

To the Graduate Council:

I am submitting herewith a thesis written by Rebecca Jackson Adcock entitled "Temporal relationships between plasma cortisol, corticosteroid-binding globulin (CBG), and the free cortisol index (FCI) in pigs." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

Henry G. Kattesh
Major Professor

We have read this thesis
and recommend its acceptance:

Judith M. Grizzle

Michael O. Smith

Accepted for the Council:

Anne Mayhew
Vice Chancellor and
Dean of Graduate Studies

(Original signatures on file with official student records.)

TEMPORAL RELATIONSHIPS BETWEEN PLASMA
CORTISOL, CORTICOSTEROID-BINDING GLOBULIN
(CBG), AND THE FREE CORTISOL INDEX (FCI) IN PIGS.

A Thesis

Presented for the Master of Science

Degree

The University of Tennessee, Knoxville

Rebecca Jackson Adcock

December, 2005

DEDICATION

This thesis is dedicated to my supportive husband, Jonathan, and my parents and husband's parents, who have encouraged and supported me to complete this educational goal.

ACKNOWLEDGEMENTS

I wish to express my thanks and appreciation to the following people, for assisting in the preparation of this thesis and in making my graduate study possible.

Dr. H. G. Kattesh, major professor and advisor, for his guidance, knowledge, and patience, and allowing me the opportunity to assist in the undergraduate anatomy and physiology class. Above all, I would like to thank him for his friendship over the past three years.

Dr. J.M. Grizzle, for serving on my committee and her friendship over the past few years.

Dr. M.O. Smith, for serving on my committee and his friendship over the past few years.

Mary Roberts, for her friendship and invaluable experience and assistance in the lab.

Dr. Jeff Carroll, for taking time out of his schedule to come to Knoxville and assist in the catheterizations.

JARTU staff, for building the pens for the pigs, as well as cleaning and feeding them.

Magdalena Rambaud and Fernando Scenna, Sara Youngerman, Angela Pollack, Kristin Rich, Tracy Livingston, Rose Clift and Jamie Gilreath for their friendship through these past three years and for making the office a pleasant place to be.

Jonathan, my husband, for all of his support and understanding through the past three years. Meeko and Merced, the dogs for always letting me realize that life is too short to pass up an opportunity to chase the Frisbee or sleep on the couch.

ABSTRACT

The free fraction of cortisol in circulation is that which is biologically available to cells. In swine, more than 60% of cortisol is bound to its carrier glycoprotein, porcine corticosteroid-binding globulin (pCBG). The Free Cortisol Index (FCI), which is the ratio of total cortisol to CBG concentrations, has been shown in humans to correlate well with free cortisol. In experiment one, the relationship between plasma free cortisol and the free cortisol index (FCI) was evaluated in 8-wk old pigs over a 24 h period and in response to the administration of saline ($n = 4$) and ACTH ($n = 4$). A high ($P < 0.001$) 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 FCI with higher values for total cortisol and the FCI occurring in the morning compared to lower pCBG values at that time. For the variables of total cortisol ($P < 0.05$), free cortisol ($P < 0.05$), and FCI ($P < 0.01$), values were elevated during the four hours following administration of ACTH. Concentrations of pCBG differed ($P < 0.001$) over time and were higher ($P < 0.01$) for ACTH treated pigs over the 24 h period. In the second study, changes in plasma cortisol, pCBG and the FCI were examined in pigs in response to adrenal stimulation or suppression. Twenty-four 8-wk old pigs allotted in equal numbers, were administered ACTH, dexamethasone, or saline, and blood samples collected every 15 min over a 6 h period. In pigs treated with ACTH, the FCI increased ($P < 0.001$) in a fashion similar to that of

total cortisol, while pCBG values remained stable. In dexamethasone treated pigs, both FCI and total cortisol decreased ($P < 0.001$) with a subsequent elevation in pCBG concentrations. The results of these two studies affirm the contention that FCI is a suitable estimate of free cortisol in swine, and that a better illustration of the temporal relationships between plasma total cortisol and pCBG may be assessed by the measurement of both.

