

Splanchnic Cortisol Production Occurs in Humans

Evidence for Conversion of Cortisone to Cortisol Via the 11- β Hydroxysteroid Dehydrogenase (11 β -HSD) Type 1 Pathway

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Glucocorticoids are potent regulators of protein, fat, and carbohydrate metabolism. To determine if cortisol production occurs within the splanchnic bed in humans, 11 nondiabetic subjects were studied using the hepatic/leg catheterization method along with an infusion of [9,11,12,12-²H₄] cortisol (D4-cortisol) as proposed by Andrews et al. In the fasting state, there was net release ($P < 0.05$) of cortisol from the splanchnic bed (6.1 ± 2.6 $\mu\text{g}/\text{min}$) and net uptake ($P < 0.05$) by the leg (1.7 ± 0.7 $\mu\text{g}/\text{min}$). This, along with cortisol production by other tissues (e.g., the adrenals), resulted in a total-body cortisol appearance rate of 18.1 ± 1.9 $\mu\text{g}/\text{min}$. Fractional splanchnic D4-cortisol extraction averaged $12.9 \pm 1.3\%$ ($P < 0.001$), splanchnic cortisol uptake 14.8 ± 2.0 $\mu\text{g}/\text{min}$ ($P < 0.001$), and splanchnic cortisol production 22.2 ± 3.3 $\mu\text{g}/\text{min}$ ($P < 0.001$). On the other hand, fractional leg D4-cortisol extraction averaged $5.6 \pm 1.8\%$ ($P < 0.02$), leg cortisol uptake 2.3 ± 0.7 $\mu\text{g}/\text{min}$ ($P < 0.01$), and leg cortisol production 0.4 ± 0.4 $\mu\text{g}/\text{min}$, which did not differ from zero. Because D4-cortisol loses a deuterium during conversion to [9,12,12-²H₃] cortisone (D3-cortisone), which in turn generates [9,12,12-²H₃] cortisol (D3-cortisol) via 11 β hydroxysteroid dehydrogenase (11 β -HSD) type 1, D3-cortisol production can be used as an index of 11 β -HSD type 1 activity. Net splanchnic D3-cortisol release (3.9 ± 0.4 $\mu\text{g}/\text{min}$) and splanchnic D3-cortisol production (7.1 ± 0.7 $\mu\text{g}/\text{min}$) occurred ($P < 0.01$) in all subjects. In contrast, there was minimal leg D3-cortisol production (0.04 ± 0.01 $\mu\text{g}/\text{min}$), resulting in a strong correlation between splanchnic D3-cortisol production and total-body D3-cortisol production in both the fasting state ($r = 0.84$; $P < 0.02$) and during an infusion of insulin ($r = 0.97$; $P < 0.01$). Thus, splanchnic production of cortisol occurs in nondiabetic humans at rates approximating that which occurs in the remainder of the

body. These data support the possibility that alterations in splanchnic cortisol production contribute to visceral fat accumulation and the hepatic insulin resistance of obesity or type 2 diabetes. *Diabetes* 53:2051–2059, 2004

Both obesity (particularly visceral) and type 2 diabetes are associated with insulin resistance (1–5). The cause(s) of these abnormalities is an area of active investigation. The recent observation that knockout (6,7) or inhibition (8,9) of 11 β hydroxysteroid dehydrogenase type 1 (11 β -HSD-1) in mice improves hepatic insulin action and protects against obesity and hyperglycemia, whereas selective overexpression of 11 β -HSD-1 in adipose tissue results in visceral obesity, hyperglycemia, and hyperlipidemia (10), has focused attention on the possibility that tissue-specific conversion of cortisone to cortisol can cause or exacerbate visceral obesity and insulin resistance.

It has long been known that 11 β -HSD is an important regulator of cortisol metabolism (11,12). More recently, it was recognized that there are at least two isoforms of this enzyme, with 11 β hydroxysteroid dehydrogenase type 2 (11 β -HSD-2) primarily converting cortisol to cortisone and 11 β -HSD-1 primarily converting cortisone to cortisol (13–19). 11 β -HSD-2 is expressed in the kidney, whereas 11 β -HSD-1 is present in both the liver and adipose tissue, with activity being higher in omental than subcutaneous fat (13,20–22). In vitro studies have reported that insulin increases 11 β -HSD-1 activity (20,22) in fat but decreases activity in the liver (23), perhaps accounting for the increase in omental but decrease in hepatic 11 β -HSD-1 activity in obese Zucker rats (24).

Although the above studies have clearly established that 11 β -HSD-1 is present in liver and fat, the magnitude, site, and importance of tissue-specific cortisol production in humans are not known. This is in large part because indirect methods such as the change in plasma cortisol concentration after ingestion of cortisone acetate (decreased by obesity and type 2 diabetes) (17,18,25,26), ratios of various urinary metabolites of cortisol and cortisone (no different in type 2 diabetic and nondiabetic subjects) (27), or the effects of carbenoxolone (a nonselective combined 11 β -HSD-1 and 11 β -HSD-2 inhibitor) have been used to assess 11 β -HSD-1 activity (28,29).

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11 β -HSD-1, 11 β hydroxysteroid dehydrogenase type 1; 11 β -HSD-2, 11 β hydroxysteroid dehydrogenase type 2; D3-cortisol: [9,12,12-²H₃] cortisol; D4-cortisol, [9,11,12,12-²H₄] cortisol.

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TABLE 1
Subject characteristics

<i>n</i>	11
Age (years)	56 ± 4
BMI (kg/m ²)	31 ± 2
Body fat (%)	40 ± 4
Fat-free mass (kg)	46 ± 3
Visceral fat (cm ²)	152 ± 31
Total abdominal fat (cm ²)	377 ± 63

Data are means ± SE.

However, Andrew et al. (30), using a novel cortisol tracer method, recently established that conversion of cortisone to cortisol occurs in humans. These authors demonstrated that infusion of [9,11,12,12-²H₄] cortisol (D4-cortisol) in fasting nondiabetic humans resulted in the formation of measurable amounts of plasma [9,12,12-²H₃] cortisol (D3-cortisol). Because conversion of D4-cortisol to D3-cortisone by 11β-HSD-2 results in the loss of the 11α deuterium and the generation of D3-cortisone that in turn forms D3-cortisol when D3-cortisone is converted back to cortisol, this observation provided strong experimental evidence of measurable 11β-HSD-1 activity in humans (30). However, neither the site nor the contribution of tissue-specific conversion of cortisone to cortisol to total-body cortisol production was determined in those studies.

