The splanchnic bed produces cortisol at rates approximating extraadrenal tissues by converting cortisone to cortisol via the 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 pathway. It is not known whether splanchnic cortisol production is regulated by nutrient ingestion and/or by the accompanying changes in hormone secretion. To address this question, 18 healthy humans were randomized to ingest either a mixed meal or to receive an intravenous saline infusion while total-body, splanchnic, and D3 cortisol production (an index of 11β-HSD type 1 activity) were measured using the combined hepatic catheterization and D4 cortisol infusion methods. Fasting glucose and insulin concentrations did not differ on the meal and saline study days. Glucose and insulin concentrations increased after meal ingestion, peaking at 11.0 ± 1.0 mmol/l and 451 ± 64 pmol/l, respectively, at 45 min, then fell to baseline thereafter. In contrast, glucose and insulin concentrations slowly fell to 5.1 ± 0.1 mmol/l and 27 ± 6 pmol/l during the 6 h of observation on the saline study day. Fasting cortisol concentration did not differ on the meal and saline study days. Cortisol increased (P < 0.05) to a peak of 353 ± 55 nmol/l after meal ingestion but did not change after saline infusion. The increase in cortisol after meal ingestion was associated with an increase in both total body cortisol (from 748 ± 63 to 1,620 ± 235 nmol/min; P < 0.01) and total body D3 cortisol (from 99 ± 11 to 143 ± 11 nmol/min; P < 0.01) production, whereas there was no change in either on the saline study day. The increase in total-body cortisol and D3 cortisol production after meal ingestion originated in extrasplanchnic tissues since splanchnic cortisol production (mean 0–360 min: 254 ± 83 vs. 262 ± 36 nmol/min) and splanchnic D3 cortisol production (mean 0–360 min: 72 ± 22 vs. 77 ± 14 nmol/min) did not differ on the meal and saline study days. We conclude that ingestion of a mixed meal does not alter either splanchnic cortisol production or the conversion of D4 cortisol to D3 cortisol or, therefore by implication, flux via the splanchnic 11β-HSD type 1 pathway. Diabetes 55:667–674, 2006

Glucocorticoids are potent regulators of protein, fat, and carbohydrate metabolism. While circulating concentrations of cortisol are comparable in lean and obese humans, recent data suggest that tissue-specific conversion of cortisone to cortisol via the 11β-hydroxysteroid dehydrogenase (11β-HSD) type 1 pathway may contribute to insulin resistance in obese individuals by generating high local cortisol concentrations (1,2). Conversely, inhibition of this pathway in mice appears to enhance insulin and protect against diet-induced obesity, diabetes, and hypertension (3,4). Of note, 11β-HSD type 1 is present in a large number of tissues, including visceral fat and the liver (1–6). We and others (7,8) have reported that substantial amounts of cortisol are produced within the splanchnic bed of humans. However, since cortisol uptake also occurs, there is minimal net splanchnic release of cortisol into the systemic circulation. Thus, while intrasplanchnic cortisone to cortisol conversion may expose tissues within the splanchnic bed (e.g., visceral fat, gut, and/or the liver) to high local cortisol concentrations, it presently is not known whether the splanchnic 11β-HSD type 1 pathway is regulated by physiologic stimuli or, if so, whether these stimuli result in parallel changes in splanchnic and extrasplanchnic cortisol production.

A variety of factors influence 11β-HSD type 1 expression and activity. Estrogen and IGF-1 decrease 11β-HSD type 1 mRNA and activity in hepatocytes and adipocytes (9,10). On the other hand, glucose, ACTH, glucocorticoids, and peroxisome proliferator–activated receptor activation increase 11β-HSD type 1 activity (5,11–13) in liver and fat. Insulin has been reported to decrease 11β-HSD type 1 activity (14), inhibit dexamethasone-induced stimulation (5), or have no effect (11) on hepatic 11β-HSD type 1. However, since these studies used in vitro systems and animal models, it presently is not known if splanchnic 11β-HSD type 1 flux in humans is regulated by physiologic changes in glucose, insulin, and other hormones that occur under the conditions of daily living.

