

## **Relationships between plasma cortisol, corticosteroid-binding globulin (CBG), and the free cortisol index (FCI) in pigs over a 24 h period**

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### **Summary**

The relationship between plasma free cortisol and the free cortisol index (FCI, the ratio of cortisol to CBG) was evaluated in eight 8-wk old pigs over a 24 h period and in response to administration of saline or ACTH. A high ( $P < .001$ ) correlation was found between actual free cortisol and the FCI in both saline ( $r = 0.73$ ) and ACTH ( $r = 0.85$ ) treated pigs. A diurnal rhythm was apparent for total cortisol, free cortisol, percent free cortisol, pCBG, and the FCI. Total cortisol ( $P < .05$ ), free cortisol ( $P < .05$ ), and the FCI ( $P < .01$ ) were elevated during the 4 h following administration of ACTH.

Concentrations of pCBG differed ( $P < .001$ ) over time and were higher ( $P < .01$ ) for ACTH treated pigs over the 24 h period. The results from this study affirm the contention that FCI is a suitable estimate of free cortisol in swine.

**Key Words:** Pig, Cortisol, CBG, FCI

### **Introduction**

A circadian pattern of total cortisol is present in many species including man, horse, and pig. For the pig this rhythm is characterized by peak amounts of circulating cortisol expressed in the morning with reduced levels during the afternoon and early evening (Whipp et al., 1970). Cortisol exists in the bloodstream in biologically active and inactive forms. The active form consists of cortisol that is “free” or unbound and that

which is loosely bound to albumin, thus allowing it to be biologically available to the cell. The majority of circulating cortisol is tightly bound to its specific carrier glycoprotein, corticosteroid-binding globulin (CBG). The percentage of CBG-bound cortisol in circulation varies among species from >60% for swine (Kattesh et al., 1990) to more than 90% for humans (Siiteri et al., 1982).

A diurnal rhythm for CBG has been reported in both humans and rats. In humans, CBG binding capacity for cortisol paralleled oscillations for the diurnal rhythm of total cortisol with a lag time of approximately four hours (Angeli et al., 1978). During stressful situations, cortisol levels can increase while CBG levels may increase or decrease, leaving the free cortisol fractions to be inadequately represented. The free cortisol index (FCI), a ratio of circulating total plasma cortisol to CBG, has been demonstrated in human studies to be a reliable and easy to use measure of plasma free cortisol (le Roux et al., 2003).

The objectives of this study were to examine the relationship between the FCI and actual levels of circulating free cortisol over a 24 h period, and to document changes in plasma total cortisol, percent free cortisol, free cortisol, pCBG and FCI in response to adrenal stimulation in pigs.

### **Methods**

Pigs (Premier x QMax 100) were weaned at 25 d of age, and housed in raised pens (2.44 m x 2.44 m) with slotted floors. Animals were given free access to a commercial diet and water. Artificial lights were provided for 13 h, starting at 0630 h, and room temperature was maintained at  $25 \pm 2^\circ\text{C}$ . A red light source was activated during the

dark period to aid in blood sampling. After catheterization, pigs were placed in individual 0.61 m x 1.22 m pens with free access to feed and water.

Eight 8-wk old female pigs ( $12.7 \pm 1.8$  kg BW) from two litters were anesthetized and fitted with an indwelling catheter. Animals were placed individually in 0.61 m x 1.22 m pens, and a 90 cm long extension tube was attached to the existing catheter/extension to allow remote access for the collection of blood samples without disturbing the pigs.

Blood samples (5 ml) were collected in heparinized tubes over a 24-h period beginning approximately 5 h post insertion of the catheter (1730 h). Plasma was collected and stored at  $-20^{\circ}\text{C}$ .

Following the fourth hourly blood sampling (2030 h), four of the eight pigs were administered saline (2.5 ml 0.9% NaCl) and the remaining four pigs administered ACTH (1 IU/kg BW) via the catheter. Samples were then collected at 30 min intervals for 4 h and then hourly for the remainder of the 24 h period. A total of 29 blood samples were collected per animal. Hematocrit values were determined on samples collected at 1730, 0230, and 1730 h.

Plasma total cortisol concentration was determined by radio-immunoassay and the concentration of porcine corticosteroid-binding globulin (pCBG) was measured by a direct ELISA. The free cortisol index was calculated using the ratio of plasma total cortisol to pCBG concentration. Percent free cortisol was determined using an ultrafiltration assay. The concentration of free cortisol was calculated using the product of percent free cortisol and total cortisol.

Data were analyzed using the MIXED procedure of SAS (SAS Inc., v9.0) for a completely randomized design. Repeated measures were utilized for differences due to time. The mean of samples taken prior to treatment administration was used as a baseline value when analyzing for differences over time. Data were presented as least squares means with standard errors, and significant differences were separated using Fisher's Least Significant Difference test. A regression analysis with log transformation, due to unequal variances, was used to estimate the correlation of free cortisol (nmol/L) and FCI.

### **Results and Discussion**

Blood samples were successfully collected from seven of the eight pigs at each of the designated sampling times. Hematocrit values measured on samples collected at the beginning and end of the experiment were not different.

The diurnal pattern for plasma cortisol and pCBG was evident in the present study (Fig. 1a). Plasma cortisol exhibited a morning peak occurring at 0730 - 0830 h and an afternoon trough at 1630 h, whereas pCBG concentrations were higher during 2100 - 2330 h and lower through the rest of the morning and afternoon. The percentage of free cortisol and FCI followed a circadian pattern like that of cortisol, increasing through the night and early morning hours before a gradual decrease through the afternoon hours (Fig 2a).