The present experiments sought to address these questions by combining the D4-cortisol infusion method with the hepatic venous and leg catheterization techniques. Splanchnic and leg cortisol balance as well as generation of D3-cortisol from D4-cortisol were measured in the fasting state to determine the contribution of these tissues to total-body cortisol production and during insulin infusion to determine whether rates of tissue-specific cortisol production can be altered. We report that the splanchnic bed produces cortisol at rates approximating that of the remainder of the body. Splanchnic cortisol production exceeds uptake after an overnight fast but not during a hyperinsulinemic clamp, resulting in net release only during the former. There was no detectable cortisol production by the leg and minimal evidence of conversion of D4-cortisol to D3-cortisol within the leg. In addition, rates of splanchnic D3-cortisol production were highly corre-

lated with total-body D3-cortisol production, implying that conversion of cortisone to cortisol via 11β-HSD-1 occurs primarily in the splanchnic bed. These data indicate that substantial cortisol production occurs within the splanchnic bed in nondiabetic humans. They also suggest that the role of splanchnic cortisol production in the pathogenesis of visceral obesity and its associated insulin resistance warrants further study.

RESEARCH DESIGN AND METHODS

After approval from the Mayo Institutional Review Board, 11 nondiabetic subjects gave informed written consent to participate in the study. All subjects were in good health and at a stable weight. None regularly engaged in vigorous physical exercise. None of the first-degree relatives of the nondiabetic subjects had a history of diabetes. Subjects were on no medications at the time of study other than thyroxine. All subjects were instructed to follow a weight maintenance diet containing 55% carbohydrate, 30% fat, and 15% protein for at least 3 days before the day of study. Subject characteristics are given in Table 1.

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. After sampling blood for baseline enrichment, 1.67 mg/kg body wt of ²H₂O was given in three divided doses at 1800, 2000, and 2200 as part of a separate protocol examining the effects of insulin on gluconeogenesis.

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 D4-cortisol (0.22 mg prime, 0.19 mg/h continuous; Cambridge Isotope Laboratories, Andover, MA) was started into a forearm vein, and at ~0700, a primed continuous infusion of [3-³H]glucose (12 μCi prime, 0.12 μCi/min continuous; New England Nuclear, Boston, MA) was also started as part of a separate protocol. Subjects were taken to an interventional radiology suite where femoral artery, femoral venous, and hepatic venous catheters were placed as previously described (3,31). At ~0930 (-60 min), an infusion of indocyanine green dye (Akorn, Buffalo Grove, IL) was started via the arterial sheath. The venous catheters and the arterial catheter were used for blood sampling.

Infusions of somatostatin (60 ng · kg⁻¹ · min⁻¹) and growth hormone (3 ng · kg⁻¹ · min⁻¹) also were started at ~1000 (time 0 min) and continued until the end of the study. Insulin was infused at a rate of 0.5 mU · kg lean body wt⁻¹ · min⁻¹ from 0 to 240 min. A dextrose infusion also was begun, and the rate was adjusted to maintain plasma glucose concentrations at ~5.0 mmol/l over the next 4 h of study.

Analytical techniques. All 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 (32). All other samples were stored at -20°C until analysis. Plasma glucose was measured by a glucose oxidase method using a YSI glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using

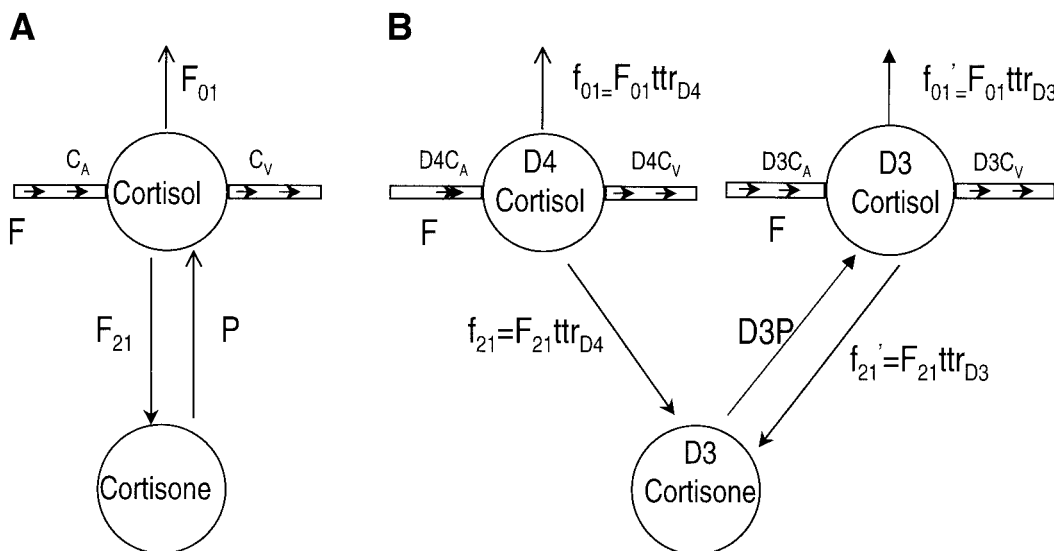


FIG. 1. Description of the model of cortisol metabolism, regional (A) and tracer (B).

a chemiluminescence method with the Access Ultrasensitive Immunoenzymatic assay system (Beckman, Chaska, MN). Plasma C-peptide and glucagon concentrations were assayed by radioimmunoassay (RIA; Linco Research, St. Louis, MO). Growth hormone was measured with the Access hGH two-site immunoenzymatic assay (Beckman).

Hepatic venous, femoral artery and femoral venous cortisol, D4-cortisol, and D3-cortisol concentrations were measured using a liquid chromatography tandem mass spectrometer as previously described (33). In brief, methylprednisolone 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, and D3-cortisol ions were generated with an 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, and 366 to 121, respectively. This approach enabled simultaneous monitoring of both the protonated parent ion and fragmented daughter ion, thereby increasing specificity. Body composition and lean body mass were measured using dual-energy X-ray absorptiometry (DPX-IQ scanner; Hologic, Waltham, MA; SmartScan version 4.6).