TABLE OF CONTENTS

CHAPTER	PAGE
I. Introduction.....	1
II. Review of Literature.....	3
III. Materials and Methods.....	18
IV. Results.....	23
V. Discussion and Implications.....	45
LITERATURE CITED.....	55
VITA.....	65

LIST OF TABLES

TABLE	PAGE
1. Least squares means $\pm$ S.E. for plasma total cortisol (nmol/L) in pigs prior to (1730 – 2030 h) and following (beginning 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples.	25
2. Least squares means $\pm$ S.E. for plasma pCBG (mg/L) in pigs prior to (1730 – 2030 h) and following (beginning 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples.	28
3. Least squares means $\pm$ S.E. for percent free cortisol (%) in pigs prior to (1730 – 2030 h) and following (beginning 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples.	30
4. Least squares means $\pm$ S.E. for plasma free cortisol (nmol/L) in pigs prior to (1730 – 2030 h) and following (beginning 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples.	33
5. Least squares means $\pm$ S.E. for free cortisol index (nmol/mg) in pigs prior to (1730 – 2030 h) and following (beginning 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples.	36

LIST OF FIGURES

FIGURE	PAGE
1. Changes in plasma total cortisol (nmol/L) in pigs sampled over 24 h following administration of adrenocorticotropin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl).	24
2. Changes in pCBG (mg/L) in pigs sampled over 24 h following administration of adrenocorticotropin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl).	27
3. Changes in percent free cortisol (%) in pigs sampled over 24 h following administration of adrenocorticotropin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl).	29
4. Changes in plasma free cortisol (nmol/L) in pigs sampled over 24 h following administration of adrenocorticotropin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl).	32
5. Changes in FCI (nmol/mg) in pigs sampled over 24 h following administration of adrenocorticotropin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl).	34
6. Relationship between the calculated free cortisol index (total cortisol/pCBG) and free cortisol for saline (0.9% NaCl) treated pigs (n = 4).	37
7. Relationship between the calculated free cortisol index (total cortisol/pCBG) and free cortisol for adrenocorticotrophin (1 IU/kg BW) treated pigs (n = 4)	38
8. Changes in plasma total cortisol (nmol/L) in pigs following administration of adrenocorticotrophin (ACTH; 1 IU/kg BW), saline (control; 2.5 ml 0.9% NaCl), or dexamethasone (Dex; 40 µg/kg BW).	40
9. Changes in pCBG (mg/L) in pigs following administration of adrenocorticotrophin (ACTH; 1 IU/kg BW), saline (control; 2.5 ml 0.9% NaCl), or dexamethasone (Dex; 40 µg/kg BW).	42
10. Changes in FCI (nmol/mg) in pigs following administration of adrenocorticotrophin (ACTH; 1 IU/kg BW), saline (control; 2.5 ml 0.9% NaCl), or dexamethasone (Dex; 40 µg/kg BW).	43

CHAPTER I

INTRODUCTION

A circadian pattern of total cortisol in a diurnal rhythm 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. 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 65% for swine (Kattesh et al., 1990) to more than 90% for humans (Siiteri, 1982). Since cortisol that is bound to CBG is deemed inactive, CBG not only transports but also modulates cortisol availability within the circulation.

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 plasma cortisol with a lag time of approximately four hours (Angeli et al., 1978). No lag time between oscillations of corticosterone and CBG were observed in the circadian pattern of CBG in rats (Hsu et al., 1988).

During stressful situations, cortisol levels can increase while CBG levels may increase or decrease, leaving the free cortisol fractions to be inadequately represented. There are a number of techniques for measuring free cortisol,

including equilibrium dialysis and gel filtration, yet these methods are costly and time consuming. The free cortisol index (FCI), a ratio of circulating total plasma cortisol to CBG, has been demonstrated to be a reliable and easy to use measure of plasma free cortisol. 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 the changes in plasma total cortisol, pCBG and FCI in pigs over a 6-h period in response to adrenal stimulation or suppression.

CHAPTER II

REVIEW OF LITERATURE

The Stress Response

Selye first introduced the theory of physiological stress in 1950, when he defined stress as any nonspecific response of the body to any demand (stressor) placed upon it. Moberg (2000) expanded on this definition by defining four biological defenses to stress: behavioral, autonomic nervous system, neuroendocrine and, immune responses. These four responses affect virtually all physiological systems in the body.

