

# Splanchnic Cortisol Production in Dogs Occurs Primarily in the Liver

## Evidence for Substantial Hepatic Specific 11 $\beta$ Hydroxysteroid Dehydrogenase Type 1 Activity

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Eight dogs underwent combined hepatic/portal vein catheterization and infusion of D4-cortisol in order to determine the relative contributions of the viscera and liver to splanchnic cortisol production. D4-cortisol concentrations progressively decreased from  $2.6 \pm 0.1$  to  $2.4 \pm 0.1$  to  $1.7 \pm 0.1$   $\mu\text{g/dl}$  ( $P < 0.001$  by ANOVA) from hepatic artery to portal vein to hepatic vein, respectively, indicating  $8 \pm 3$  and  $28 \pm 3\%$  extraction across the viscera and liver, respectively. On the other hand, hepatic artery, portal vein, and hepatic vein cortisol concentrations did not differ ( $0.31 \pm 0.12$  vs.  $0.28 \pm 0.11$  vs.  $0.27 \pm 0.10$   $\mu\text{g/dl}$ , respectively), indicating zero net cortisol balance. This meant that  $1.0 \pm 0.1$   $\mu\text{g/min}$  of cortisol was produced within the splanchnic bed, all of which occurred within the liver ( $1.2 \pm 0.1$   $\mu\text{g/min}$ ). On the other hand, visceral cortisol production did not differ from zero ( $-0.2 \pm 0.2$   $\mu\text{g/min}$ ;  $P < 0.001$  vs. liver). Flux through the 11 $\beta$  hydroxysteroid dehydrogenase (HSD) type 1 pathway can be measured by determining the rate of conversion of D4-cortisol to D3-cortisol. D3-cortisol concentrations were lower in the portal vein than hepatic artery ( $0.45 \pm 0.03$  vs.  $0.48 \pm 0.02$ , respectively;  $P < 0.01$ ) but did not differ in the portal vein and hepatic vein, indicating net uptake across the viscera but zero balance across the liver. D3-cortisol production with the viscera and liver averaged  $0.2 \pm 0.1$   $\mu\text{g/min}$  ( $P = \text{NS}$  vs. zero production) and  $0.6 \pm 0.1$   $\mu\text{g/min}$  ( $P < 0.001$  vs. zero production;  $P < 0.001$  vs. viscera production), respectively. We conclude that most, if not all, of splanchnic cortisol production occurs within the liver. Taken together, these data suggest that the high local cortisol concentrations generated via the 11 $\beta$  HSD type 1 pathway within the liver likely contribute to the regulation of hepatic glucose, fat, and protein metabolism. *Diabetes* 55:3013–3019, 2006

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HSD, hydroxysteroid dehydrogenase.

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**G**lucocorticoids are potent regulators of carbohydrate, fat, and protein metabolism. Many tissues can convert the inactive metabolite cortisone to the active glucocorticoid cortisol via the 11 $\beta$  hydroxysteroid dehydrogenase (HSD) type 1 pathway (1–3). Overexpression of 11 $\beta$  HSD in fat or liver in mice can cause obesity, hyperglycemia, and hypertension (4–6). Conversely, knockout or inhibition of 11 $\beta$  HSD type 1 protects against obesity, lowers hepatic glucose production, and reduces glucose and triglyceride concentrations in diabetic mice (7–11). Targeted reduction of glucocorticoid receptors in the liver has similar effects, suggesting that it may be normal for these tissues to be exposed to high local concentrations of cortisol (12,13). Taken together, these data suggest that tissue-specific production of cortisol likely is of biologic importance.

We (14–16) and others (17) have recently established that cortisol is produced within the splanchnic bed of humans at rates approximating those that occur in extrasplanchnic tissues (e.g., the adrenals). However, the relative contribution of the various tissues within the splanchnic bed to cortisol production is currently not known. In vitro studies indicate that visceral fat possesses considerable 11 $\beta$  HSD type 1 activity (18–20). If cortisol is produced by visceral fat in humans and if the resultant cortisol is released into the portal circulation, then liver of individuals with large amounts of visceral fat may be exposed to high glucocorticoid concentrations. If so, this could contribute to or cause many of the metabolic abnormalities (e.g., hepatic deposition of fat, elevated plasma triglyceride, increased gluconeogenesis, and hepatic insulin resistance) that are associated with visceral obesity (18,21). On the other hand, since 11 $\beta$  HSD type 1 also is present in the liver, intrahepatic cortisol production could also expose hepatocytes to high local cortisol concentrations (2,3,6,10,12). Therefore, visceral fat and/or the liver could be the site of splanchnic cortisol production.