Calculations. The model of Fig. 1A was used to describe cortisol kinetics at a regional level. At steady state, the net cortisol balance across the region is the difference between regional cortisol uptake (U) because of the conversion to cortisone (F_{21}) and irreversible loss (F_{01}), and regional cortisol production (P):

$$\Phi(C_a - C_v) = U - P = F_{01} + F_{21} - P \quad (1)$$

where Φ is blood flow across the region, and C_a and C_v are arterial and venous cortisol concentrations.

The model of tracer cortisol kinetics is shown in Fig. 1B: D4-cortisol (D4C) loses one carbon atom in the conversion to D3 cortisone, which can be converted to D3-cortisol (D3C) via 11 β -HSD-1. Mass balance equations for D4-cortisol and D3-cortisol are as follows:

$$\Phi(D4C_a - D4C_v) = f_{01} + f_{21} \quad (2)$$

$$\Phi(D3C_a - D3C_v) = f_{01}' + f_{21}' - D3P \quad (3)$$

where D3P is the regional production of D3-cortisol, f_{01} and f_{01}' are irreversible loss of D4-cortisol and D3-cortisol, respectively, f_{21} and f_{21}' are the interconversion of D4-cortisol and D3-cortisol, respectively, to tracer D3-cortisone. The tracer-tracee indistinguishability principle provides the link between tracee and tracer fluxes.

$$f_{01}/F_{01} = ttr_{D4} \quad f_{21}/F_{21} = ttr_{D4} \quad (4)$$

$$f_{01}'/F_{01}' = ttr_{D3} \quad f_{21}'/F_{21}' = ttr_{D3} \quad (5)$$

where ttr_{D4} and ttr_{D3} denote the arterial tracer-to-tracee ratio of D4-cortisol and D3-cortisol, respectively.

By using equalities of Eq. 4, Eq. 2 becomes:

$$\Phi(D4C_a - D4C_v) = (F_{01} + F_{21})ttr_{D4} \quad (6)$$

By using equalities of Eq. 5, Eq. 3 becomes:

$$\Phi(D3C_a - D3C_v) = (F_{01}' + F_{21}')ttr_{D3} - D3P \quad (7)$$

Eq. 6 provides an expression for regional cortisol uptake (U):

$$U = F_{01} + F_{21} = \frac{\Phi(D4C_a - D4C_v)}{ttr_{D4}} \quad (8)$$

By using Eq. 8 into Eq. 1, the expression for regional cortisol production (P) is derived:

$$P = \frac{\Phi(D4C_a - D4C_v)}{ttr_{D4}} - \Phi(C_a - C_v) \quad (9)$$

By using Eq. 8 into Eq. 7, the expression for regional D3-cortisol production D3P is derived:

$$D3P = \frac{\Phi(D4C_a - D4C_v)}{ttr_{D4}} ttr_{D3} - \Phi(D3C_a - D3C_v) \quad (10)$$

Application of Eqs. 8–10 at splanchnic level, using hepatic vein measurements of C_v and D3C $_v$ and the median of quadruple determinations of splanchnic blood flow (see below) for Φ , enables calculation of splanchnic cortisol uptake and production and of splanchnic D3-cortisol production. Similarly, their application at leg level, by using femoral vein measurements of C_v and D3C $_v$ and the median of quadruple determinations of leg blood flow for Φ , enables calculation of leg cortisol uptake and production and of leg D3-cortisol production.

Splanchnic plasma flow was calculated by dividing the indocyanine green infusion rate by the arterial hepatic venous concentration gradient of the dye, and leg plasma flow was calculated by dividing the dye infusion rate by the concentration gradient across the leg (32,34). The corresponding blood flows were calculated by dividing the respective plasma flow by (1 – hematocrit).

Finally, whole-body cortisol (P_{WB}) and D3-cortisol production ($D3P_{WB}$) were calculated as follows:

$$P_{WB} = \frac{F_{D4}}{ttr_{D4}} \quad (11)$$

$$D3P_{WB} = \frac{F_{D4}}{D4Ca/D3Ca} \quad (12)$$

where F_{D4} is the infusion rate of D4-cortisol.

Statistical analysis. Data in the text and figures are expressed as means \pm SE. Rates are expressed as micromoles per minute. Responses during the period before insulin infusion and during the insulin infusion were determined by averaging the results present from –30 to 0 min and from 210 to 240 min, respectively. Student's paired t test was used to determine if rates differed from zero and if rates differed before and after the insulin infusion. A P value of <0.05 was considered statistically significant.

RESULTS

Plasma glucose, insulin, and C-peptide concentrations. Fasting plasma glucose concentration averaged 5.2 ± 0.1 mmol/l before the insulin infusion and was maintained constant at 5.1 ± 0.1 mmol/l during the insulin infusion (Fig. 2A). Fasting plasma insulin concentrations averaged 37 ± 9 pmol/l before the insulin infusion and increased to 133 ± 11 pmol/l during the insulin infusion (Fig. 2B). Fasting C-peptide (Fig. 2C) concentrations averaged 0.46 ± 0.1 nmol/l before the insulin infusion and were suppressed to 0.03 ± 0.0 nmol/l during the insulin infusion, thereby indicating that endogenous insulin secretion was inhibited.

Plasma cortisol, D4-cortisol, and D3-cortisol concentrations. Hepatic venous cortisol concentrations (Fig. 3A) were greater ($P < 0.001$) than femoral artery cortisol concentrations before (9.9 ± 1.2 vs. 9.5 ± 1 μ g/dl) but not during (9.9 ± 2.1 vs. 10.1 ± 2.2 μ g/dl) the insulin infusion. On the other hand, femoral arterial cortisol concentrations were no different than femoral venous concentrations before the insulin infusion (9.5 ± 1 vs. 9.4 ± 1.3 μ g/dl) but were greater ($P < 0.05$) than femoral venous cortisol concentrations during the insulin infusion (9.9 ± 1.2 vs. 9.6 ± 2.0 μ g/dl).

