increased twofold during exercise in both insulin-deficient PX dogs, leads to an increase in net FFA release from mesenteric fat that is equal in magnitude to the response in PX dogs, and nondiabetic dogs; during exercise, a stimulus that enhances FFA flux. Second, it was studied while insulin was being acutely infused to achieve levels normally present during exercise, a treatment that may be expected to inhibit lipolysis. For this purpose, liver and gut arteriovenous differences were used during rest and 2.5 h of treadmill exercise in insulin-deficient (n = 6) and acutely insulin-infused (n = 4) depancreatized (PX) dogs. The data show that 1) exercise, in insulin-deficient PX dogs, leads to an increase in net FFA release from mesenteric fat that is equal in magnitude to the response in nondiabetic dogs; 2) net hepatic fractional FFA extraction is increased twofold during exercise in both insulin-deficient PX dogs and nondiabetic control dogs; 3) during exercise, ~40 and 75% of the FFA consumed by the liver is effectively transferred from fat stores mobilized from splanchnic adipose tissue in insulin-deficient PX and nondiabetic dogs, respectively; 4) hepatic ketogenic efficiency is elevated during rest three- to fourfold in insulin-deficient PX dogs compared with nondiabetic control dogs and remains elevated during exercise; and 5) surprisingly, acute insulin replacement is ineffective in normalizing net gut, hepatic, or splanchnic FFA or ketone body balances in PX dogs.

METHODS

Animal maintenance and surgical procedures. Ten experiments were performed on mongrel dogs (mean wt 21.8 ± 0.8 kg) of either gender that had been fed a standard diet (Kal Kan beef dinner, Vernon, CA, and Wayne Lab Blox: 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry weight; Allied Mills, Chicago, IL) and housed in a facility that met American Association for the Accreditation of Laboratory Animal Care guidelines. The protocols were approved by the Vanderbilt University Animal Care Committee. At least 16 days before each study, a laparotomy was performed with animals under anesthesia (pentobarbital sodium; 25 mg/kg). The vessels perfusing and draining the pancreas were isolated, ligated, and severed, and the pancreas was removed. Silastic catheters (0.03 in. ID) were inserted into the vena cava for indocyanine green (ICG) infusion. Silastic catheters (0.04 in. ID) were inserted into the portal vein and left common hepatic vein for sampling. This sampling configuration was used to assess the effects of diabetes on splanchnic fat metabolism by using the depancreatized (PX) dog. Studies were conducted with animals in the diabetic state when coupled with exercise, a stimulus that enhances FFA flux, and acute infusion of insulin to exercise levels, a treatment that may be expected to inhibit lipolysis. The purpose was to establish the interaction of diabetes and exercise with the contributions of the gut and liver to the net splanchnic balance of FFA and ketone body.

ONE OF THE HALLMARKS of poor metabolic control in people with insulin-dependent diabetes mellitus is an excessive level of circulating free fatty acids (FFA). The increased FFA concentrations contribute to the deranged metabolism caused by insulin deficiency, in part, by promoting an increase in hepatic fat oxidation. Elevated rates of hepatic fat oxidation increase the formation of ketone bodies and fuel the excessive rates of gluconeogenesis that characterize poorly controlled diabetes.

Studies that have attempted to examine the effects of exercise on hepatic FFA metabolism in humans with diabetes have been limited by difficulties associated with distinguishing between hepatic and extrahepatic splanchnic tissues. Prior studies in humans have as-sessed balances across the splanchnic bed by use of sampling from an artery and the hepatic vein. This measurement alone does not account for the contribution of adipose tissue within the splanchnic bed to the hepatic FFA load. This is a critical deficit, especially for an understanding of hepatic fat metabolism during exercise because studies in normal dogs have shown that exercise stimulates FFA release from splanchnic adipose tissue, masking increases in net hepatic FFA uptake (27). As a consequence, measurements of net splanchnic FFA uptake will not reflect the effects of exercise on hepatic FFA metabolism. An extension of this is that the hepatic ketogenic efficiency (the ratio of net hepatic ketone body output to FFA uptake), an index of the ability of the liver to convert FFA to ketones, will be overestimated.