When an animal perceives a situation as stressful, the hypothalamus is stimulated through a series of neural pathways, humoral substances, and local mediators to release corticotrophin-releasing hormone (CRH) (Buckingham et al., 1997). Transported by the hypothalamic-hypophyseal portal system, CRH enters the pituitary gland activating corticotrope cells to release adrenocorticotrophic hormone (ACTH) into the general circulation. From the bloodstream, ACTH stimulates the zona fasciculata of the cortical region in the adrenal gland to increase production and release of cortisol (von Borell, 1995). Cortisol then travels through the blood to various effector tissues and organs including, but not limited to, muscle, adipose and the liver (as reviewed by Munck et al., 1984). Plasma cortisol levels may remain elevated during the initial stress response, but can decline if the stressor persists for long periods of time (Hauger et al., 1990).

Role of Cortisol

Cortisol, a glucocorticoid, has various roles to help maintain homeostasis. Through a primary role to aid metabolism, cortisol has mostly an anabolic effect on the liver, while in other tissues such as muscle and adipose tissue, cortisol is catabolic and anti-anabolic (Baxter and Rousseau, 1979). In the liver, cortisol increases glucose production through activation of gluconeogenic enzymes (P-enolpyruvate carboxykinase and glucose-6-phosphatase). These enzymes effectively increase gluconeogenesis during periods of starvation or diabetes. In individuals with adrenal corticosteroid deficiencies, cortisol replacement therapy aids in maintaining the release of gluconeogenic enzymes and amino acids from skeletal muscle during the fasting state (Exton, 1979). Cortisol mobilizes energy during fasting periods by stimulating lipolysis and promoting the lipolytic effect of epinephrine to increase production of lactate (Baxter and Rousseau, 1979). Glycerol and lactate released from adipose tissue contribute to gluconeogenesis to provide an alternative energy source for glucose (Baxter and Rousseau, 1979). Cortisol also decreases protein synthesis in muscle by inducing the breakdown of protein and nucleic acids (Exton, 1979).

Immunosuppressive and Anti-inflammatory Actions of Cortisol

Cortisol has a profound, mostly negative affect on immunosuppressive and inflammatory responses. Mediated primarily on cell movement and availability at sites of inflammation, cortisol decreases neutrophil accumulation. Cortisol is an effective vasoconstrictor of capillary beds, which slows cells and

fluid from migrating into areas of inflammation (Fauci, 1979). However, in the presence of cortisol, movement of neutrophils out of bone marrow is increased (Fauci, 1979). Cortisol's effect on monocytes is more sensitive than its effect on neutrophils. Exposure of monocytes to cortisol results in their redistribution out of circulation and into tissues along with inhibition of their functional capabilities of phagocytosis and destruction of pathogens (Fauci, 1979).

Many pro-inflammatory cytokines, including several interleukins (IL-1, IL-2, IL-3, IL-6, IL-8, and IL-12), tumor necrosis factor and interferon- γ , are inhibited by cortisol. Cortisol activates a negative feedback response by stimulating the production of anti-inflammatory cytokines, such as IL-10, IL-4, and transforming growth factor to suppress inflammation (Elenkov and Chrousos, 2002).

Expression and activity of proteolytic enzymes elastase and collagenase are inhibited by cortisol resulting in reduced degradation of connective tissue at sites of inflammation (Goulding and Flower, 1997).

Glucocorticoid Effect on Organ Maturation

Increased plasma cortisol levels have been associated with normal organ maturation in the mammalian fetus (Baxter and Rousseau, 1979), and can induce specialized functions, which acts as a catalyst for terminal events in differentiation. These events can include the onset of labor in sheep due to activation of placental enzymes resulting that contribute to the initiation of uterine contractions (reviewed by Ballard, 1979). In the fetal rabbit, cortisol helps to enhance digestive functions and decrease absorption of intact proteins in the

intestine (Ballard, 1979). Studies indicate that administration of cortisol accelerates this process and, conversely, deficiencies during development may retard maturation (Ballard, 1979). Maternal and fetal plasma cortisol concentrations in swine increase sharply two days prior to parturition and then decrease the following day (First and Bosc, 1979). Randall (1983) observed an increase in fetal plasma cortisol concentrations in swine from 16 to 6 days prepartum, which then rapidly increased during the last 8 hours prior to delivery. Maternal cortisol concentrations increased slightly during the four days prior to parturition. In a later study, plasma total cortisol, percent free and free cortisol concentrations in fetal pigs were greater on day 110 compared to day 110 of gestation (Kattesh et al., 1997). Maternal free cortisol levels were two to three times greater on days 110 to 112 compared to that estimated prior to day 105 of gestation (Kattesh et al., 1997). Studies in the fetal lamb, rat and rabbit indicated that cortisol helps to accelerate the appearance of surfactant in lung tissue, which aids in reducing the surface tension of the alveoli during expiration (Ballard, 1979).