The present studies were undertaken to address this question. Total-body and splanchnic cortisol production were measured in healthy nondiabetic humans using the hepatic venous catheterization technique in combination with an intravenous infusion of D4 cortisol (7,8). In addition, since D4 cortisol loses a single deuterium when it is converted to D3 cortisone, which in turn can be converted to D3 cortisol via the 11β-HSD type 1 pathway, this approach also allows the simultaneous assessment of both total-body and splanchnic D3 cortisol production (7,8,15).

Because humans are in the postprandial state for the...
TABLE 1
Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Meal</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.3 ± 4.5</td>
<td>53.3 ± 4.7</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/5</td>
<td>4/4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.8 ± 0.97</td>
<td>31.8 ± 0.97</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>52.3 ± 2.7</td>
<td>50.6 ± 4.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>36.6 ± 2.8</td>
<td>39.6 ± 3.6</td>
</tr>
<tr>
<td>Visceral fat (cm²)</td>
<td>179.5 ± 19.8</td>
<td>168.9 ± 20.2</td>
</tr>
</tbody>
</table>

Data are means ± SE.

majority of their lives, and since ingestion of food results in an increase in a variety of hormones and substrates (e.g., insulin, incretins, growth hormone, glucose, and amino acids) that modulate cortisol production (10,16,17), rates observed after ingestion of a mixed meal were compared with those observed in subjects fasted for the same period of time.

RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 18 non diabetic subjects gave informed written consent to participate in the study. All subjects were in good health, clinically euthyroid, and at a stable weight. None regularly engaged in vigorous physical exercise. None of the first-degree relatives of the subjects had a history of diabetes. At the time of the study, the subjects were on no medications other than thyroxine. All subjects were regularly engaged in vigorous physical exercise. None of the first-degree relatives of the subjects had a history of diabetes. At the time of the study, the subjects were on no medications other than thyroxine. All subjects gave informed written consent to participate in the study. All subjects were regularly engaged in vigorous physical exercise.

Experimental design. Subjects were admitted to the Mayo Clinic General Clinical Research Center at 1700 on the evening before the study. A standard 10 cal/kg meal (55% carbohydrate, 30% fat, and 15% protein) was eaten between 1730 and 1800. Subjects were randomized to receive either a mixed meal or intravenous saline the following morning.

At 0500 on the morning after admission, an intravenous catheter was placed in a forearm vein in the left arm for infusions of saline, isotopes, and hormone solutions. A urinary bladder catheter was placed at ~0530. At ~0600, a primed-continuous infusion of [0,11,12,12-H²] cortisol (0.22 mg prime, 0.19 mg/h continuous) was begun in 95% ethanol diluted in 80 ml 0.9% normal saline infused 0.1 ml/min (Cambridge Isotope Laboratories, Andover, MA) was started into a forearm vein for both study groups. Primed-continuous infusions of [6,6-H²] glucose, [6-H²] glucose, [1-15C] oleate, and [9,10-H²] (N) oleyltriolein were also infused as part of separate protocols to examine glucose and fat metabolism. Subjects were taken to an intervention laboratory suite where femoral arterial, femoral venous, and hepatic venous catheters were placed as previously described (18). At ~0900 (~60 min), indocyanine green dye (Akorn, Buffalo Grove, IL) was infused through the arterial sheath. The venous catheters and the arterial catheter were used for blood sampling. At 1000 (0 time), one group of subjects ate a mixed meal (~450 kcal; 46% carbohydrate, 15% protein, and 40% fat) prepared as previously described (19), whereas the other group remained fasting throughout the study.