The present study accounts for the contribution of the splanchnic adipose tissue by sampling from the portal vein, which drains the extrahepatic splanchnic tissue and perfuses the liver, in the chronically catheterized dog. The contributions of hepatic and extrahepatic splanchnic tissues to fat metabolism can then be assessed by combining portal vein with arterial and hepatic vein sampling. This sampling configuration was used to assess the effects of diabetes on splanchnic fat metabolism by using the depancreatized (PX) dog. Studies were conducted with animals in the diabetic state when coupled with exercise, a stimulus that enhances FFA flux, and acute infusion of insulin to exercise levels, a treatment that may be expected to inhibit lipolysis. The purpose was to establish the interaction of diabetes and exercise with the contributions of the gut and liver to the net splanchnic balance of FFA and ketone body.
was made in the neck region, the carotid artery was isolated, and a Silastic catheter (0.04 in. ID) was inserted into the vessel for sampling. After insertion, the catheters were filled with saline containing heparin (200 U/ml; Abbott Laboratories, North Chicago, IL), and their free ends were knotted. The knotted free catheter ends were stored under the skin of the abdominal region (except for the carotid artery catheter, which was stored in a pocket under the skin of the neck), and the incisions were closed completely. Catheter placement was verified on autopsy.

PX dogs were treated daily with subcutaneous insulin injection ~20 min before feeding. The insulin dose was adjusted to minimize glycosuria. Insulin requirements varied among animals but averaged 12 U each of regular and NPH insulin (Eli Lilly, Indianapolis, IN). The last injection of NPH insulin was administered 48 h before an experiment and was replaced with extra regular insulin because the latter is cleared more rapidly from its subcutaneous injection site (~5–8 h). Exocrine pancreatic enzymes (McNeil Pharmaceuticals, Springhouse, PA) were replaced orally, and dogs were treated with cimetidine (SmithKline Beecham Pharmaceuticals, North Chicago, IL), and their free ends were knotted. Silastic tubing was connected to the arterial vessel for sampling. After insertion, the catheters were filled with saline containing heparin (200 U/ml; Abbott Laboratories, North Chicago, IL), and their free ends were knotted. Saline was infused in the arterial exposed catheters, brought to the back of the dog, and secured with quick-drying glue. Saline was infused in the arterial catheter during experiments (0.1 ml/min).

Starting 1 wk after surgery, dogs were accustomed to running on a treadmill. Dogs were not exercised during the 48 h preceding an experiment. The animals consumed all of the daily food ration and had a leukocyte count <18,000/mm³ 3 days before experimentation.

Studies were conducted after an 18-h fast because this allows for complete meal absorption in the dog (9). On the day of the experiment, the free catheter ends were accessed through small skin incisions made under local anesthesia (2% lidocaine; Astra Pharmaceutical Products, Worcester, MA). The contents of each catheter were aspirated, and catheters were flushed with saline. Silastic tubing was connected to the exposed catheters, brought to the back of the dog, and secured with quick-drying glue. Saline was infused in the arterial catheter during experiments (0.1 ml/min).

Experimental procedures. Experiments consisted of an equilibration period (~160 to ~40 min), a basal sampling period (~40 to 0 min), a period of moderate-intensity (100 m/min, 12% grade) exercise (0–150 min), and a period of moderate-intensity (100 m/min, 12% grade) exercise (0–150 min), and a period of exercise recovery (160–240 min). The exercise intensity used in these experiments has been shown to result in a twofold increase in heart rate (26) and an increase in O₂ uptake to ~50% of maximum (17). An infusion of ICG (0.1 mg·m⁻²·min⁻¹) was also started at the beginning of the equilibration period and continued for the duration of the experiment. One group of PX dogs (n = 6) was studied with a saline infusion. Another group of PX dogs was studied as described above but with an intraportal insulin infusion (200 μU·kg⁻¹·min⁻¹), commencing at time (t) = −160 min. Arterial, portal vein, and hepatic vein samples were taken at t = −40, −20, 0, 10, 30, 60, 90, 120, 150, 160, 180, and 240 min. Data published previously from a group of nondiabetic control dogs studied in an identical manner to the insulin-deprived PX dogs (28) are presented in RESULTS for comparison with the PX dogs. The experiments in nondiabetic control dogs were conducted within 18 mo of those in the experimental groups. Nondiabetic animals were in the same weight range as the PX dogs, and the same health criteria were used in all dogs.