Distribution of Cortisol within the Circulation

Cortisol exists in the bloodstream in biologically active and inactive forms (Westphal, 1983; Hammond, 1990). 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 inactive form of cortisol is tightly bound to

its specific carrier glycoprotein, corticosteroid-binding globulin (CBG), which has one binding site per molecule (Ballard, 1979).

Total plasma cortisol concentrations vary among species from 0.01 to 0.05 μM in ewes, dogs and cows; 0.05 to 0.2 μM for horses; 0.1 to 0.4 μM for humans; and 0.5 to 0.7 μM for old world primates and 1.5 to 5.5 μM for new world primates (reviewed by Gayrard et al., 1996). Research by Rafai and Fodor (1980) determined that plasma cortisol concentrations in 50-day-old-pigs ranged from 20.2 to 39.2 ng/ml, and 130 day old pigs had a less variable range of 27.8 to 39.2 ng/ml. The majority of circulating cortisol is bound to CBG. This percentage varies among species, from 65% for swine (Kattesh et al., 1990) to more than 90% for humans (Siiteri et al., 1982). The availability of unbound cortisol is influenced by the distribution of its binding proteins, CBG and albumin. In humans, CBG and albumin concentrations average 0.71 μM and 550 μM , respectively (Ballard, 1979). Although albumin is in greater concentration and has up to 20 binding sites per molecule, its binding affinity for cortisol (0.5×10^4 L/mol) is considerably less than that of CBG (3.0×10^7 L/mol) (Ballard, 1979).

Plasma cortisol concentration and its distribution among protein- bound and unbound forms exhibit significant changes in the pre- and post-partum animal. Plasma cortisol binding capacity in the sheep fetus rose steadily from day 15 (23.6 ng/ml) to term (86.5 ng/ml) and decreased rapidly to less than 15 ng/ml by 5 days after birth (Berdusco et al., 1995). Corticosteroid binding capacity in the rat increased by more than 97% from day 6 to day 90 after birth (Viau et al.,

1996). In a study reported by Kattesh et al. (1990), total cortisol and percent unbound cortisol were higher in pigs at birth and steadily decreased in a linear fashion over a 42 day sampling period. The percentage of CBG-bound cortisol was lowest on days 3 through 21 compared to that measured on day 42, but actual CBG-bound cortisol decreased after day 1 postpartum and remained unchanged. The authors concluded that by day 28 of age the majority of circulating cortisol in the pig has shifted from unbound and albumin bound to CBG bound. While total cortisol levels are relatively similar in 50 to 130 day old pigs, a significant age-related increase in CBG concentration has been reported to occur in pigs between 21 and 84 days of age (Roberts et al., 2003).

In a recent study, postnatal gene expression of hepatic CBG mRNA in the pig increased from day 3 to day 40. The authors concluded that because this increase was unrelated to plasma CBG concentrations, actual circulating CBG levels may be determined by factors such as metabolic clearance, transfer to extra-vascular spaces, and/or post-translation modifications (Heo et al., 2003).

Corticosteroid-binding globulin (CBG)

Corticosteroid-binding globulin (CBG) is a monomeric glycoprotein, produced in the liver, having a molecular weight of ~ 50 to 60 kDa with one corticosteroid binding site per molecule (Mickelson et al., 1982; Westphal, 1983;). Two electrophoretic variants of CBG have been isolated in many species including the human (Mickelson et al., 1982), rabbit (Seralini et al., 1990), rat (Ali and Bassett, 1995) and pig (Roberts et al., 2003). Removal of sialic acid by