Hepatic venous and femoral venous D4-cortisol concentrations (Fig. 3B) were lower ($P < 0.001$) than femoral arterial D4-cortisol concentrations before (1.6 ± 0.3 vs. 1.7 ± 0.3 vs. 1.8 ± 0.3 μ g/dl, respectively) and during (1.3 ± 0.2 vs. 1.5 ± 0.3 vs. 1.6 ± 0.3 μ g/dl, respectively) the insulin infusion. On the other hand, hepatic venous D3-cortisol concentrations (Fig. 3C) were greater ($P < 0.001$) than femoral arterial D3-cortisol concentrations both before (2.1 ± 0.3 vs. 1.7 ± 0.3 μ g/dl) and during (2.1 ± 0.4 vs. 1.7 ± 0.3 μ g/dl) the insulin infusion. In contrast, femoral arterial D3-cortisol concentrations did not differ from femoral venous D3-cortisol concentrations either before

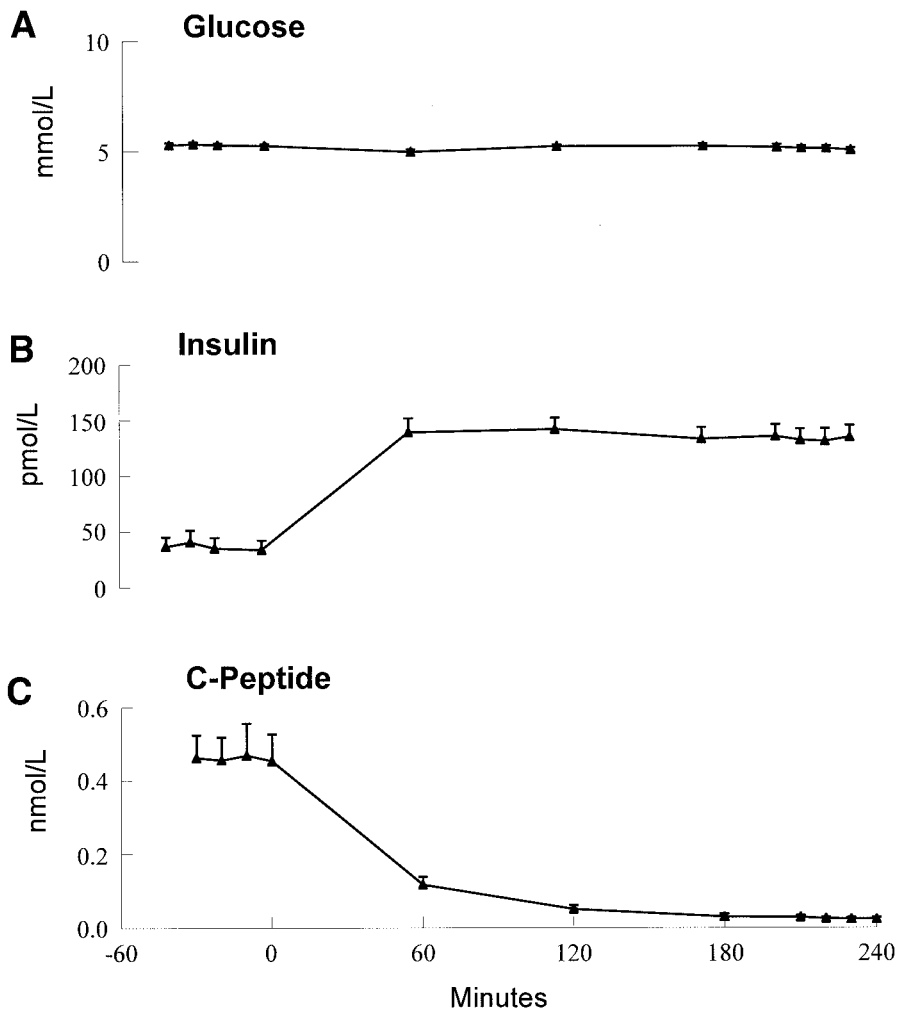


FIG. 2. Plasma glucose (A), insulin (B), and C-peptide (C) concentrations observed before and during infusion of insulin. The insulin infusion was started at time 0.

($1.6 \pm 0.3 \mu\text{g/dl}$) or during ($1.6 \pm 0.3 \mu\text{g/dl}$) the insulin infusion.

Arterial D4-cortisol enrichment and D4-cortisol-to-D3-cortisol ratio. Arterial D4-cortisol enrichment (Fig. 4A) and the arterial D4-cortisol-to-D3-cortisol ratio (Fig. 4B) remained constant before and during the insulin infusion, permitting accurate calculation of total-body cortisol and D3-cortisol production.

Total-body cortisol production, net splanchnic cortisol balance, splanchnic cortisol uptake, and splanchnic cortisol production. Total-body cortisol production (Fig. 5A) averaged $18.1 \pm 1.9 \mu\text{g/min}$ before the insulin infusion and increased slightly but not significantly ($P = 0.3$) to $24.0 \pm 5.2 \mu\text{g/min}$ during the insulin infusion. Splanchnic blood flow did not differ before ($1,376 \pm 105 \text{ ml/min}$) and during ($1,252 \pm 104 \text{ ml/min}$) the insulin infusion. Net splanchnic balance of cortisol (Fig. 5B) averaged $-6.1 \pm 2.6 \mu\text{g/min}$ (i.e., net release; $P < 0.05$ vs. 0) before the insulin infusion and decreased ($P < 0.01$) to rates that no longer differed from zero during the insulin infusion ($0.8 \pm 3.0 \mu\text{g/min}$). Fractional splanchnic D4-cortisol extraction averaged $13 \pm 1\%$ ($P < 0.001$ vs. 0) before the insulin infusion and increased slightly but not significantly to $15 \pm 1\%$ ($P < 0.001$ vs. 0) during the insulin infusion. This resulted in a slight but nonsignificant increase in splanchnic cortisol uptake (Fig. 5D) from $15.0 \pm 2.0 \mu\text{g/min}$ ($P < 0.001$ vs. 0) before the insulin infusion to $18.0 \pm$

$3.4 \mu\text{g/min}$ during the insulin infusion ($P < 0.001$ vs. 0). Splanchnic cortisol production averaged $22.2 \pm 3.3 \mu\text{g/min}$ ($P < 0.001$ vs. 0) before the insulin infusion and decreased slightly but not significantly to $17.2 \pm 3.2 \mu\text{g/min}$ ($P < 0.001$ vs. 0) during the insulin infusion (Fig. 5C).