Processing of blood samples. Whole blood β-hydroxybutyrate concentrations were determined in samples deproteinized with 4% perchloric acid (0.5 ml whole blood in 1.5 ml perchloric acid) by an enzymatic method developed for the Technicon Autoanalyzer (15). For determination of acetoacetate, the perchloric acid-deproteinized blood samples were neutralized with potassium hydroxide on the day of each study and assayed enzymatically the following day (18). Plasma FFA concentrations were determined by the method described by Ho (12). This assay has a coefficient of variation of 4.0%. The assay was linear over the range of the standard curve (r² = 0.992 ± 0.002). Samples exceeding the range of the standards were diluted as necessary. Plasma immunoreactive insulin was measured by using the Sephadex-bound antibody procedure (29) or by a double-antibody system (16). Immunoreactive glucagon concentrations were measured in plasma samples containing 50 μl of 500 kalikrein inhibitor units/ml Trasylol (FBA Pharmaceuticals, NY) by radioimmunoassay with the use of a 30,000 antisera (2). Plasma glucose concentration was measured by using the glucose oxidase method on a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Plasma ICG was determined spectrophotometrically (805 nm) in arterial and hepatic vein samples immediately after the study.

Calculations. Net gut FFA balances were calculated as PVF × ([P] − [A]) and net hepatic FFA balances were calculated by the formula HAF × ([A] − [H]) + PVF × ([P] − [H]), where [A], [P], and [H] are the arterial, portal vein, and hepatic vein substrate concentrations, and HAF and PVF are the hepatic artery and portal vein blood flows, respectively. The calculations were the same for the calculation of ketone balances, except that the sign (±) was reversed. Net splanchnic balance was calculated as the sum of net gut and hepatic balances. These calculations were performed with flows measured by using the ICG-extraction technique (14). The ICG-extraction technique measures total hepatic plasma flow but does not differentiate between inputs from the portal vein and hepatic artery. The proportions of the hepatic blood supply provided by the hepatic artery and portal vein were assumed to be 20 and 80%, respectively, based on flow measurements.

Fig. 1. Effect of exercise on arterial plasma free fatty acid (FFA) levels in insulin-deficient depancreatized dogs (n = 6), acute insulin-replaced (n = 4), and nondiabetic control dogs (n = 6). Values are means ± SE. Values in insulin-deprived depancreatized dogs were significantly greater than in insulin-replaced depancreatized dogs (P < 0.05) during the recovery period and during the basal, exercise and recovery periods in nondiabetic control dogs (P < 0.005).
made in our laboratory by using the Doppler technique in dogs exercising at the same exercise intensity and duration as in the present study (7). Plasma levels and flows were used for the calculation of net free fatty acids balances, whereas blood levels and flows were used to calculate net ketone balances.

Statistics were performed by using SuperAnova and Statview (Abacus Concepts, Berkeley, CA) on a Macintosh PowerPC. Statistical comparisons between groups and over time were made by using an analysis of variance designed to account for repeated measures. Between-group comparisons were tested for significance by using Fisher’s protected least significant difference. Within-group comparisons were examined for significance by using contrasts solved by univariate repeated measures. Statistics are reported in the text and corresponding table or figure legend for each variable. Differences were considered significant when P values were < 0.05. Data are expressed as means ± SE.

RESULTS

Arterial plasma insulin, glucagon, and glucose levels. Data for arterial insulin, glucagon, and glucose comprise a part of earlier communications (24, 25) and therefore are not presented here in detail. Insulin was undetectable in arterial blood of insulin-deprived PX dogs throughout the experiment. Acute insulin infusion led to insulin levels of 6 ± 1 µU/ml in the basal period. Insulin levels did not significantly change during exercise or exercise recovery. Insulin levels were 13 ± 2 µU/ml at rest and 5 ± 1 µU/ml at 150 min of exercise in nondiabetic control dogs. Arterial glucagon levels were 63 ± 8 and 69 ± 9 pg/ml at rest in insulin-deprived and insulin-infused PX dogs, respectively, and levels were unaffected by exercise. Glucagon levels were 62 ± 5 pg/ml at rest and 104 ± 20 pg/ml during exercise in nondiabetic control dogs. Arterial plasma glucose levels were 464 ± 38 mg/dl at rest in insulin-deprived dogs. Levels were not significantly affected by exercise in this group. Plasma glucose levels were 372 ± 35 mg/dl in insulin-infused PX dogs at rest and fell by 140 mg/dl over the 150-min exercise period (P < 0.05). Plasma glucose levels were 106 ± 1 mg/dl at rest in nondiabetic control dogs and 99 ± 2 mg/dl by the end of exercise.