Analytical techniques. Samples were placed in ice, centrifuged at 4°C, and separated. Plasma indocyanine green concentration was measured spectrophotometrically at 805 nm on the day of study as previously described (20). All other samples were stored at ~20°C until analysis. Plasma glucose was measured using a glucose oxidase method using a YSI glucose analyzer (Yellow Springs, OH). Plasma insulin was measured using a chemiluminescence method with the Access Ultraseensitive Immunoenzymatic assay system (Beckman, Chaska, MN). Hepatic venous, femoral arterial, and femoral venous cortisol, D₄ cortisol, and D₃ cortisol concentrations were measured using a liquid chromatography tandem mass spectrometer as previously described (7,21). In brief, prednisolone was added as an internal standard, and methylene chloride was used to extract the relevant steroids. The dried extract was then reconstituted and injected into a liquid chromatography tandem mass spectrometer. Cortisol, D₄ cortisol, D₃ cortisol, cortisone, and D₃ cortisone ions were generated with electrospray source in positive mode and were detected with multiple reaction monitoring using the specific transitions for m/z 363 to 121, 367 to 121, 366 to 121, 361 to 163, and 364 to 164, respectively. This approach enabled simultaneous monitoring of both the protonated parent ion and fragmented daughter ion, thereby increasing specificity. The relative extraction efficiency was ~97%. Lean body mass was measured using dual-energy X-ray absorptiometry (DPX-IQ scanner, SmartScan Version 4.6; Hologic, Waltham, MA).

Calculations. Splanchnic plasma flow was calculated by dividing the indocyanine green infusion rate by the arterial hepatic venous concentration gradient of the dye. Calculation of splanchnic net cortisol balance, cortisol uptake, and cortisol production has been previously described in detail (7). In brief, net cortisol and D₄ cortisol balance were calculated by multiplying the arterial venous difference in cortisol and D₄ cortisol concentrations across the splanchnic bed by blood flow. Cortisol and D₃ cortisol uptake was calculated by multiplying the fractional extraction of D₄ cortisol across the splanchnic bed times the product of blood flow and either cortisol or D₃ cortisol concentration. Cortisol and D₃ cortisol production were calculated by subtracting cortisol or D₃ cortisol uptake from net balance. Total-body cortisol and D₃ cortisol production were calculated by dividing the D₃ cortisol infusion rate by plasma D₃ cortisol enrichment or the plasma ratio of D₃ cortisol to D₄ cortisol.

Statistical analysis. Data in the text and figures are expressed as means ± SE. Rates of cortisol production are expressed as micrograms per minute. Values from ~30 to 0 min were means and considered as basal. Areas above basal were calculated using the trapezoidal rule. Student’s paired and nonpaired t tests were used to compare results within and between groups, respectively. P < 0.05 was considered statistically significant.

RESULTS

Femoral artery and hepatic venous glucose and insulin concentrations. Fasting hepatic venous glucose concentrations were greater (P < 0.01) than femoral artery glucose concentrations on both the meal (5.7 ± 0.1 vs. 5.3 ± 0.1 mmol/l) and saline (5.8 ± 0.2 vs. 5.4 ± 0.1 mmol/l) study days, indicating net release of glucose from the splanchnic bed (Fig. 1). The difference between the hepatic venous and femoral arterial glucose concentration became greater during the 60–90 min after ingestion of the carbohydrate-containing meal, indicating net release of glucose from the splanchnic bed. The difference between the hepatic venous and femoral arterial glucose concentration decreased thereafter, returning to preprandial values by approximately 4 h. On the other hand, both hepatic venous and femoral arterial glucose concentrations remained essentially constant on the saline study day throughout the 6 h of observation.

Fasting hepatic venous insulin concentrations were greater (P < 0.01) than femoral artery insulin concentrations on both the meal (58 ± 8 vs. 40 ± 6 pmol/l) and saline (68 ± 14 vs. 40 ± 7 pmol/l) study days, indicating net release of insulin from the splanchnic bed (Fig. 1). This difference increased as insulin secretion increased during the 60 min after meal ingestion, then decreased over the succeeding 2 h as insulin secretion decreased. Hepatic venous insulin concentrations fell more rapidly than femoral artery insulin concentrations presumably because of the slower clearance of insulin from the larger systemic pool. On the other hand, hepatic venous and arterial insulin concentrations decreased minimally on the saline study day, with the former remaining higher than the latter throughout the 6 h of study.