Leg cortisol balance, leg cortisol uptake, and leg cortisol production. Leg blood flow did not differ before and during the insulin infusion (575 ± 71 vs. $501 \pm 67 \text{ ml/min}$). Net leg cortisol balance averaged $1.7 \pm 0.7 \mu\text{g/min}$ per leg before the insulin infusion ($P < 0.05$ vs. 0) and increased slightly but not significantly ($P = 0.1$) to $2.8 \pm 0.8 \mu\text{g/min}$ per leg ($P < 0.01$ vs. 0) during the insulin infusion (Fig. 6A). Leg D4-cortisol extraction averaged $6 \pm 2\%$ ($P < 0.02$ vs. 0) before the insulin infusion and increased ($P < 0.05$) to $9 \pm 2\%$ ($P < 0.001$ vs. 0) during the insulin infusion. This was associated with leg cortisol uptake of $2.3 \pm 0.7 \mu\text{g/min}$ per leg ($P < 0.001$ vs. 0) before the insulin infusion and a slight but not significant increase ($P = 0.2$) to $3.2 \pm 0.8 \mu\text{g/min}$ per leg ($P < 0.01$ vs. 0) during the insulin infusion (Fig. 6B). On the other hand, leg cortisol production did not differ from zero either before ($0.5 \pm 0.4 \mu\text{g/min}$ per leg) or during ($0.2 \pm 0.2 \mu\text{g/min}$ per leg) the insulin infusion (Fig. 6C).

Splanchnic and leg D3-cortisol balance and D3-cortisol production. There was net release ($P < 0.001$) of D3-cortisol from the splanchnic bed (Fig. 7A) both before ($-3.92 \pm 0.4 \mu\text{g/dl}$) and during ($-3.68 \pm 0.8 \mu\text{g/dl}$) the

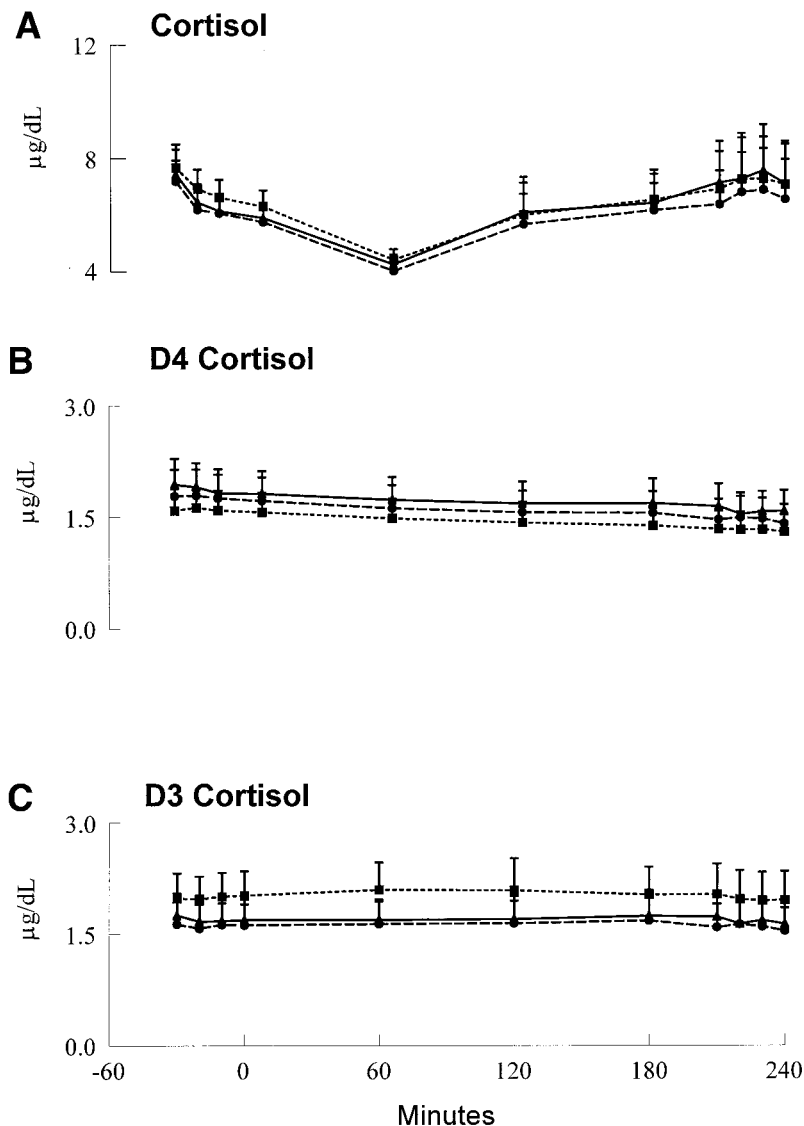


FIG. 3. Plasma cortisol (A), D4-cortisol (B), and D3-cortisol (C) concentrations observed before and during infusion of insulin in the femoral artery (\blacktriangle), hepatic vein (\blacksquare), and femoral vein (\bullet). The insulin infusion was started at time 0.

insulin infusion. On the other hand, there was net uptake of D3-cortisol by the leg (Fig. 7B) both before ($0.4 \pm 0.1 \mu\text{g/dl}$) and during ($0.5 \pm 0.1 \mu\text{g/dl}$) the insulin infusion. Splanchnic D3-cortisol production was present ($P < 0.001$ vs. 0) both before ($7.0 \pm 0.7 \mu\text{g/min}$) and during ($6.6 \pm 1.2 \mu\text{g/min}$) the insulin infusion (Fig. 7C). Leg D3-cortisol production did not differ from zero before ($0.04 \pm 0.08 \mu\text{g/min}$) the insulin infusion but was greater than zero ($P < 0.01$) during ($0.2 \pm 0.05 \mu\text{g/min}$) the insulin infusion (Fig. 7D).

Splanchnic D3-cortisol production was strongly correlated with total-body D3-cortisol production both before ($r = 0.84$; $P < 0.001$) and during ($r = 0.97$; $P < 0.001$) the insulin infusion (Fig. 8). In contrast, leg D3-cortisol production did not correlate with total-body D3-cortisol production either before ($r = 0.14$; $P = 0.7$) or during ($r = 0.33$; $P = 0.35$) the insulin infusion.