Portal vein FFA responses essentially paralleled the arterial responses. Portal vein FFA levels did not change with exercise in either insulin-deficient (2,465 ± 297 µeq/l in the basal state vs. 2,400 ± 0.3 µeq/l in insulin-deprived PX dogs (Fig. 1). Levels did not change in response to exercise but fell during exercise recovery (P < 0.05). Acute insulin infusion did not significantly alter the arterial FFA levels in the basal state or the FFA responses to exercise and recovery. Both groups of PX dogs had arterial FFA levels that were approximately twofold those in nondiabetic control dogs (P < 0.01). FFA levels rose approximately twofold during exercise in control dogs (P < 0.001) and, as a consequence, were not significantly different from those in PX dogs at t = 150 min of exercise. Portal vein FFA responses essentially paralleled the arterial responses. Portal vein FFA levels did not change with exercise in either insulin-deficient (2,465 ± 297 µeq/l in the basal state vs.
2,663 ± 326 µeq/l during the last 60 min of exercise) or acutely insulin-infused (1,949 ± 197 µeq/l in the basal state vs. 2,246 ± 290 µeq/l during the last 60 min of exercise) PX dogs. Portal vein FFA levels doubled (943 ± 117 µeq/l in the basal state vs. 1,926 ± 210 µeq/l during the last 60 min of exercise) with exercise in control dogs (P < 0.01). At rest, portal vein levels were marginally decreased in insulin-infused PX dogs compared with insulin-deficient PX dogs (P = 0.052). Portal vein FFA levels in insulin-deficient PX dogs were almost threefold those of control dogs at rest (P < 0.01). During exercise, only the difference between insulin-deprived PX dogs and control dogs was significantly different (P < 0.01). Hepatic vein FFA levels also did not change with exercise in either insulin-deficient (2,077 ± 248 µeq/l in the basal state vs. 2,063 ± 271 µeq/l during the last 60 min of exercise) or acutely insulin-infused (1,672 ± 172 µeq/l in the basal state vs. 1,600 ± 161 µeq/l during the last 60 min of exercise) PX dogs. In nondiabetic control dogs, hepatic vein FFA levels were increased ~1.8 times in response to exercise (805 ± 100 µeq/l in the basal state vs. 1,396 ± 113 µeq/l during the last 60 min of exercise) (P < 0.01). At rest, hepatic vein levels were marginally decreased in insulin-infused compared with insulin-deficient PX dogs (P = 0.053). Hepatic vein FFA levels in resting insulin-deficient PX dogs were ~2.5 times greater than those in resting control dogs (P < 0.01). During exercise, hepatic vein FFA levels in insulin-deficient PX dogs were greater than those in insulin-infused PX dogs (P < 0.05). The difference between the hepatic vein FFA responses during exercise in insulin-deficient PX dogs and control dogs was significantly different (P < 0.01).

Net extrahepatic splanchnic FFA output (Fig. 2) rose from 0.4 ± 0.8 to 4.2 ± 0.9 µmol·kg⁻¹·min⁻¹ by 150 min of exercise in insulin-deficient PX dogs (P < 0.02). Interestingly, acute insulin increased basal net extrahepatic splanchnic FFA output to 4.1 ± 0.6 µmol·kg⁻¹·min⁻¹. Rates in this group, however, increased no further in response to exercise. In the nondiabetic control dogs, net extrahepatic splanchnic FFA output rose from 0.1 ± 0.7 to 6.8 ± 1.5 µmol·kg⁻¹·min⁻¹ (P < 0.05). Rates in control dogs were not significantly different from those in insulin-deprived PX dogs. Net extrahepatic splanchnic FFA output decreased in the postexercise state in all protocols (P < 0.05).

Net hepatic FFA uptake (Fig. 3) was elevated by approximately threefold in insulin-deprived PX dogs compared with nondiabetic control dogs in the basal state (P < 0.01). Although rates did not change in response to exercise in insulin-deprived PX dogs, control dogs were characterized by an approximately threefold increase (P < 0.02). Consequently, net hepatic FFA uptake was not significantly different during exercise or exercise recovery. Acute insulin infusion did not significantly affect basal net hepatic FFA uptake in PX dogs, and rates remained above those in nondiabetic control dogs (P < 0.05). In contrast to insulin-deprived PX dogs, insulin-infused PX dogs had a significant increase in net hepatic FFA uptake in response to exercise (P < 0.05), corresponding to the respective difference between hepatic vein FFA concentrations.
Differences in net hepatic FFA uptake between PX and control dogs corresponded to the differences in arterial FFA levels. During exercise, net hepatic fractional FFA extraction significantly rose \((P < 0.05)\) in response to exercise in all protocols (Table 1). Because net hepatic FFA uptake exceeded net extrahepatic FFA output, the splanchnic bed was a net consumer of FFAs. Because net extrahepatic splanchnic and hepatic FFA balances generally changed in opposite directions during exercise, net splanchnic FFA uptake doubled during exercise in the insulin-infused control groups (data not shown). Net splanchnic FFA uptake and acetoacetate levels (Fig. 4 and Fig. 5) in insulin-deprived PX and control groups (data not shown). Net splanchnic FFA uptake doubled during exercise in the insulin-infused PX groups (data not shown).