Femoral artery and hepatic venous cortisol, D₄ cortisol, and D₃ cortisol concentrations. Fasting hepatic venous and femoral artery cortisol concentrations did not differ on either the meal (7.1 ± 0.5 vs. 7.0 ± 0.5 µg/dl) or saline (6.0 ± 0.5 vs. 6.4 ± 0.8 µg/dl) study days, indicating that there was no net release of cortisol from the splanchnic bed (Fig. 2). Hepatic venous and femoral artery cortisol concentrations continued to fall as glucose concentrations rose during the first hour after meal ingestion. Both hepatic venous and femoral arterial cortisol began to rise 60–90 min after meal ingestion, coincident with the fall in glucose concentrations back toward basal levels. Hepatic venous and...
Femoral arterial cortisol concentrations also fell during the first few hours of the saline study day, then remained essentially constant thereafter. Of note, the femoral arterial to hepatic venous cortisol gradient did not increase after meal ingestion, indicating that the rise in plasma cortisol concentrations was not due to an increased release of cortisol from the splanchnic bed.

Hepatic venous $D_4$ cortisol concentrations were consistently lower ($P < 0.001$) than femoral artery $D_4$ cortisol concentrations on both the meal and saline study days, indicating extraction of cortisol by the splanchnic bed. In contrast, hepatic venous $D_3$ cortisol concentrations were consistently higher ($P < 0.01$) than femoral artery $D_3$ cortisol concentrations, indicating splanchnic synthesis of cortisol via the 11$\beta$-HSD type 1 pathway. The femoral arterial to hepatic venous gradients of $D_4$ cortisol and $D_3$ cortisol concentrations remained constant over the 6 h of observation on both the meal and saline study days, indicating that meal ingestion did not alter net splanchnic $D_4$ cortisol or $D_3$ cortisol release.

**Femoral artery and hepatic venous cortisone and $D_3$ cortisone concentrations.** Fasting hepatic venous cortisone concentrations were lower ($P < 0.001$) than femoral artery cortisone concentrations on both the meal (0.27 ± 0.2 vs. 1.6 ± 0.2 μg/dl) and saline (0.11 ± 0.1 vs. 1.2 ± 0.2 μg/dl) study days, indicating extensive splanchnic cortisone extraction (Fig. 3). Femoral artery cortisone concentrations continued to fall during the first hour after meal ingestion then tended ($P = 0.06$) to increase during the following hour, coincident with the increase in femoral artery cortisol concentration. In contrast, femoral artery cortisone concentration remained essentially flat from 60 min onward on the saline study day. Hepatic venous cortisone concentrations were persistently low throughout the meal and saline study days.

Fasting hepatic venous $D_3$ cortisone concentrations were lower ($P < 0.001$) than femoral artery $D_3$ cortisone concentrations on both the meal (0.02 ± 0.01 vs. 0.24 ± 0.03 μg/dl) and saline (0.1 ± 0.1 vs. 0.34 ± 0.1 μg/dl) study days, indicating extensive splanchnic uptake of $D_3$ cortisone. There was no suggestion of a change in femoral artery or hepatic venous $D_3$ cortisone concentrations on either the meal or saline study days, consistent with an unchanged rate of conversion of the infused $D_4$ cortisol to $D_3$ cortisone.

**Femoral artery $D_4$ cortisol enrichment and $D_4$ cortisol-to-$D_3$ cortisol ratio.** Fasting femoral artery $D_4$ cortisol enrichment did not differ on the meal and saline study days (Fig. 4). Femoral artery $D_4$ cortisol enrichment changed minimally during the first hour after meal ingestion, then fell slightly during the next 2 h, increasing back toward basal thereafter.