neuroaminidase, or the removal of the carbohydrate by glycosylation did not eliminate the doublet band (Mickelson et al., 1982). Mickelson et al. (1982) suggested that the doublet was due to differences in heterogeneity that exist in the protein makeup of the molecule. Research by Ali and Bassett (1995) supported the idea that variations in either the sugar or protein moieties of the molecule were responsible for the doublet. Differences could also be due to variation in post-translation processing of the protein sequence, conformation, or a combination of both (Ali and Bassett, 1995). The electrophoretic variants may also be explained by modifications to CBG mRNA causing the structure of CBG to differ according to its tissue source (Ali and Bassett, 1995). This theory is supported by the presence of tissue specific CBG variants found in various glucocorticoid and progesterone target organs such as the pituitary (De Kloet et al., 1975; Koch et al., 1976), kidney (Feldman et al., 1973), muscle (Mayer et al., 1975), and lungs (Giannopoulos, 1976).

Although CBG is primarily produced in the liver, CBG mRNA has been isolated from spleen and ovary in adult rabbits (Seralini et al., 1990), and lung, kidney and testis from the rhesus monkey (Hammond et al., 1987). In swine, liver, lung, kidney and adrenal gland were tested in post-partum pigs on day 3 and day 40 (Heo et al., 2003). Results indicated that only the liver expressed CBG mRNA in 40-day-old pigs. While glucocorticoids are a major regulator of CBG, other factors including estrogen (Bonte et al., 1999), pregnancy (Bertherat et al., 2002), and anti-epileptic drugs (Coolens et al., 1987) cause an increase in

CBG concentrations. Hyperthyroidism, liver cirrhosis, and renal diseases reduce CBG concentrations (Coolens et al., 1987).

Function of CBG

It is believed that the primary function of CBG is to act as a reservoir to replenish the free fraction of cortisol. Under this hypothesis, bound cortisol remains unavailable to tissues. This hypothesis is supported by the work of Mendel (1992) reporting that the plasma concentration of free hormone and not protein-bound hormone is the limiting factor for intracellular hormone concentrations. Mendel reasoned that without the circulatory binding proteins such as CBG, steroid hormones like cortisol would readily leave the circulation and move into cell membranes due to their natural lipophilic properties.

CBG may also serve in the intracellular transport of cortisol. Kuhn (1988) isolated CBG within cells of various target tissues including liver, kidney, uterus, pituitary gland and fat. Extraction of radiolabeled CBG indicated no degradation resulted following its recruitment from circulation. Internalization of CBG by target cells may further aid cortisol transport through protection against cellular metabolism and thus insure delivery to nuclear receptors (Kuhn, 1988).

Acting as a reservoir, CBG can store cortisol in the plasma for release at specified target sites, such as those experiencing inflammation or tissue damage. Mammalian CBG is a member of the serine protease inhibitor (SERPIN) superfamily. The common characteristic of SERPIN's is that they are cleaved by serine proteases secreted by activated neutrophils at sites of inflammation

(Hammond et al., 1991). By cleaving CBG at sites of inflammation, local concentrations of cortisol can become greater than that available in the general circulation (Breuner and Orchinik, 2002). It has been shown through in vitro studies that the addition of elastase results in the release of more than 80% of CBG-bound cortisol (Hammond et al., 1990). Bartalena et al. (1993) demonstrated that the main mediator of the acute phase inflammatory response, IL-6, had an inhibitory dose and time effect on CBG synthesis by hepatic cells in culture. This in vitro study suggests that IL-6 may help to reduce the biosynthesis of CBG during inflammation.

Diurnal Variation of Cortisol

A diurnal variation of total cortisol in a circadian pattern has been demonstrated in many species including man (Weitzman et al., 1971), horse (Bottoms et al., 1972) and pig (Whipp et al., 1970). These patterns closely follow the sleep/wake or locomotor activity rhythm, and arise differently depending on whether the species is diurnal or nocturnal. In diurnal species this pattern is characterized by peak total cortisol in the morning, decreasing through the day, and reaching a nadir in later afternoon. Conversely, nocturnal animals display peak cortisol levels in early evening that decrease through the night to their lowest levels toward morning (Edwards et al., 2001). In rats, plasma corticosterone (the major circulating glucocorticoid) is low at birth and gradually increases over time to establish a circadian rhythm by 4 weeks of age (Takahashi et al., 1979). Pigs have initially high levels of cortisol at birth that

decrease over the developing weeks, establishing a circadian rhythm by 8 weeks of age (Evans et al., 1988). This pattern is characterized by peak amounts of cortisol expressed in the morning and reduced levels during the afternoon and early evening (Whipp et al., 1970; Bottoms et al., 1972).