DISCUSSION

The present study confirms the previous report by Andrew et al. (30) that D3-cortisol is formed during D4-cortisol infusion, indicating that cortisone is converted to cortisol via the 11β -HSD-1 pathway in nondiabetic humans. It

extends that report by demonstrating that the vast majority of cortisone to cortisol conversion occurs within the splanchnic bed. Perhaps even more remarkable, these data indicate that the rate of production of cortisol within the splanchnic bed is equal to if not greater than that produced by nonsplanchnic tissues (e.g., the adrenals). Infusion of insulin was accompanied by a decrease in net splanchnic cortisol release to rates that no longer differed from zero, thereby establishing that splanchnic cortisol production and/or uptake is not constant. Taken together, these data indicate that substantial local cortisol production occurs within the splanchnic bed of humans.

We are unaware of any previous study that has measured splanchnic cortisol production in humans. The high rates of splanchnic cortisol production observed in the present studies were somewhat surprising. Therefore, it was reassuring that there was consistent evidence of splanchnic cortisol production, whether assessed by simply measuring the arterial to hepatic venous gradient of unlabeled cortisol, by using the fractional splanchnic extraction of D4-cortisol to calculate the actual rate of splanchnic cortisol production, by measuring the arterial

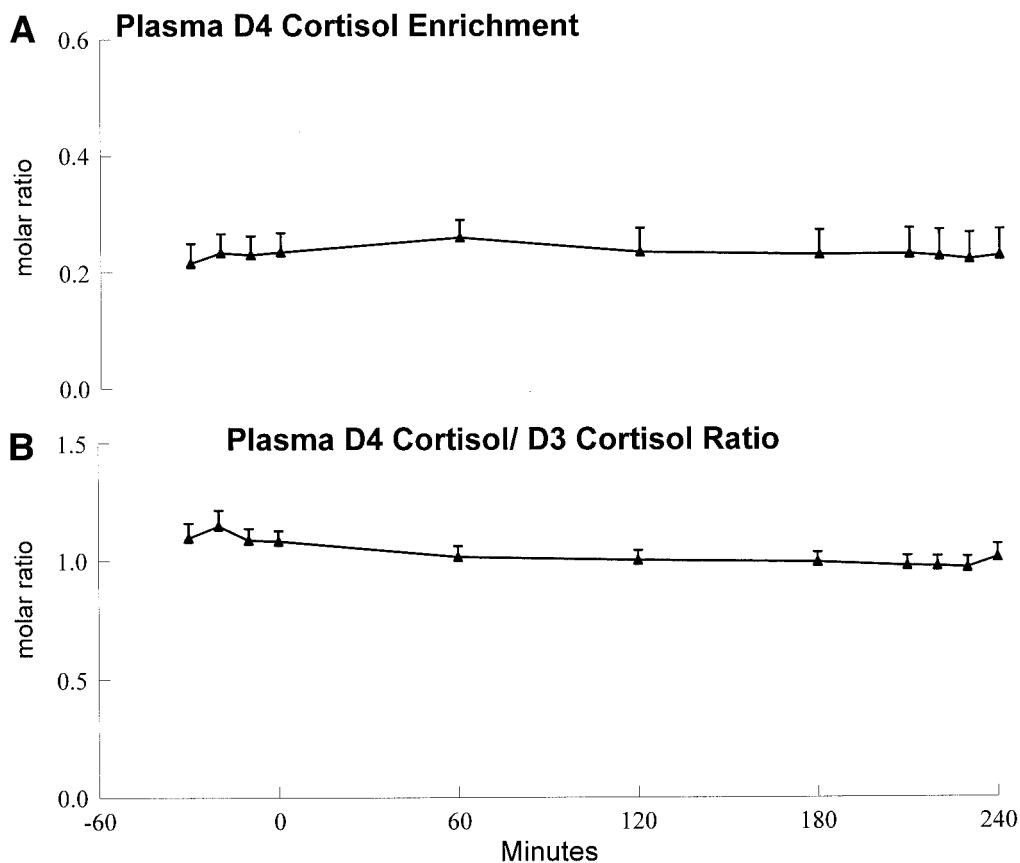


FIG. 4. Arterial plasma D4-cortisol enrichment (A) and D4-cortisol-to-D3-cortisol ratio (B) observed before and during the infusion of insulin. The insulin infusion was started at time 0.

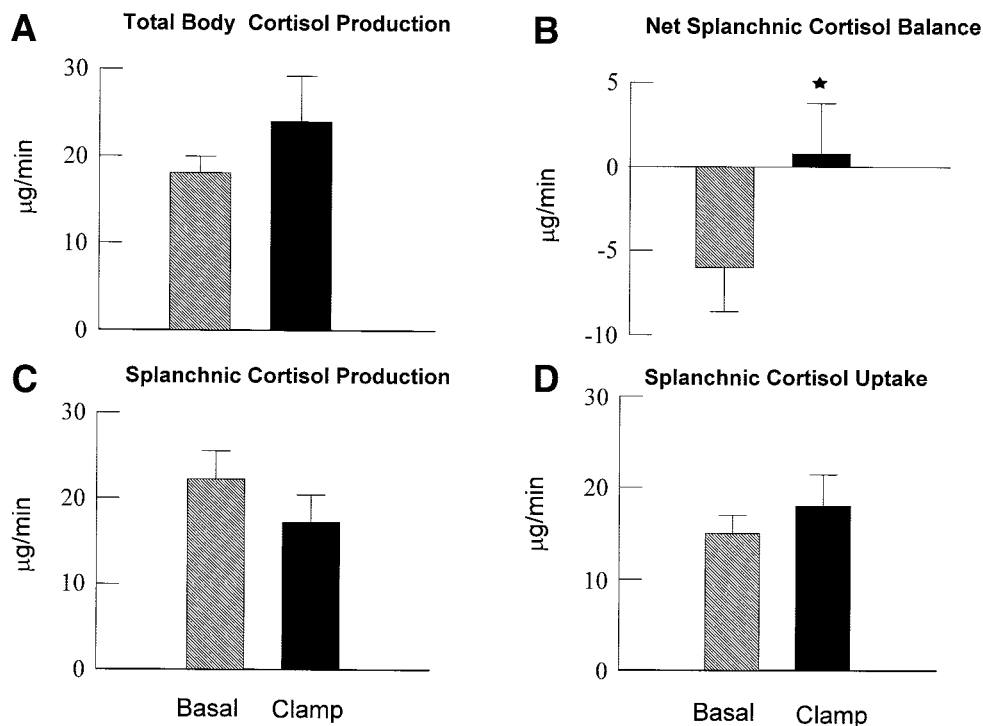


FIG. 5. Rates of total-body cortisol production (A), net splanchnic cortisol balance (B), splanchnic cortisol production (C), and splanchnic cortisol uptake (D) observed after an overnight fast before (basal) and during the final 40 min of a 4-h insulin infusion (clamp). A negative balance indicates net splanchnic cortisol release, whereas splanchnic cortisol production is represented as a positive number. * $P < 0.05$ vs. basal.