![Image](https://example.com/fig1.png)  
**FIG. 1.** Femoral arterial and hepatic venous concentrations of glucose and insulin observed on the meal study (**left panels**) and intravenous saline study (**right panels**). A mixed meal was ingested at time 0 on the meal study day.
The fasting femoral artery D₄ cortisol–to–D₃ cortisol ratio also did not differ on the meal and saline study day. The femoral artery D₄ cortisol–to–D₃ cortisol ratio remained essentially constant on the saline study day but fell on the meal study day particularly during the first 2 h after meal ingestion.

**Total-body and splanchnic cortisol and D₃ cortisol production.** Basal rates of total-body cortisol production did not differ on the meal and saline study days (Fig. 5). Total-body cortisol production fell slightly during the first hour after meal ingestion, then increased (P < 0.05) during the succeeding 2 h. In contrast, splanchnic cortisol production tended to fall throughout the entire period of observation, with no evidence of an increase after meal ingestion. Both total-body and splanchnic cortisol production on the saline study day decreased slightly during the first hour of observation then remained essentially constant thereafter.

Basal rates of total-body D₃ cortisol production also did not differ on the saline and meal study days. Total-body D₃
cortisol production did not change over time on the saline study day but increased \((P < 0.01)\) on the meal study day, reaching a peak \(\sim 3\) h after meal ingestion. In contrast, splanchnic \(D_3\) cortisol production did not differ on the saline and meal study days and did not differ before or after meal ingestion.

**DISCUSSION**

Cortisol is synthesized by the adrenal glands and is then released into the systemic circulation. Once in the systemic circulation, cortisol can be inactivated by conversion to cortisone by \(11\beta\)-HSD type 2, an enzyme that is located in numerous tissues, including the kidney, vascular endothelium, and the brain (1–6,10). Cortisone can be further metabolized via \(5\alpha\)-reductase or converted back to cortisol by \(11\beta\)-HSD type 1 (5,22–26). The present data confirm previous reports that a substantial amount of cortisol is produced within the splanchnic bed of humans via the \(11\beta\)-HSD type 1 pathway (7,8). They extend these data by demonstrating that rates of splanchnic cortisol production are not altered by nutrient ingestion or the associated changes in the hormonal milieu. They also indicate that the previously reported (16) postprandial increase in plasma cortisol concentration is due to an increase in extrasplanchnic rather than splanchnic cortisol production, implying differential regulation of these two processes.

In the present study, ingestion of the meal resulted in a doubling of hepatic venous glucose concentrations and an eightfold increase in hepatic insulin concentrations, reflecting an even greater increase in portal venous concentrations. We presume that the concentration of a variety of incretin hormones also increased since the meal contained protein and fat as well as carbohydrate. While we were able to detect a small but measurable increase in total-body cortisol production after meal ingestion, there was no evidence of an increase in either splanchnic cortisol or \(D_3\) cortisol production, thus arguing against an acute effect of insulin and other postprandial hormones on splanchnic \(11\beta\)-HSD type 1 flux. There also was no evidence of a change in leg cortisol or \(D_3\) cortisol release, which—consistent with our previous report (7)—was negligible on both study days (data not shown). As discussed in detail elsewhere, the current approach measures flux across the entire splanchnic bed (7). Therefore, we cannot determine whether cortisone-to-cortisol conversion occurred in gut, visceral fat, and/or the liver. In theory, insulin and other postprandial hormones could acutely increase cortisone-to-cortisol conversion in one tissue and acutely decrease by the same amount conversion in another tissue. Since

![FIG. 3. Femoral arterial and hepatic venous concentrations of cortisone and \(D_3\) cortisone observed on the meal study (left panels) and intravenous saline study (right panels). A mixed meal was ingested at time 0 on the meal study day.](image-url)
ethical considerations preclude sampling of portal venous blood in humans for research purposes, studies in animals likely will be required to rule out what we believe is a remote possibility.