Andrew et al. (17) recently reported that visceral cortisol production is increased in healthy humans. However, since access to the portal vein is not ethically possible in healthy humans, the method used in those studies was indirect, introducing uncertainty as to the validity of this conclusion. On the other hand, we have reported that splanchnic cortisol production does not differ in lean and

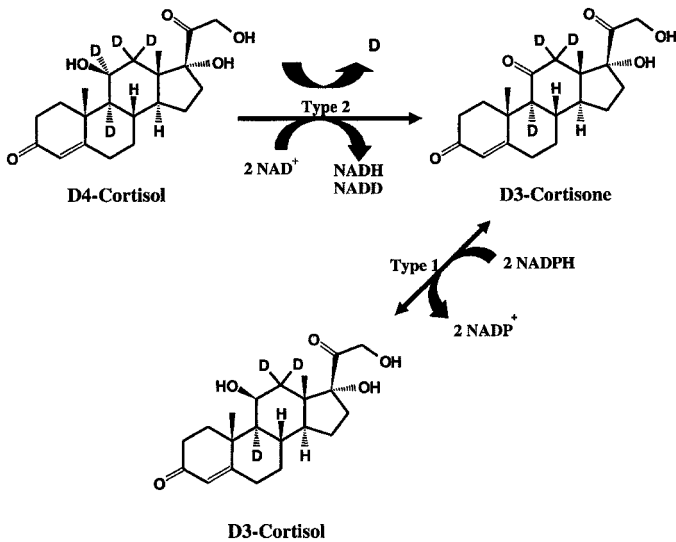


FIG. 1. Schematic representation of D4-cortisol conversion to D3-cortisol via the 11β HSD pathway.

obese nondiabetic humans or in obese diabetic humans despite marked differences in visceral fat (14). Furthermore, Aldhahi et al. (22) reported that peripheral venous and portal venous cortisol concentrations were equal in severely obese humans at the time of bariatric surgery, implying a negligible rate of release of cortisol from visceral fat. However, our studies did not directly measure visceral and hepatic cortisol production so that, at least in theory, increased production of cortisol by visceral fat in the obese individuals could have been offset by decreased production of cortisol by the liver, resulting in no overall change in splanchnic cortisol production (15). Furthermore, the lack of difference in peripheral venous and portal venous cortisol concentrations in the study of Aldhahi et al. (22) could be explained by concurrent uptake and production of cortisol by visceral fat.

Since the site(s) of splanchnic cortisol production has important theoretical and therapeutic implications, the current studies utilized the well-established awake hepatic/portal venous catheterized dog model combined with a D4-cortisol infusion to concurrently measure overall splanchnic, visceral, and hepatic cortisol production and 11β HSD type 1 flux (Fig. 1). We report that cortisol is produced within the splanchnic bed of the dog with the majority of cortisol production occurring within the liver with little, if any, being produced by the viscera. On the other hand, since there is concurrent uptake of cortisol by both the viscera and the liver, cortisol production within the splanchnic bed does not alter the exposure of extra-splanchnic tissues to cortisol. Thus, intrahepatic conversion of cortisone to cortisol via the 11β HSD type 1 pathway appears to result in selective exposure of the liver to high local cortisol concentrations. Taken together, these data suggest that intrahepatic cortisol production likely contributes to the regulation of hepatic glucose, fat, and protein metabolism.

RESEARCH DESIGN AND METHODS

Experiments were conducted on eight 42-h fasted, conscious, lean mongrel dogs of either sex (18–25 kg). Housing and diet have been previously described (23). The surgical facility met the standards published by the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. All dogs underwent a laparotomy 2 weeks before the

experiment in order to implant an infusion catheter into the duodenum (for purposes unrelated to the current study) and sampling catheters into the femoral artery and the portal and left common hepatic veins, as well as Transonic flow probes around the hepatic artery and portal vein, as described elsewhere (23). Each dog was used for only one experiment. All dogs studied were healthy, as indicated by 1) leukocyte count <18,000/mm<sup>3</sup>, 2) a hematocrit value >35%, 3) a good appetite, and 4) normal stools.

Angiocaths (Deseret Medical, Becton Dickinson, Sandy, UT) were inserted percutaneously into leg veins for infusion of isotopes and indocyanine green (ICG; Sigma, St. Louis, MO), the latter to provide a secondary means of determining hepatic plasma flow in case of Transonic failure. Animals were allowed to rest quietly in a Pavlov harness for 30 min before the experiments started. The protocol consisted of an equilibration period (–150 to 0 min) and a 30-min sampling period. At –90 min, a primed continuous infusion of [9,11,12,12-<sup>2</sup>H<sub>4</sub>]cortisol (0.0188 mg prime, 0.0164 mg/h continuous; Cambridge Isotope Laboratories, Andover, MA) was started.

**Analytical techniques.** Samples were placed in ice, centrifuged at 4°C, and separated. Arterial, portal venous, and hepatic venous cortisol, D4-cortisol, and D3-cortisol concentrations were measured using liquid chromatography–mass spectrometer as previously described (15,24). In brief, prednisolone was added as an internal standard and methylene chloride used to extract the relevant steroids. The dried extract was then reconstituted and injected into a liquid chromatography–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 charge/mass ratio (m/z) 363:121, 367:121, 366:121, 361:163, and 364:164, 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%.