Injection of ACTH temporarily interrupted the circadian rhythm of plasma cortisol for 4 h before returning to values similar to control pigs (Fig. 1b). The administration of ACTH at 2030 h, resulting in elevated plasma cortisol values from 2100 to 0030 h, appeared to have suppressed the morning cortisol peak, but the afternoon trough was

similar to that in control pigs. Plasma pCBG concentrations in ACTH treated pigs fluctuated over time in a fashion similar to that in saline treated pigs (Fig. 1b). Following injection of ACTH, percent free cortisol remained similar to that in the saline treated pigs (Fig. 2b). However, actual free cortisol (percent free x total cortisol), as well as FCI, was elevated. This was due to plasma total cortisol being elevated while pCBG concentrations remained constant. Total cortisol and actual free cortisol decreased over the remainder of the 24 h period. At 0230 h, cortisol values in the ACTH pigs were lowest while pCBG values were at their highest concentrations. Once the FCI peaked in pigs administered ACTH, it then fell below baseline concentrations for 2 h before returning to values similar to that in saline treated pigs. The FCI in ACTH treated pigs did not increase like that in saline pigs during the morning hours.

### **Conclusions**

To our knowledge this is the first experiment to measure plasma total cortisol, pCBG, percent cortisol, plasma free cortisol and FCI over a 24 h period. Our results indicate that when pCBG concentrations remain relatively stable over a 24 h period, the FCI will change according to fluctuations in total cortisol. By factoring in CBG values through the application of the FCI, a more accurate portrayal of the availability of circulating cortisol is possible.

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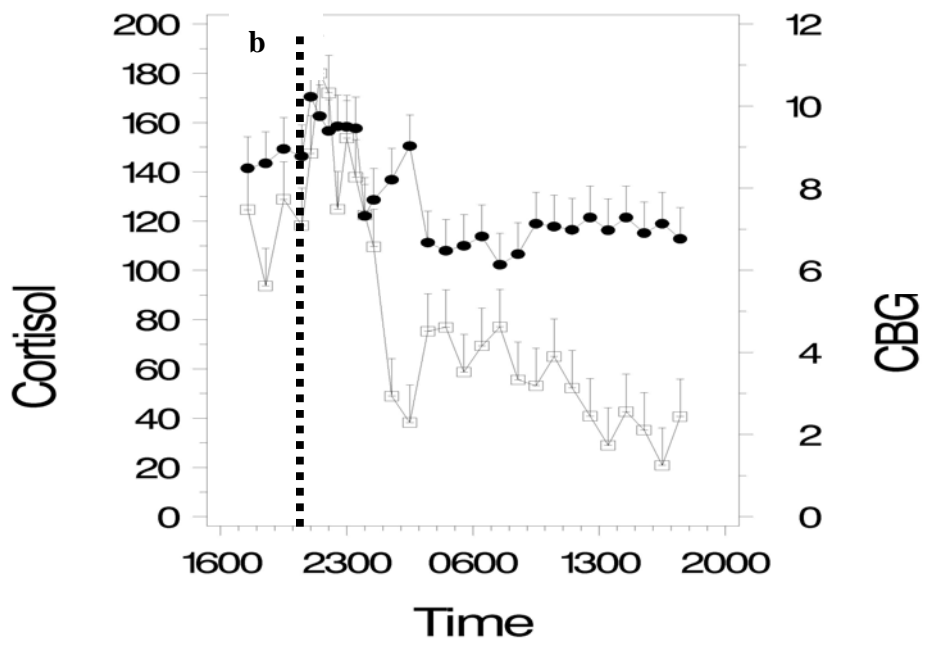
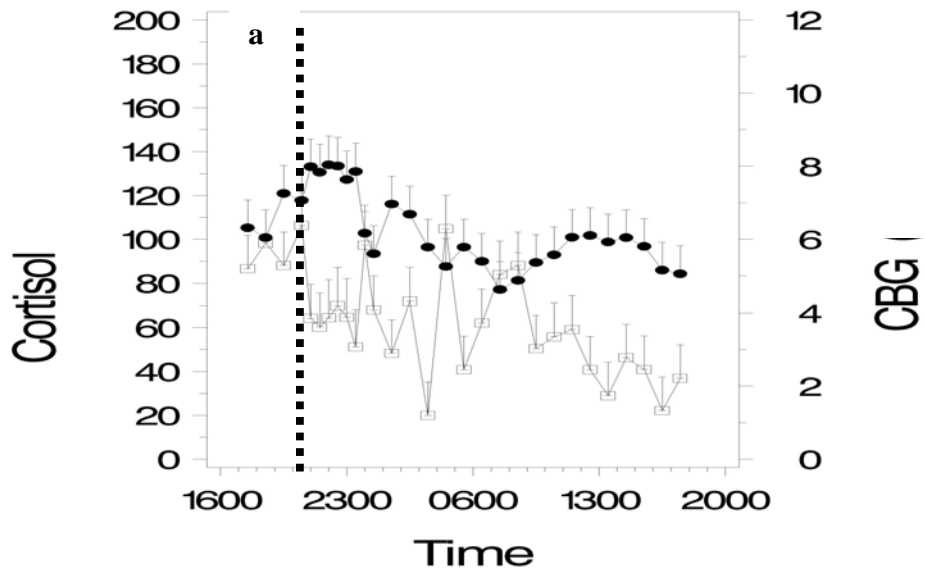


Fig. 1. Relationship between total cortisol ( $\square$ ; nmol/L) and pCBG ( $\bullet$ ; mg/L) in pigs sampled over 24 h following administration of saline (**a**; 0.9% NaCL) or adrenocorticotrophin (**b**; 1 IU/kg BW). Each point represents the mean value (+ S.E., n = 4). Treatment was administered immediately following 2030 h sample (vertical dotted line).