Benedict et al. (16) have recently reported that intraduodenal amino acid administration increased plasma cortisol concentration, whereas plasma cortisol concentration remained unchanged after intravenous amino acid infusion, indicating that cortisol metabolism is modulated by an intestinally derived signal. In those experiments, intraduodenal infusion of amino acids also increased plasma ACTH, suggesting involvement of the pituitary hypothalamic axis (16). Previous studies have shown that gastric inhibitory polypeptide receptors are present in the adrenal and may account for meal-induced hypercortisolemia in some forms of adrenal hyperplasia (27). In addition, cortisol concentrations increase during intravenous infusion of glucagon-like peptide 1 (28,29). The present data, taken together with those of Benedict et al., lend further support to the concept that nutrient ingestion, either directly or indirectly (e.g., by stimulating incretin or ACTH release), is a physiologic regulator of adrenal function.

Of note, plasma cortisol concentrations were slightly but not significantly higher before study in the meal than in the saline study groups. Since the subjects were randomly assigned to the groups and since all studies were identical, we suspect this was due to chance alone. This slightly higher baseline may have led to an underestimation of the subsequent postprandial increase in plasma cortisol concentration. It is possible that differences in plasma estrogen in the women could have blurred changes in 11β-HSD activity. However, we doubt if this is the case since no women were on

FIG. 4. Arterial plasma $d_{4}$ cortisol enrichment and plasma $d_{4}$ cortisol-to-$d_{3}$ cortisol ratio observed on the meal study and intravenous saline study. A mixed meal was ingested at time 0 on the meal study day.
estrogen replacement, all but two were postmenopausal, and the two women who were menstruating were equally distributed between the meal and saline groups.

$D_3$ Cortisone derived from the infused $D_4$ cortisol can be converted to $D_3$ cortisol via the $11\beta$-HSD type 1 pathway (15). Therefore, once steady state is reached, an increase in the rate of appearance of $D_3$ cortisol indicates an increase in $11\beta$-HSD type 1 flux. Increased conversion of $D_3$ cortisone to $D_3$ cortisol could be caused by increased precursor availability, increased activity of the enzyme, or a combination of both. As is evident in Fig. 5, $D_3$ cortisol production increased after ingestion of the mixed meal but did not increase over the same interval of time on the saline study day. This was not due to an increase in precursor availability since arterial $D_3$ cortisone concentrations did not differ on the meal and saline study days (Fig. 3). Furthermore, splanchnic $D_3$ cortisol production did not increase and did not differ on the two study days. This indicates that the increase in $11\beta$-HSD type 1 flux did not originate in the splanchnic bed. Numerous tissues, including fat and muscle, possess $11\beta$-HSD type 1 activity (1–6). Presumably, if the increase in total-body production was caused by an increase in cortisone-to-cortisol conversion within either fat or muscle, we would have detected increased release from the leg, which we did not. This leaves open the intriguing possibility that meal ingestion increases flux in other extrasplanchnic tissues that possess $11\beta$-HSD type 1 activity (e.g., the brain). It also leaves open the perhaps more important question as to what, if any, is the biologic significance of this postprandial increase in cortisol production.

In summary, the present data indicate that the postprandial increase in plasma cortisol concentrations that occur after ingestion of a mixed meal was due to an increase in extrasplanchnic cortisol production. In contrast, ingestion of a mixed meal and its associated changes in the hormonal milieu did not alter splanchnic cortisol or splanchnic $D_3$ cortisol production, thus arguing against an acute effect of insulin and/or incretin hormones on splanchnic $11\beta$-HSD flux. The effect, if any, of the postprandial increase in total-body cortisol production and tissue-specific cortisone-to-cortisol conversion on postprandial nutrient metabolism remains to be determined.

FIG. 5. Rates of total-body cortisol production (upper left panel), splanchnic cortisol production (upper right panel), total-body $D_3$ cortisol production (lower left panel), and splanchnic $D_3$ cortisol production (lower right panel) observed after ingestion of a mixed meal or intravenous infusion of saline.
ACKNOWLEDGMENTS
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