Arterial ketone body levels, net balances, and net hepatic ketogenic efficiency. Arterial \(\beta\)-hydroxybutyrate and acetoacetate levels (Fig. 4 and Fig. 5) in insulin-deprived and insulin-infused PX dogs were, as expected, substantially higher than levels in nondiabetic control dogs \((P < 0.02)\). Because of an increase in ketone body utilization, \(\beta\)-hydroxybutyrate and acetoacetate levels fell with exercise in PX dogs. Although \(\beta\)-hydroxybutyrate and acetoacetate levels rose during exercise in control dogs, levels were still considerably lower than in both PX groups \((P < 0.05)\). These differences were sustained during exercise recovery. In addition to the differences between PX and control dogs, the insulin-deprived PX dogs had higher arterial \(\beta\)-hydroxybutyrate and acetoacetate levels than did PX animals in which insulin had been infused acutely \((P < 0.05)\).

Net extrahepatic splanchnic \(\beta\)-hydroxybutyrate and acetoacetate uptakes (Table 2) were generally higher in both PX groups than in nondiabetic control dogs \((P < 0.05)\). Only in the case of net extrahepatic splanchnic \(\beta\)-hydroxybutyrate uptake in insulin-deprived PX dogs, which was characterized by great variability, were rates not uniformly higher than in the control dogs. The higher rates of uptake that existed occurred as a result of the differences in circulating levels. In response to exercise, net extrahepatic splanchnic acetoacetate uptake fell by \(\sim 50\%\) in both PX groups \((P < 0.05)\). Recovery from exercise led to restoration of preexercise rates.

Not surprisingly, differences in net hepatic \(\beta\)-hydroxybutyrate and (Fig. 6) and acetoacetate (Fig. 7) outputs between groups were relatively similar to their respective arterial levels. Net hepatic \(\beta\)-hydroxybutyrate and

Table 2. Net extrahepatic splanchnic \(\beta\)-hydroxybutyrate and acetoacetate uptake in insulin-deprived and insulin-replaced depancreatized and nondiabetic control dogs

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Basal</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>160</th>
<th>180</th>
<th>240</th>
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</thead>
<tbody>
<tr>
<td>Insulin-deprived PX</td>
<td>0.7 ± 0.5</td>
<td>0.6 ± 0.2</td>
<td>0.9 ± 0.3</td>
<td>0.7 ± 0.3</td>
<td>1.2 ± 0.3</td>
<td>0.4 ± 0.2</td>
<td>1.6 ± 0.7</td>
<td>1.3 ± 0.6</td>
<td>0.0 ± 0.5</td>
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<tr>
<td>Insulin-replaced PX</td>
<td>1.1 ± 0.3</td>
<td>0.7 ± 0.2</td>
<td>1.1 ± 0.4</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.1</td>
<td>1.4 ± 0.4</td>
<td>0.5 ± 0.1</td>
<td>1.3 ± 0.6</td>
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<tr>
<td>Nondiabetic control</td>
<td>0.2 ± 0.1*</td>
<td>0.1 ± 0.01†</td>
<td>0.2 ± 0.04†</td>
<td>0.3 ± 0.1†</td>
<td>0.3 ± 0.1†</td>
<td>0.2 ± 0.1</td>
<td>0.7 ± 0.2†</td>
<td>0.4 ± 0.1†</td>
<td>0.4 ± 0.1</td>
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<tr>
<th>Experimental Group</th>
<th>160</th>
<th>180</th>
<th>240</th>
</tr>
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<tbody>
<tr>
<td>Insulin-deprived PX</td>
<td>0.8 ± 0.4</td>
<td>0.9 ± 0.2</td>
<td>0.4 ± 0.2*</td>
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<tr>
<td>Insulin-replaced PX</td>
<td>0.6 ± 0.2*</td>
<td>0.7 ± 0.2*</td>
<td>0.8 ± 0.1*</td>
</tr>
<tr>
<td>Nondiabetic control</td>
<td>0.1 ± 0.1†</td>
<td>0.1 ± 0.1†</td>
<td>0.1 ± 0.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE; \(n = 6, 4,\) and 5 insulin-deprived PX, insulin-replaced PX, and nondiabetic control dogs, respectively. *Significantly different from corresponding basal measurement, \(P < 0.05–0.02.\) †Significantly different from corresponding value in insulin-deprived PX dogs, \(P < 0.05–0.002.\)
acetoacetate outputs were markedly higher in insulin-deprived and acutely insulin-infused PX dogs than in nondiabetic control dogs in the basal state ($P < 0.01$). Exercise did not significantly affect net hepatic output of the ketone bodies in either PX group but increased these variables by approximately threefold in control dogs ($P < 0.02$). Net hepatic ketogenic efficiency was approximately fourfold greater in insulin-deprived PX dogs compared with control dogs ($P < 0.01$). Exercise did not significantly affect this variable in insulin-deprived PX dogs or control dogs. Acute insulin in PX dogs had no effect on net hepatic ketogenic efficiency in the basal state but resulted in a decrease in this variable in response to exercise (Table 3) ($P < 0.05$).