The circadian rhythm of cortisol in swine is altered due to many factors including photoperiod and environment. The average morning total cortisol concentration in hogs following a 14 hour exposure to natural light was higher than that measured in hogs following exposure to 10 hours of natural light, or 9 or 15 hours of artificial light (Barnett et al., 1981b). Research conducted by Griffith and Minton (1991) concluded that a circadian rhythm of a morning increase and afternoon decrease in plasma cortisol levels existed in pigs regardless of exposure to a constant light or dark photoperiod.

Moving gilts from one pen to another resulted in animals exhibiting peak cortisol concentrations within 0.5 hour following relocation (Becker et al., 1985). The peak response quickly diminished and cortisol levels returned to normal circadian rhythm within 2 hours after transport (Becker et al., 1985). In barrows restrained by immobilization for 20 minutes, a 2-3 fold increase in total cortisol was observed by the end of restraint. Once restraint was removed, cortisol levels continued to increase to peak values 40 minutes after the onset of restraint, and quickly returned to baseline levels within 20 minutes of the peak (Klemcke et al., 1989).

Chronic stress has been shown to totally disrupt circadian rhythms. In pubertal rats, 5 minutes of immobilization for 5 days disrupted the circadian rhythm of corticosterone (Paris and Ramaley, 1974). By day 5 of immobilization, daily rhythm was no longer evident due to elevated corticosterone levels in both the morning and evening (Paris and Ramaley, 1974). Janssens et al. (1995) documented that tether housing of gilts for 11 weeks resulted in an increase in evening but not morning plasma cortisol concentrations. Morning cortisol concentrations for tether-housed gilts were not elevated and did not change throughout the experiment, resulting in a flattened diurnal pattern. Pigs raised in barren pen environments with little room exhibited a blunted cortisol diurnal rhythm at 22 weeks of age compared to that of pigs housed in larger, environmentally enriched pens (de Jong et al., 2000).

Abrupt environment alterations, resulting in an acute stress response, can also influence cortisol circadian rhythm. Moving pigs from individual pens to a group pen for 8 days disrupted circadian rhythm, but only on the day of transfer. During the day of transfer to the group pen, the cortisol concentration was significantly higher compared to the other days. Movement back to individual pens resulted in a non-significant increase in cortisol concentration (Barnett et al., 1981a). Kattesh et al. (1995) compared total and percent unbound cortisol in gilts housed in fixed versus moveable-sided stalls, the latter permitting gilts to turn completely around. Gilts housed in fixed-sided stalls had higher total cortisol for the first 8 hours of the 14- day treatment period and a higher percentage of

unbound cortisol for the first 4 days of treatment. The authors concluded that restricting the movement of gilts, which had previously been allowed to turn around, can elicit a short-term stress response that disrupts the diurnal variation of cortisol release.

A circadian rhythm has also been observed for free cortisol. The circadian rhythm of salivary cortisol, which is considered free cortisol, was examined in neonatal pigs (Gallagher et al., 2002). A circadian rhythm was evident in female pigs from day 6 and male pigs from day 10. When a stressor consisting of 4 hours of isolation was examined in pigs from 12 to 24 weeks of age, the amplitude of the free cortisol circadian rhythm was increased in barrows but not in gilts (Ruis et al., 1997). The increase in barrows may be attributed to castration that occurred 2 to 4 days after birth, and may have significantly influenced the hypothalamic-pituitary-adrenal (HPA) axis. It was also noted that the free cortisol rhythm was unstable, and maximum values shifted in all pigs when the stressor was applied in the morning than compared to evening isolation. The authors concluded that this was most likely due to basal cortisol levels already being elevated during the morning (Ruis et al., 1997).

Diurnal Variation of CBG

Diurnal variation of plasma CBG concentration has been observed in humans (Angeli et al., 1978) and rats (Calvano et al., 1984). In humans, CBG binding capacity for cortisol paralleled oscillations for the diurnal pattern of total plasma cortisol with a lag time of approximately 4 hours (Angeli et al., 1978).