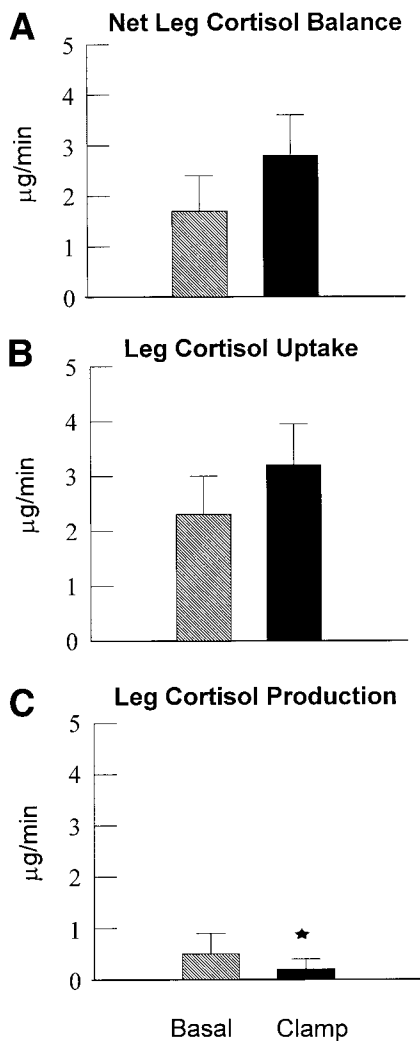


FIG. 6. Net leg cortisol balance (A), leg cortisol uptake (B), and leg cortisol production (C) observed after an overnight fast (basal) and during the final 40 min of a 4-h insulin infusion (clamp). A negative balance indicates net splanchnic cortisol release, whereas splanchnic cortisol production is represented as a positive number. * $P < 0.05$ vs. basal.

to hepatic venous gradient of D3-cortisol, or by measuring the rate of splanchnic D3-cortisol production. Net splanchnic cortisol release, by definition, means that cortisol was being produced within the splanchnic bed. After an overnight fast, net splanchnic cortisol release ($\sim 6 \mu\text{g}/\text{min}$) accounted for approximately one-third of total-body cortisol production ($\sim 18 \mu\text{g}/\text{min}$). However, it should be noted that these measurements were made at a time of day when cortisol production presumably was close to its maximum daily rate (35). Therefore, we do not know whether the relative contribution of splanchnic and non-splanchnic tissues to total-body cortisol production is the same at other times of day, since we do not know if there is a diurnal variation in splanchnic cortisol production.

The tracer data also strongly support splanchnic cortisol production. Calculation of splanchnic cortisol production assumes that deuterated cortisol is cleared at the same rate as unlabeled cortisol. This same assumption is made when any deuterated tracer is used to measure tracee turnover. Perhaps of greater importance from the prospective of the present experiments, slower clearance of D4-cortisol than

unlabeled cortisol would underestimate rather than overestimate splanchnic cortisol production. Furthermore, there was net release of D3-cortisol by the splanchnic bed. D3-cortisol can only be synthesized through the conversion of D3-cortisone to D3-cortisol (30). Therefore, conversion of unlabeled cortisone to unlabeled cortisol also must have been occurring. Because D3-cortisol can be subsequently converted to D3-cortisone by $11\beta\text{-HSD-2}$ or irreversibly degraded via the 5α reductase pathway to other metabolites, net D3-cortisol release underestimates the actual rate of splanchnic D3-cortisol production.

It should be noted that the present experiments do not permit determination of the site (e.g., visceral fat vs. liver) of D3-cortisol synthesis within the splanchnic bed. However, the actual rate of splanchnic D3-cortisol production can still be calculated, since this calculation only assumes that, with the exception that D3-cortisone can be converted back to D3-cortisol (which is the process being measured), once D3-cortisol enters the blood, the metabolism of D3-cortisol and D4-cortisol are the same. Because deuterated water also was given as part of a separate protocol directed at measuring the effects of insulin on gluconeogenesis, in theory, deuterium could be reincorporated onto the fourth carbon, yielding D4-cortisol when D3-cortisone was converted to cortisol via $11\beta\text{-HSD-1}$. If this were to occur, this would underestimate fractional extraction of D4-cortisol and, therefore, both splanchnic cortisol and D3-cortisol production rates. Thus, the above data provide strong experimental evidence that the splanchnic bed converts cortisone, a metabolically inactive precursor, to cortisol, a potent glucocorticoid.

The data regarding cortisol production by the leg are less definitive. In vitro studies have established that peripheral fat also possesses $11\beta\text{-HSD-1}$ activity, albeit at a lower level than that present in visceral fat (20). However, in contrast to the splanchnic bed, there was net uptake of cortisol across the leg. Leg fractional D4-cortisol extraction was approximately one-half of that of the splanchnic bed. This resulted in leg cortisol production rates that did not differ from zero. Net release of D3-cortisol from the leg also was zero. On the other hand, leg D3-cortisol production, while very low, was statistically different from zero, leaving open the possibility that small amounts of cortisone can be converted to cortisol within the leg. However, it remains possible that study of a larger number of subjects with a wider range of obesity may allow detection of leg cortisol production.