Estimated hepatic plasma flow (EHPF). Basal hepatic plasma flow was significantly higher in insulin-replaced PX dogs and significantly lower in nondiabetic control dogs compared with insulin-deprived PX dogs (Table 4). During exercise, EHPF values converged so that differences were minimal between groups. Differences that were present between groups at rest were also present during recovery. Insulin-deprived and insulin-replaced PX dogs had decreased EHPF while exercising compared with at rest.

**DISCUSSION**

Experiments conducted in humans have defined the role of splanchnic fat metabolism in adaptations to muscular work in healthy subjects and in individuals with diabetes by sampling from an artery and the hepatic vein (20, 22, 23). The measurement of total splanchnic arteriovenous differences gives a value that is the sum of balances from all tissues drained by the hepatic vein (gastrointestinal tract, adipose tissue, pancreas, spleen, liver). Use of dogs allows for sampling from the portal vein, which drains extrahepatic tissues and perfuses (with the hepatic artery) the liver. As a consequence, hepatic and extrahepatic balances can be distinguished. The present study demonstrates that the presence of extrahepatic splanchnic FFA output and ketone body uptake can result in considerably higher rates of net hepatic FFA uptake and ketone body output than corresponding net splanchnic values. Moreover, the magnitude of the difference between splanchnic and hepatic balances is affected, to some extent, by two factors that lead to marked adjustments in lipid metabolism, i.e., exercise and diabetes.

Wahren and colleagues (22, 23) and Ahlborg et al. (3) have shown that the specific activity of infused [14C]oleic acid is lower in the hepatic vein than in the artery, even in the presence of net splanchnic oleic acid uptake, in both healthy subjects and subjects with diabetes. This indicates that oleic acid is released from some tissues of the splanchnic bed at the same time as it is being consumed by others. It was further shown that splanchnic oleic acid release is increased by exercise in healthy subjects. In subjects with diabetes deprived of insulin for 24 h and having a blood glucose level of $\approx 310$ mg/dl,
the basal and exercise-induced increment in splanchnic oleic acid release was similar to what was seen in the healthy subjects (22). The responses of net extrahepatic FFA balance presented here for dogs are consistent, from a qualitative standpoint, with the responses for splanchnic oleic acid release reported for humans. In both poorly controlled PX and normal dogs, net extrahepatic FFA output increased similarly with exercise. In people with diabetes deprived of insulin for 24 h, but having a blood glucose level of ~240 mg/dl (compared with ~310 mg/dl in the study described above), splanchnic oleic acid release was elevated at rest but did not increase with exercise (23). Similarly, when the blood glucose level was reduced by ~70 mg/dl in PX dogs with acute basal insulin replacement, basal extrahepatic FFA release was increased but did not rise further during exercise.

It may seem paradoxical at first that basal net extrahepatic FFA release is higher with insulin infusion (27). Net extrahepatic splanchnic FFA release is a function of both gut FFA uptake and release. Either a reduction in gut FFA uptake or increase in lipolysis could explain the higher basal net extrahepatic FFA release with insulin infusion. A decrease in gut FFA uptake could result if the reduction in glucose levels leads to a decrease in re-esterification of FFAs in the adipose tissue of the gut. A reduction in gut FFA uptake could also result if insulin facilitates the intestinal metabolism of alternative fuels. It is also possible that the reduction in blood glucose level that accompanies insulin infusion facilitates the stimulation of splanchnic lipolysis (24).