Conversely, maximum CBG levels in the rat occurred at the beginning of the dark period with lowest concentrations at the end of the dark period and beginning of the light period (Hsu et al., 1988). There was no lag time between oscillations of corticosterone and CBG concentrations. The authors concluded that fluctuations in CBG concentrations were due to a decrease in the binding protein's concentration. When endogenous corticosteroids were removed as a result of adrenalectomy, CBG levels remained similar to the nocturnal peaks observed in non-adrenalectomized rats indicating a direct relationship between corticosterone and its binding protein (Hsu et al., 1988). The authors concluded that this relationship helps to decrease the availability of corticosterone to target cells.

ACTH Stimulation/Dexamethasone Suppression

Adrenocorticotrophic hormone (ACTH) and dexamethasone can be used to stimulate or suppress adrenal response. Parrott et al. (1989) reported finding a significant increase in plasma cortisol concentration within 30 minutes of an intravenous administration of ACTH (50 to 200 IU, 250 µg/ml) in pre-pubertal pigs. Plasma cortisol exhibited a maximum increase 75 minutes post injection and a 230% increase over baseline levels. Plasma cortisol concentrations in 3-month-old Holstein calves administered ACTH (0.6 IU per kg BW) reached maximal levels at 40 minutes following injection and remained elevated for 3 hours (Negrão et al., 2004).

In contrast to the stimulatory effects of ACTH, dexamethasone will suppress the adrenal response. In rats given dexamethasone 90 minutes prior to

acute restraint, corticosterone was 80% less compared to that in rats not given dexamethasone prior to restraint. In subsequent challenges, dexamethasone administered to rats prior to the administration of CRH was able to block the induced corticosterone secretion. The authors suggested that dexamethasone is able to inhibit the HPA axis by blocking the glucocorticoid receptors primarily in the pituitary (Cole et al., 2000).

Plasma concentrations of CBG are also influenced by administration of ACTH or dexamethasone. CBG levels were measured in 2-month-old rats injected over a 13 day period with saline, ACTH or a single injection of ACTH on day 13. Rats administered ACTH daily had significantly lower CBG concentrations compared to the saline or single ACTH injected animals. The authors concluded that the decrease in CBG concentration was the result of sustained adrenal stimulation by ACTH resulting in increased corticosterone levels (Armario et al., 1994). Zhao et al. (1997) observed that in rats treated with dexamethasone for five days, serum CBG, hepatic and renal CBG mRNA was significantly higher than that in control rats.

Free Cortisol Index (FCI)

As stated previously, the free fraction of cortisol is that which is biologically available to the cells. There are a number of techniques for measuring free cortisol including equilibrium dialysis and gel filtration, yet these methods are costly and time consuming. Due to these constraints, many researchers choose to measure total cortisol concentration instead. However, by measuring total

cortisol, it is difficult to determine how much free cortisol is readily available to cells. Since 60 to 90% of circulating cortisol is bound to CBG, free cortisol is greatly affected by the availability of CBG (Dhillon et al., 2002). Situations such as stress can cause total cortisol levels to increase while CBG levels may increase or decrease, leaving the free fraction of cortisol to be misrepresented (Le Roux et al., 2002 and 2003).

Coolens et al. (1987) devised a formula for the measurement of free cortisol, based on total cortisol, CBG and albumin concentrations and their affinity constants. However, due to the difficulty in performing this calculation, it is seldom used. The free cortisol index (FCI), the ratio of plasma total cortisol to CBG concentration, may serve as an alternative for measuring actual free cortisol. The only requirements for its determination are having a means to quantify total cortisol and CBG. Le Roux et al. (2002) observed a high correlation ($R=0.90$) between the FCI and free cortisol obtained through the steady-state gel filtration, as well as a correlation of 0.98 between the FCI and free cortisol values obtained using the more complex calculation formulated by Coolens et al. (1987). In a recently conducted experiment in humans, a similarly high correlation ($R^2=0.82$) was found between the FCI and plasma free cortisol determined using an ultrafiltration assay (Lewis et al., 2003). The authors concluded that the FCI could be useful in assessing adrenal function for situations where CBG values are not stable.