**Calculations.** Portal, hepatic arterial, and hepatic venous plasma flow were determined by the ultrasonic flow probes. Calculation of splanchnic net cortisol balance, cortisol uptake, and cortisol production has been previously described in detail (15). In brief, fractional extraction of D4-cortisol across the liver was calculated as:

$$\text{Liver ER } D4_{\text{cort}} = \frac{[(A_{\text{cort}} \times \text{HAPF}) + (\text{PoV}_{\text{cort}} \times \text{PoV PF})] - (\text{HV}_{\text{cort}} \times \text{Liver PF})}{[(A_{\text{cort}} \times \text{HAPF}) + (\text{PoV}_{\text{cort}} \times \text{PoV PF})]} \quad (1)$$

where ER is extraction ratio, A is arterial, HV is hepatic venous, PoV is portal venous, HAPF (ml/min) is hepatic artery plasma flow, PoV PF (ml/min) is portal venous plasma flow, and Liver PF (ml/min) is liver plasma flow, which equals APF + PoV PF.

Fractional extraction across the viscera was calculated as:

$$\text{Visceral ER } D4_{\text{cort}} = \frac{A D4_{\text{cort}} - \text{PoV } D4_{\text{cort}}}{A D4_{\text{cort}}} \quad (2)$$

Viscera net cortisol balance was calculated as:

$$N_{\text{Visceral CB}} = (A_{\text{Tcort}} - \text{PoV}_{\text{Tcort}}) \times \text{PoV PF} \quad (3)$$

where N is net and CB is cortisol balance.

Liver net cortisol balance was calculated as:

$$N_{\text{Liver CB}} = [(A_{\text{Tcort}} \times \text{HAPF}) + (\text{PoV}_{\text{Tcort}} \times \text{PoV PF})] - (\text{HV}_{\text{Tcort}} \times \text{Liver PF}) \quad (4)$$

Liver cortisol uptake was calculated as:

$$\text{Liver CU} = (A_{\text{Tcort}} \times \text{Liver PF}) \times \text{Liver ER } D4_{\text{cort}} \quad (5)$$

where CU is cortisol uptake.

Visceral cortisol uptake was calculated as:

$$\text{Visceral CU} = (\text{PoV}_{\text{Tcort}} \times \text{PoV PF}) \times \text{Visceral ER } D4_{\text{cort}} \quad (6)$$

Visceral and liver cortisol production were calculated:

$$\text{Liver CP} = N_{\text{Liver CB}} - \text{Liver CU} \quad (7)$$

$$\text{Visceral CP} = N_{\text{Visceral CB}} - \text{Visceral CU} \quad (8)$$

For the sake of clarity of presentation, cortisol production is expressed as a positive number in the figures and the text.

D3 visceral and liver net balance, uptake, and production were calculated similar to the calculations for cold cortisol (Eqs. 3–8). Overall splanchnic net balance, uptake, and production were calculated as the algebraic sum of viscera and liver net balance, uptake, and production.

Total-body cortisol production (TBCP) and D3-cortisol production (TBD3CP) were calculated as:

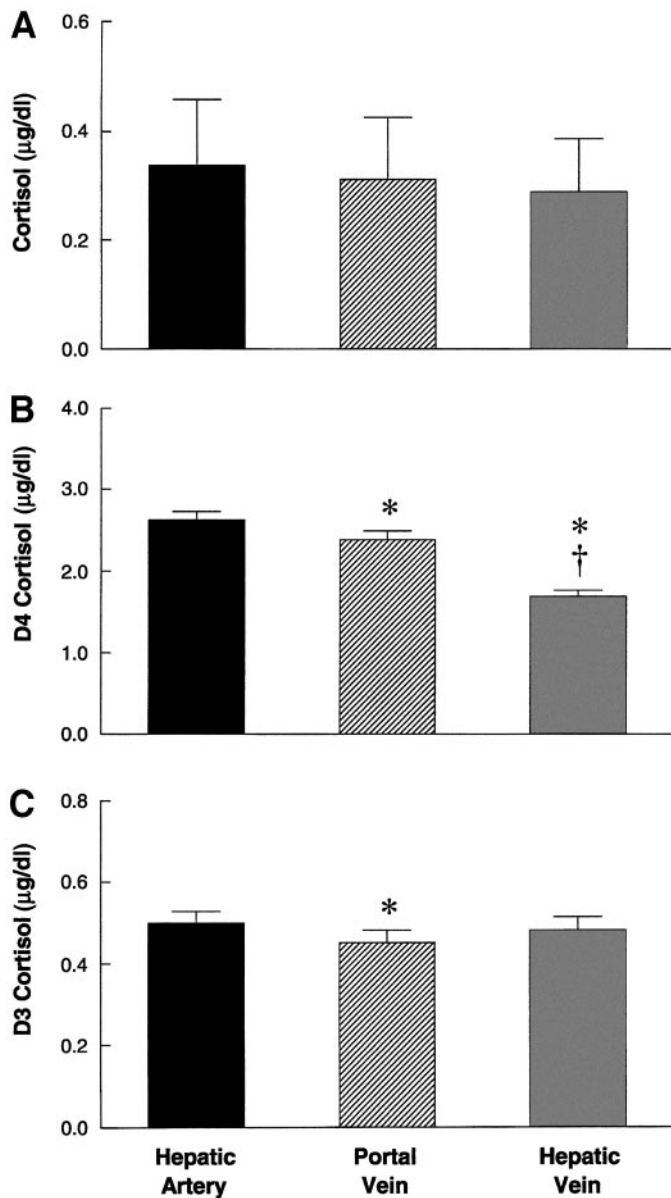


FIG. 2. Concentrations of cortisol (A), D4-cortisol (B), and D3-cortisol (C) in the hepatic artery, portal vein, and hepatic vein. \* $P < 0.01$  vs. hepatic artery; † $P < 0.005$  vs. portal vein.

$$\text{TBCP} = \left( \frac{F D4_{\text{cort}}}{A D4_{\text{cort}} / A_{\text{Tcort}}} \right) - F D4_{\text{cort}} \quad (9)$$

$$\text{TB D3 CP} = \frac{F D4_{\text{cort}}}{A D4_{\text{cort}} / A D3_{\text{cort}}} \quad (10)$$

where  $T_{\text{cort}}$  ( $\mu\text{g/ml}$ ) equals cortisol + D4-cortisol + D3-cortisol concentrations, and  $F D4_{\text{cort}}$  ( $\mu\text{g/min}$ ) 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 micrograms per minute. Since steady state was achieved at  $\sim 90$  min, values from 90, 105, and 120 min were averaged and considered as basal. ANOVA followed by Student's paired test were used to determine if concentrations and rates differed across the splanchnic bed, viscera, and liver and to determine if rates of uptake and production differed from zero. A  $P$  value  $< 0.05$  was considered statistically significant.

## RESULTS

**Arterial, portal venous, and hepatic venous cortisol, D4-cortisol, and D3-cortisol concentrations.** Arterial, portal, and hepatic venous cortisol concentrations (Fig. 2)

did not differ ( $0.31 \pm 0.12$  vs.  $0.28 \pm 0.11$  vs.  $0.27 \pm 0.10$   $\mu\text{g/dl}$ , respectively). In contrast, portal venous D4-cortisol concentrations ( $2.4 \pm 0.1$   $\mu\text{g/dl}$ ) were lower ( $P < 0.01$ ) than arterial D4-cortisol concentrations ( $2.6 \pm 0.1$   $\mu\text{g/dl}$ ), and hepatic venous D4-cortisol concentrations ( $1.7 \pm 0.1$   $\mu\text{g/dl}$ ) were lower ( $P < 0.001$ ) than portal D4-cortisol concentrations, indicating  $8 \pm 3$  and  $28 \pm 3\%$  extraction of cortisol by the viscera and liver, respectively ( $P < 0.001$  for viscera vs. liver).

Portal venous D3-cortisol concentrations were lower ( $P < 0.01$ ) than arterial D3-cortisol concentrations ( $0.45 \pm 0.03$  vs.  $0.48 \pm 0.02$   $\mu\text{g/dl}$ , respectively) but did not differ from hepatic venous D3-cortisol concentrations ( $0.47 \pm 0.03$   $\mu\text{g/dl}$ ). Hepatic venous D3-cortisol concentrations also did not differ from arterial D3-cortisol concentrations.

Total portal cortisol (sum of unlabeled cortisol, D4-cortisol, and D3-cortisol) concentrations were slightly lower ( $P = 0.05$ ) than total arterial cortisol concentrations ( $3.1 \pm 0.2$  vs.  $3.4 \pm 0.2$   $\mu\text{g/dl}$ , respectively). In addition, total hepatic venous cortisol concentrations ( $2.4 \pm 0.2$   $\mu\text{g/dl}$ ) were lower ( $P < 0.001$ ) than total portal concentrations.

Arterial cortisol concentrations present before initiation of the D4-cortisol infusion ( $4.3 \pm 0.7$   $\mu\text{g/dl}$ ) did not differ from total arterial cortisol (i.e., sum of unlabeled cortisol, D4-cortisol, and D3-cortisol) concentrations present during the D4-cortisol infusion ( $3.5 \pm 0.2$   $\mu\text{g/dl}$ ).

**Arterial, portal venous, and hepatic venous cortisone and D3-cortisone concentrations.** Portal venous and arterial unlabeled cortisone concentrations (Fig. 3) ( $0.25 \pm 0.10$  vs.  $0.24 \pm 0.10$   $\mu\text{g/dl}$ , respectively) did not differ, indicating negligible extraction of the precursor cortisone by the viscera. In contrast, hepatic venous cortisone concentrations ( $0.07 \pm 0.06$   $\mu\text{g/dl}$ ) were markedly lower ( $P < 0.005$ ) than portal venous cortisone concentrations due to extensive extraction of cortisone by the liver.