Studies in Zucker rats have suggested that insulin increases $11\beta\text{-HSD-1}$ activity in omental fat but decreases $11\beta\text{-HSD-1}$ activity in the liver (24). Because measurement across the splanchnic bed assesses the effect of insulin on both of these tissues, it is perhaps not surprising that there was, at most, a small decrease in splanchnic cortisol production during the insulin infusion. On the other hand, splanchnic uptake tended to increase during the insulin infusion. Although neither of these changes was statistically significant, when combined, they resulted in a significant decrease in net splanchnic cortisol balance to rates that no longer differed from zero. A similar pattern was also observed across the leg. However, none of the changes within the leg, including net balance, were significant. Suppression of splanchnic cortisol release during the insulin

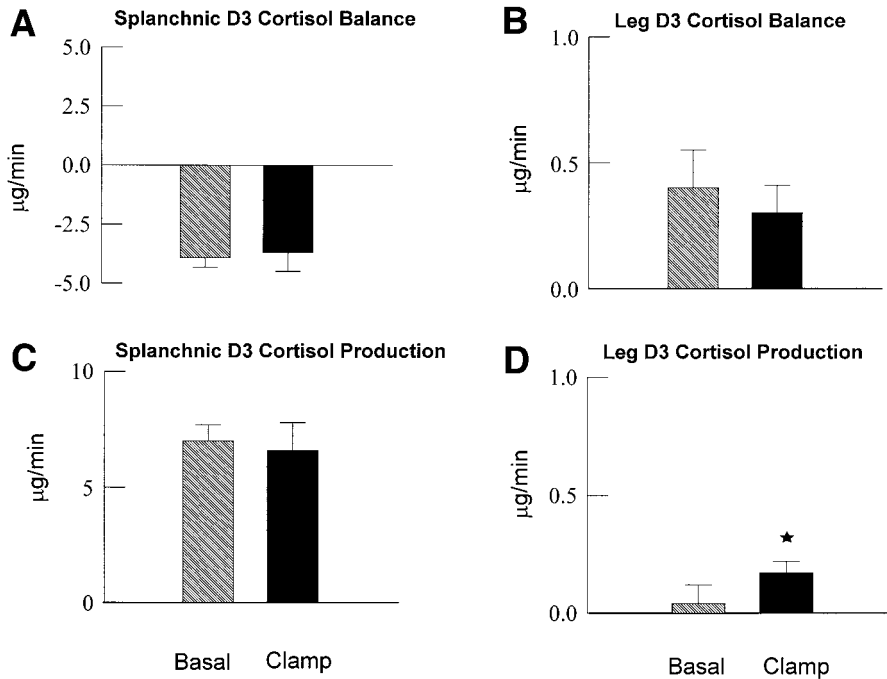


FIG. 7. Net splanchnic D3-cortisol balance (A), net leg D3-cortisol balance (B), splanchnic D3-cortisol production (C), and leg D3-cortisol production (D) observed after an overnight fast before (basal) and during the final 40 min of a 4-h insulin infusion (clamp). A negative balance indicates net splanchnic cortisol release, whereas splanchnic cortisol production is represented as a positive number. **P* < 0.05 vs. basal.

infusion is intriguing because it would suggest that an increase in insulin of a magnitude similar to that which occurs when a carbohydrate-containing meal is eaten for breakfast (36) causes the splanchnic bed to stop releasing cortisol. However, this conclusion remains tentative because control experiments were not performed in which insulin was maintained at basal levels throughout the study. Therefore, the present experimental design cannot distinguish an effect of insulin from that which may have been observed with the passage of time alone. Additional experiments will be required to clarify this issue.

In summary, the splanchnic bed produces cortisol in nondiabetic humans at rates that are equal to if not greater

than those produced by nonsplanchnic tissues (e.g., the adrenals). In addition, rates of splanchnic cortisol production are sufficiently high after an overnight fast and they exceed uptake resulting in net splanchnic release of cortisol. Extensive conversion of D4-cortisol to D3-cortisol occurs within the splanchnic bed indicating that splanchnic cortisol production results, at least in part, from conversion of the inactive precursor cortisone to the active glucocorticoid cortisol via the 11β-HSD-1 pathway (30). In contrast, the leg appears to produce little if any cortisol. Infusion of insulin was accompanied by a decrease in net splanchnic cortisol release, indicating that splanchnic cortisol production and/or uptake are not constant. In view of the potential impact of

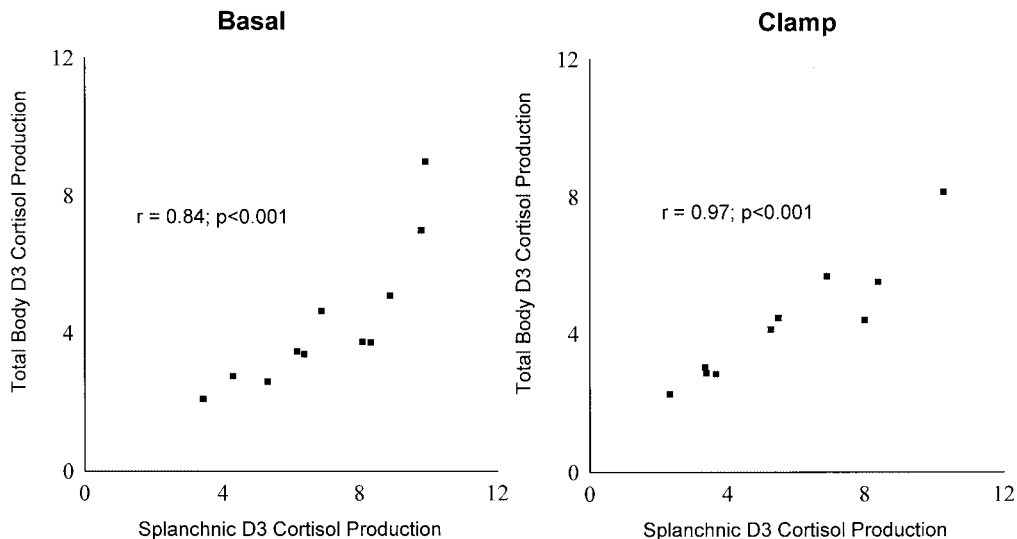


FIG. 8. The correlation observed between splanchnic (x-axis) and total-body (y-axis) D3-cortisol production after an overnight fast (basal) and during the final 40 min of a 4-h insulin infusion (clamp).

splanchnic cortisol production on visceral fat accumulation and hepatic insulin action, future studies examining its role in the pathogenesis of obesity and type 2 diabetes will be of considerable interest.

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