The mass action effect of the higher arterial FFA levels in insulin-dependent and insulin-infused PX dogs led to a greater net hepatic FFA uptake compared with that in nondiabetic dogs. Hepatic fractional FFA extraction, a concentration- and flow-independent index of hepatic FFA uptake, was similar in PX and normal dogs throughout the experiments. Hepatic fractional FFA extraction was increased by exercise in all groups. This finding is generally similar to the results obtained by using [14C]oleic acid to measure splanchnic fractional oleic acid extraction during rest and exercise in humans (22, 23). The net release of FFA from the gut counterbalanced the exercise-induced increase in net hepatic fractional FFA extraction and, as a consequence, net splanchnic fractional FFA extraction was unchanged. The presence of net FFA release from extrahepatic tissues may explain the absence of an exercise-induced increase in net splanchnic fractional FFA extraction in some healthy and diabetic people (20). Finally, it can be calculated by comparing net gut to net hepatic FFA balances that ~40 and 70% of the FFAs taken up by the liver, in PX and normal dogs, respectively, are effectively shuttled from mesenteric fat to the liver without contributing to the systemic FFA pool. The hepatic utilizations of glucose (1) and leucine (6) will increase to a greater extent if the hepatic delivery of these substrates is elevated by a selective increase in their portal vein concentrations. If such is also the case for the utilization of FFA by the liver, then the exercise-induced release of FFA into the portal vein may have a greater impact on hepatic FFA utilization than does a similar increase from peripheral fat depots.

Table 3. Net hepatic ketogenic efficiency in insulin-deprived and insulin-replaced depancreatized and nondiabetic control dogs

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Basal</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>Recovery, min</th>
<th>160</th>
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<tbody>
<tr>
<td>Insulin-deprived PX</td>
<td>0.38±0.03</td>
<td>0.39±0.05</td>
<td>0.36±0.04</td>
<td>0.38±0.04</td>
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<td>0.61±0.19</td>
<td>0.48±0.11</td>
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</tr>
<tr>
<td>Insulin-replaced PX</td>
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<td>0.40±0.10</td>
<td>0.22±0.01*†</td>
<td>0.34±0.06</td>
<td>0.28±0.06†</td>
<td>0.22±0.03*</td>
<td>0.41±0.10</td>
<td>0.23±0.05†</td>
<td>0.38±0.14</td>
<td></td>
</tr>
<tr>
<td>Nondiabetic control</td>
<td>0.10±0.03†</td>
<td>0.07±0.04†</td>
<td>0.06±0.03†</td>
<td>0.07±0.02†</td>
<td>0.16±0.05†</td>
<td>0.13±0.02†</td>
<td>0.12±0.04†</td>
<td>0.17±0.05†</td>
<td>0.25±0.11</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6, 4, and 5 insulin-deprived PX, insulin-replaced PX, and nondiabetic control dogs, respectively. *Significantly different from corresponding basal measurement, P < 0.05–0.02. †Significantly different from corresponding value in insulin-deprived PX dogs, P < 0.05–0.005.

Table 4. Estimated hepatic plasma flow in insulin-deprived and insulin-replaced depancreatized and nondiabetic control dogs

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Basal</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>150</th>
<th>Recovery, min</th>
<th>160</th>
<th>180</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin-deprived PX</td>
<td>27.1±2.1</td>
<td>15.8±1.0*</td>
<td>18.5±1.4*</td>
<td>19.5±2.1*</td>
<td>20.0±1.6*</td>
<td>18.6±2.7*</td>
<td>27.6±1.9</td>
<td>29.1±2.8</td>
<td>29.7±3.8</td>
<td></td>
</tr>
<tr>
<td>Insulin-replaced PX</td>
<td>33.9±1.2†</td>
<td>18.0±0.6†</td>
<td>26.0±4.2†</td>
<td>21.0±1.1*</td>
<td>23.1±0.8*</td>
<td>23.2±1.8*</td>
<td>31.9±1.3</td>
<td>34.5±2.3†</td>
<td>35.1±2.4†</td>
<td></td>
</tr>
<tr>
<td>Nondiabetic control</td>
<td>20.2±0.9†</td>
<td>19.8±1.5</td>
<td>18.9±1.3</td>
<td>19.4±1.8</td>
<td>18.3±1.6</td>
<td>18.9±1.4</td>
<td>21.8±2.3†</td>
<td>22.2±1.7†</td>
<td>21.0±1.2†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6, 4, and 6 insulin-deprived PX, insulin-replaced PX, and nondiabetic control dogs, respectively. Estimated hepatic flow is expressed as ml·kg⁻¹·min⁻¹. *Significantly different from corresponding basal measurement, P < 0.05–0.001. †Significantly different from corresponding value in insulin-deprived PX dogs, P < 0.05–0.001.
Acetoacetate and β-hydroxybutyrate were readily taken up by extrahepatic splanchic tissue and were presumably oxidized. The higher arterial ketone body levels led to higher rates of net hepatic ketone body uptake in insulin-deprived and insulin-infused PX dogs compared with nondiabetic dogs. Gut fractional extraction of the ketone bodies was, nevertheless, actually reduced in the two groups of PX dogs. This finding extends previous observations that showed that ketone body clearance is reduced at high ketogenic concentrations (4, 10, 13, 19) by demonstrating that gut fractional ketone removal is also reduced at high ketone levels. It is possible that the impaired gut fractional ketone body extraction is a function of some aspect of the diabetic state other than the elevated ketone body levels. This is not likely to be the case because ketone clearance is impaired when ketone levels are increased by prolonged fasting and not by diabetes (10).