A similar pattern was observed for D3-cortisone. Portal venous and arterial D3-cortisone concentrations did not differ ( $0.46 \pm 0.04$  vs.  $0.40 \pm 0.06$   $\mu\text{g/dl}$ , respectively), whereas hepatic venous D3-cortisone concentrations ( $0.03 \pm 0.00$   $\mu\text{g/dl}$ ) were markedly lower ( $P < 0.005$ ) than portal venous D3-cortisone concentrations.

Total arterial cortisone (cortisone and D3-cortisone) concentrations did not differ from total portal venous cortisone concentrations ( $0.64 \pm 0.13$  vs.  $0.71 \pm 0.11$   $\mu\text{g/dl}$ , respectively). In contrast, total hepatic venous cortisone concentrations ( $0.10 \pm 0.10$   $\mu\text{g/dl}$ ) were lower ( $P < 0.001$ ) than total portal venous cortisone concentrations.

**Splanchnic, visceral, and hepatic net cortisol balance, cortisol uptake, and cortisol production.** Arterial, portal venous, and total splanchnic plasma flow (Fig. 4) averaged  $61 \pm 6$ ,  $320 \pm 27$ , and  $381 \pm 28$   $\text{ml/min}$ , respectively. Net splanchnic cortisol balance ( $3.8 \pm 0.4$   $\mu\text{g/min}$ ) was positive ( $P < 0.0001$  vs. zero balance), indicating net cortisol uptake across the entire splanchnic bed. This was due to a positive net cortisol balance (i.e., net uptake) across the liver ( $2.8 \pm 0.2$   $\mu\text{g/min}$ ;  $P < 0.0001$  vs. zero). On the other hand, net cortisol balance across the viscera did not differ from zero ( $1.0 \pm 0.4$   $\mu\text{g/min}$ ).

Cortisol uptake across the splanchnic bed averaged  $4.8 \pm 0.4$   $\mu\text{g/min}$ . Of this,  $0.8 \pm 0.3$   $\mu\text{g/min}$  occurred within the viscera and  $4.0 \pm 0.2$   $\mu\text{g/dl}$  within the liver. Cortisol uptake across both the viscera and liver were greater than zero ( $P < 0.05$  and  $P < 0.0001$ , respectively), and cortisol uptake across the liver was greater than that across the viscera ( $P < 0.001$ ). Cortisol production within the

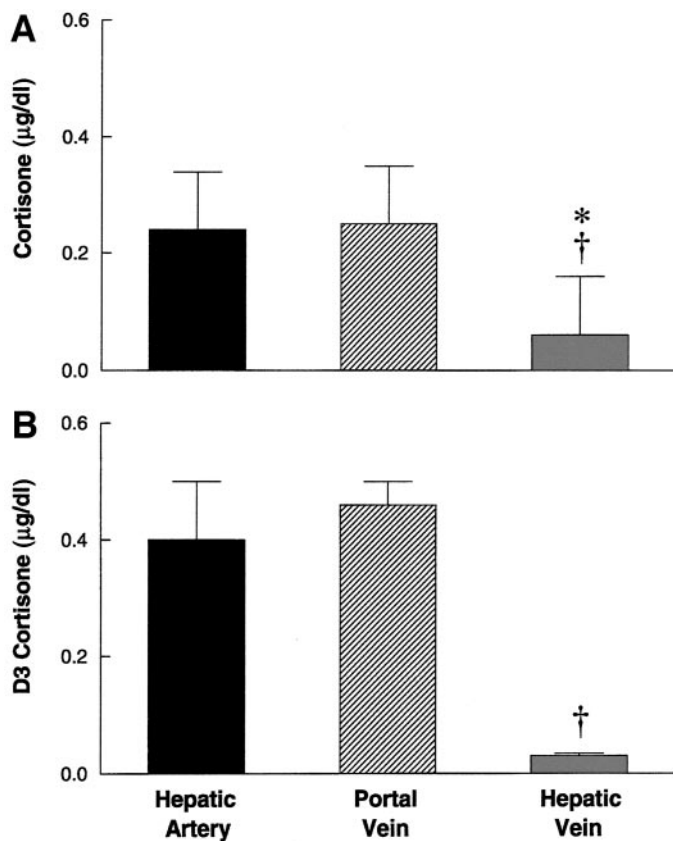


FIG. 3. Concentrations of cortisone (A) and D3-cortisone (B) in the hepatic artery, portal vein, and hepatic vein. \* $P < 0.01$  vs. hepatic artery; † $P < 0.005$  vs. portal vein.

splanchnic bed averaged  $1.0 \pm 0.1 \mu\text{g}/\text{min}$  ( $P < 0.0001$  vs. zero production), of which  $-0.2 \pm 0.2 \mu\text{g}/\text{min}$  was produced within the viscera ( $P = \text{NS}$  vs. zero production) and  $1.2 \pm 0.01 \mu\text{g}/\text{min}$  within the liver ( $P < 0.0001$  vs. zero production and  $P < 0.001$  vs. viscera).

**Splanchnic, visceral, and hepatic net D3-cortisol balance, D3-cortisol uptake, and D3-cortisol production.** D4-cortisol is converted to D3-cortisone by  $11\beta$  HSD type 2, which in turn is converted to D3-cortisol by  $11\beta$  HSD type 1. Therefore, measurement of D3-cortisol production provides an index of flux through the  $11\beta$  HSD pathway. Net D3-cortisol balance across the entire splanchnic bed ( $-0.2 \pm 0.1 \mu\text{g}/\text{min}$ ), across the viscera ( $-0.1 \pm 0.1 \mu\text{g}/\text{min}$ ), and across the liver ( $-0.1 \pm 0.1 \mu\text{g}/\text{min}$ ) did not differ from zero, indicating that rates of D3-cortisol uptake equaled production across those tissues (Fig. 4B).

D3-cortisol uptake across the splanchnic bed averaged  $0.6 \pm 0.1 \mu\text{g}/\text{min}$ . Of this,  $0.1 \pm 0.0 \mu\text{g}/\text{min}$  occurred within the viscera and  $0.5 \pm 0.2 \mu\text{g}/\text{min}$  within the liver. D3-cortisol uptake across both the viscera and liver was greater than zero ( $P < 0.05$  and  $P < 0.001$ , respectively), and cortisol uptake across the liver was greater than that across the viscera ( $P < 0.0001$ ).

D3-cortisol production within the splanchnic bed averaged  $0.8 \pm 0.2 \mu\text{g}/\text{min}$  ( $P < 0.001$  vs. zero production). Of this,  $0.2 \pm 0.1 \mu\text{g}/\text{min}$  occurred within the viscera ( $P = \text{NS}$  vs. zero production) and  $0.6 \pm 0.1 \mu\text{g}/\text{min}$  within the liver ( $P < 0.001$  vs. zero production). The rate of D3-cortisol production that occurred within the liver was greater ( $P < 0.001$ ) than that which occurred in the viscera.

**Total-body cortisol and D3-cortisol production.** Total-body cortisol and D3-cortisol production averaged  $1.7 \pm$

$0.2$  and  $1.0 \pm 0.10 \mu\text{g}/\text{min}$ , respectively ( $P < 0.001$  vs. zero production).

## DISCUSSION

Previous studies (14–17) have established that substantial amounts of cortisol are produced within the splanchnic bed of humans via conversion of the inactive metabolite cortisone to the active glucocorticoid cortisol. The present study indicates that cortisol is also produced within the splanchnic bed of dogs with most, if not all, of the cortisol production occurring within the liver. The relative contribution of the viscera and liver to total splanchnic cortisol production was calculated in two ways. In the first, the total amount of cortisol (cortisol, D4-cortisol, and D3-cortisol) entering and leaving the viscera and liver was determined by measuring arterial, portal, and hepatic venous concentrations. As shown in Fig. 1, D4-cortisol is an irreversible tracer (i.e., once converted to D3-cortisone, it cannot be converted back to D4-cortisol). Therefore, fractional extraction of cortisol can be separately calculated across the viscera and liver (15,25). Fractional extraction of cortisol was three to four times higher (28 vs. 8%) in the liver than the viscera. Since the decrease in cortisol concentrations from the hepatic artery to the portal vein to the hepatic vein was negligible (Fig. 2A), this indicated that the liver was producing more cortisol than the viscera in order to offset the far higher rate of extraction.

The above analysis provides qualitative, albeit irrefutable, proof that more cortisol was being produced within the liver than viscera. Since the concentrations of viscera cortisol, D4-cortisol, and D3-cortisol entering and leaving the splanchnic bed, viscera, and liver are all known, the relative contributions of the viscera and liver to splanchnic cortisol production can be calculated. As evident in Fig. 5, net visceral cortisol balance ( $\sim 1.0 \mu\text{g}/\text{min}$ ) and cortisol uptake ( $\sim 0.8 \mu\text{g}/\text{min}$ ) closely approximated one another, meaning that viscera cortisol production did not differ significantly from zero. In contrast, net cortisol balance across the liver ( $\sim 2.8 \mu\text{g}/\text{min}$ ) was less than cortisol uptake across the liver ( $\sim 4.0 \mu\text{g}/\text{min}$ ), indicating that cortisol was produced within the liver at a rate of  $\sim 1.2 \mu\text{g}/\text{min}$ , which entirely accounted for splanchnic cortisol production ( $\sim 1.0 \mu\text{g}/\text{min}$ ). A similar pattern emerged when D3-cortisol production rates were measured. D3-cortisol is only generated when D3-cortisone is converted to D3-cortisol (Fig. 1). Therefore, measurement of the rate of production of D3-cortisol provides an index of flux via the  $11\beta$  HSD pathway. The calculation of D3-cortisol production is essentially the same as that of cortisol production in that the concentrations of D3-cortisol entering and leaving the various tissue beds, as well as plasma flow and fractional extraction of cortisol, were all known. Once again, splanchnic D3-cortisol production was entirely accounted for by the liver; visceral D3-cortisol production was not statistically different from zero (Fig. 4B).