The markedly elevated net hepatic β-hydroxybutyrate and acetoacetate output rates in insulin-deprived PX dogs were not further increased by exercise. This contrasts with findings in dogs with intact pancreata because net hepatic output of the ketone bodies increased with prolonged exercise in these animals. The presence of these increases in normal dogs is the result of a greater increase in hepatic FFA load during exercise and an increase in plasma glucagon (20, 28). Acute insulin caused a small fall in net hepatic β-hydroxybutyrate output at the end of exercise and during exercise recovery but did not alter the response of net hepatic acetoacetate output. Compared with insulin-deficient PX dogs, in insulin-infused animals net rates of hepatic ketone output were not significantly different. The presence of net gut ketone body uptake resulted in a ~10–15% lower net rate of ketone release from the splanchnic bed compared with the liver alone. As a result, estimates of ketogenesis in human subjects made by using arteriovenous differences are probably low because net splanchnic, but not hepatic, ketone body output can be obtained.

Hepatic ketogenic efficiency, the fraction of the net hepatic FFA uptake that can be accounted for by net hepatic ketone output, was approximately fourfold higher in insulin-deprived PX dogs compared with nondiabetic control dogs during both rest and exercise. Intraportal insulin infusion in PX dogs had no effect on this variable. Hepatic ketogenic efficiency was not increased during exercise in any of the three protocols. This was surprising in light of previous studies in humans that show that splanchnic ketogenic efficiency is generally increased by exercise in nonketotic diabetic (22, 23) and healthy (23) subjects. The reason for the difference may relate to measurement differences related to the inability to measure hepatic balances in humans. Although PX dogs have normal basal glucagon levels, they do not exhibit an increase with exercise (21). The increase in glucagon has been shown to be an important determinant of the increase in hepatic ketogenic efficiency during exercise (28). The absence of this increase in PX dogs could be the reason that efficiency does not rise in PX dogs.

Despite a higher EHPF in resting PX dogs, rates were not different during exercise. The higher basal EHPF in PX dogs is consistent with the greater portal vein blood flow in alloxan-diabetic dogs measured by using Doppler techniques (8). We observed a higher EHPF in PX dogs previously, but the difference was not significant (25). These results, however, contrast with those obtained in humans, which show no effect of diabetes on hepatic blood flow (20, 22, 23). The difference between the present study in dogs compared with earlier studies in humans probably relates to the induction of poor metabolic control in the dogs. PX dogs had a pronounced fall in EHPF with exercise. This is consistent with the exaggerated splanchic bed norepinephrine spillover observed in exercising alloxan-diabetic dogs compared with control dogs (8). The mechanism behind the further increase in EHPF with acute insulin infusion is unclear. Insulin has vascular effects, leading to increased muscle blood flow (5). The insulin levels necessary for these effects are much higher than those present in our study. In addition, these effects probably do not extend to the splanchic bed. A fall in glucose resulting in hypoglycemia also increases EHPF (11). It is conceivable that the acute fall in glucose, observed with acute insulin, could increase EHPF even without hypoglycemia.

The data presented herein show that 1) exercise, in insulin-deficient PX dogs, leads to an increase in net gut FFA output that is equal in magnitude to the increase seen in nondiabetic control dogs; 2) during exercise, ~40 and 75% of the FFA consumed by the liver is effectively transferred from mesenteric fat stores to the liver in insulin-deficient PX and nondiabetic dogs, respectively; 3) hepatic fractional FFA extraction is increased twofold during exercise in both insulin-deficient PX dogs and nondiabetic control dogs; 4) hepatic ketogenic efficiency is elevated during rest in insulin-deficient PX dogs compared with control dogs and, even though it rises no further, remains elevated during exercise; and 5) surprisingly, acute insulin infusion is ineffective in normalizing net gut, hepatic, or splanchic balances of FFA and ketones in PX dogs.

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