$11\beta$  HSD type 1 is an intracellular enzyme. Conversion of cortisone to cortisol within the cell would be detected with the tracer method used in the current experiments only if the cortisol that was produced diffused across the plasma membrane and equilibrated with cortisol present in the extracellular space. Previous experiments in using the microdialysis technique indicate that there is rapid equilibrium between cortisol produced within the cell and

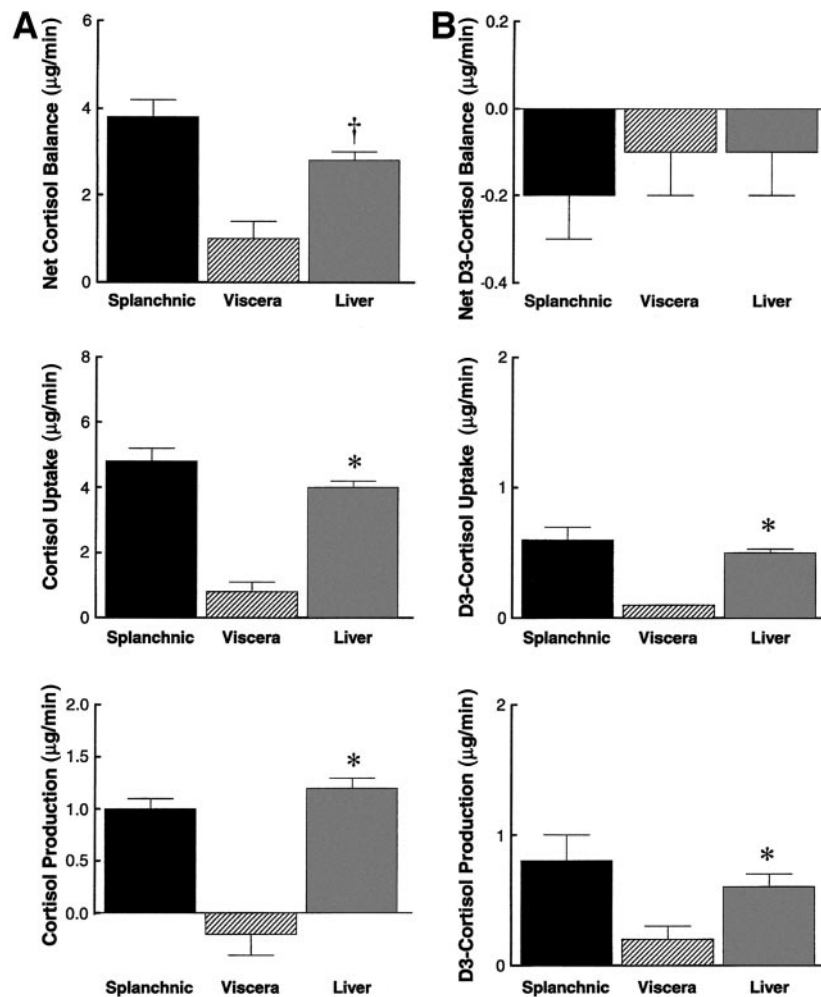


FIG. 4. Total splanchnic, visceral, and hepatic net balance, uptake, and production of cortisol (A) and D3-cortisol (B). \* $P < 0.001$  vs. viscera; † $P < 0.01$  vs. viscera.

cortisol within interstitial fluid (26). Infusion of [ $^3\text{H}$ ]cortisone into the interstitial fluid of adipocytes resulted in a prompt increase in interstitial [ $^3\text{H}$ ]cortisol, the concentration of which rapidly changed during insulin infusion. This indicates that [ $^3\text{H}$ ]cortisone diffused into the cell and was converted to [ $^3\text{H}$ ]cortisol by intracellular  $11\beta$  HSD type 1, which was then diffused back into the interstitial space. On the other hand, since the kinetics and distribution volume of locally produced cortisol are not known, the present study does not exclude the possibility that small rates of production of cortisol with the viscera result in high local concentrations. However, they do indicate that net release of cortisol from the viscera does not occur in dogs; therefore, production of cortisol within the viscera does not increase hepatic glucocorticoid exposure.

The present experiments suffer from certain limitations. While the data indicate that concurrent cortisol uptake and production occurs within the liver, we do not know the location or the biologic significance of these processes. The dogs used in the present experiments were not obese. Therefore, it is possible visceral cortisol production would have been greater if they were obese and had more visceral fat. However, we doubt if this would be the case, since splanchnic cortisol production did not differ in lean and obese humans, despite marked differences in visceral fat (14). Furthermore, peripheral venous and portal venous cortisol concentrations did not differ in severely

obese subjects at the time of bariatric surgery (22). As in humans (14–16), splanchnic and total-body cortisol production are approximately equal in the dog. However, cortisol production rates in the dog ( $\sim 0.05 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) are approximately one-quarter those in humans ( $\sim 0.2 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ), perhaps because dogs have very little cortisol-binding globulin (27). However, in vitro studies suggest that free cortisol concentrations do not differ from those present in humans (27). The extent to which differences in cortisol-binding globulin influences flux via the  $11\beta$  HSD type 1 pathway remains to be determined. Due to the high rates of cortisol clearance, substantial amounts of D4-cortisol had to be infused in order to be able to accurately measure plasma D4-cortisol and D3-cortisol concentrations. This resulted in suppression of endogenous cortisol concentrations. However, total cortisol concentration (i.e., the sum of unlabeled cortisol, D4-cortisol, and D3-cortisol) during the D4-cortisol infusion did not differ from those present before the D4-cortisol infusion ( $3.5 \pm 0.2$  vs.  $4.3 \pm 0.7 \mu\text{g}/\text{dl}$ ) and therefore did not alter the systemic glucocorticoid concentrations to which the viscera and liver were exposed. D4-cortisol can be converted to D3-cortisone via the  $11\beta$  HSD type 2 pathway or due to dehydrogenase activity of  $11\beta$  HSD type 1. The methods used in the present experiments cannot distinguish between these two possibilities. However, the source of D3-cortisone does not influence

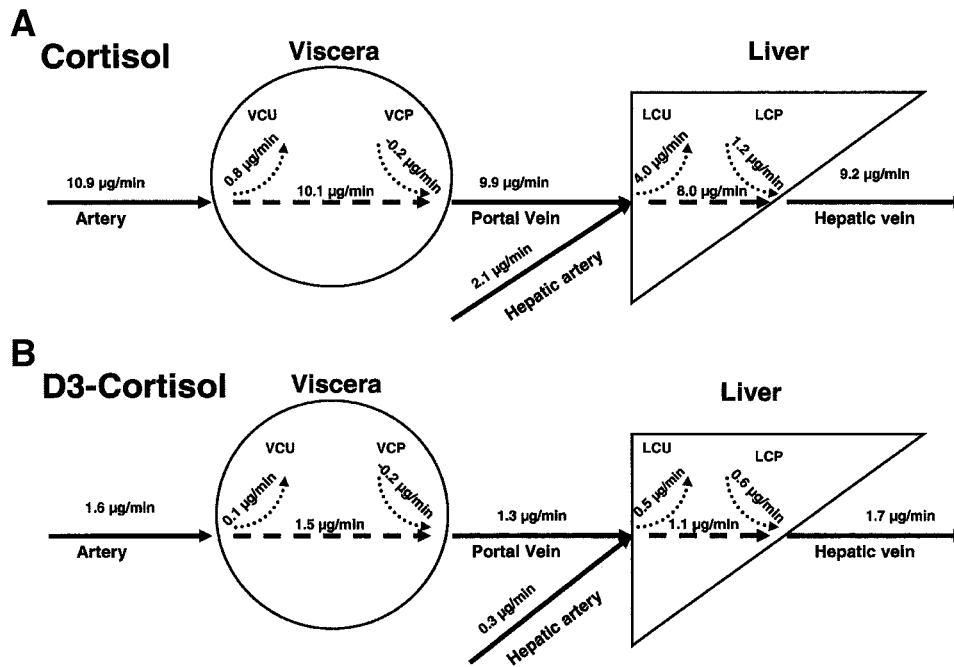


FIG. 5. Schematic representation of the calculation of viscera and liver cortisol (A) and D3-cortisol (B) uptake and production. LCP, liver cortisol production; LCU, liver cortisol uptake; VCP, viscera cortisol production; VCU, viscera cortisol uptake.

the calculation of D3-cortisol uptake and production, since this calculation only requires knowledge of the fractional extraction of D4-cortisol across the organ of interest and the amount of D3-cortisol entering and leaving that organ.

In summary, we report that as in humans, substantial amounts of cortisol are produced within the splanchnic bed of the dog with most, if not all, being produced within the liver. Little, if any, cortisol is released by the viscera into the portal vein. On the other hand, since there is concurrent uptake of cortisol by both the viscera and the liver, cortisol production within the splanchnic bed does not alter the exposure of extrasplanchnic tissues to cortisol. Taken together, these data suggest that the high local cortisol concentrations generated via the  $11\beta$  HSD type 1 pathway within the liver likely contributes to the regulation of hepatic glucose, fat, and protein metabolism.

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