CHAPTER III

MATERIALS AND METHODS

Animals and Housing

Animals used in both experiments were housed at the Johnson Animal Research and Teaching Unit (JARTU). Pigs (Premier x QMax 100) were weaned at 25 days of age, and housed in raised pens (2.44 m x 2.44 m) with slotted floors. Animals were given ad libitum access to a commercial diet (3.315 kcal/kg of ME, 18% CP, 1.15% lysine; as fed basis) and water. Artificial lights were provided 13 h, starting at 0630 h. 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 ad libitum access to feed and water. The University of Tennessee Animal Care and Use Committee approved these studies.

Experimental Design

Catheterization: Pigs were fitted with an indwelling catheter according to the procedure of Carroll et al. (1998). Briefly, animals were anesthetized using halothane administered with oxygen via a facemask attached to a closed circuit anesthesia machine. The jugular vein was located using a 21-ga access needle, and a guide wire was inserted through the access needle into the jugular vein. The access needle was removed, leaving the guide wire inside the jugular vein. A polyurethane catheter (Global Veterinary Products, New Buffalo, MI) was

inserted over the guide wire into the jugular vein approximately 7 to 8 cm in depth and the guide wire removed. The catheter was secured at the site of insertion using tissue adhesive sutures placed on either side of the catheter collar. A 20 cm extension was attached to the catheter and routed to the dorsal surface of the neck and secured with flexible bandaging tape. An additional 90 cm long extension was attached to the existing catheter/extension to allow remote access for the collection of blood samples without disturbing the pigs. The entire catheterization process took approximately 20 min per animal.

Blood Collection: Immediately prior to collection of each blood sample, 2 ml of blood was drawn from the catheter and discarded. A blood sample (5 ml) was collected in a heparinized syringe (Li-Heparin LH/4.5ml, Sarstedt Monovette, Newton, NC), and the catheter was flushed with 2.5 ml of 0.9% NaCl followed by 0.5 ml of heparinized saline (50 IU/ml in 0.9% NaCl). Samples were centrifuged at 1520 x g for 15 min, the plasma allocated to 2- 1.5 ml vials (Wheaton, Millville, NJ) and stored at -20°C.

Experiment 1: Eight 8-wk old female pigs (12.7 + 1.8 kg BW) from two litters were catheterized and placed individually in 0.61 m x 1.22 m pens. Blood samples were collected over a 24-h period beginning approximately 5 h post insertion of the catheter (1730 h). Following the fourth hourly blood sampling (2030 h), four of the eight pigs were administered ACTH (1 IU/kg BW, 10 IU/ml in 0.9% NaCl) and the remaining pigs administered saline (2.5 ml 0.9% NaCl) via the catheter. Samples were then collected at 30 min intervals for 4 h and then

hourly samples resumed 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.

Experiment 2: Twenty-four 8-wk old female pigs selected from 8 litters were assigned to 2 replicate groups of twelve pigs, with 3 pigs per litter receiving one of three treatments. Three days prior to initiation of the experiment, pigs from group one (15.7 + 2.4 kg) and group two (22.2 + 2.8 kg) were placed in individual 0.61 m x 1.22 m pens. On the morning of the experiment, each pig was fitted with a catheter, returned to its pen and assigned to one of three treatments; ACTH (1 IU/kg BW, 10 IU/ml in 0.9% NaCl), dexamethasone (40 µg/kg BW, 500 µg/ml 5% ethanol 0.9% NaCl solution), or saline (2.5 ml 0.9% NaCl). Blood sampling began at 1430 h, a minimum of 4 h after the catheter was inserted. Blood samples were collected over a 6-h period every 15 min, beginning at -1 h. Treatments were administered via the catheter immediately following the 0 h blood sampling. A total of 25 samples were collected per animal. Hematocrit values were determined on samples collected at the beginning (-1.0 h) and end of the experiment (5.0 h).

Plasma Analysis

Cortisol. Plasma total cortisol concentration was determined by radioimmunoassay as reported previously (Scroggs et al., 2002). Cross-reactivity as listed by the manufacturer was less than 0.10% for dexamethasone. Intra- and inter- assay CV were 3.6 and 13.5% for low (110 nmol/L), 4.4 and 15.7% for

medium (325 nmol/L), and 3.3 and 12.2 % for high (772 nmol/L) cortisol standards.

Corticosteroid-Binding Globulin. The concentration of porcine corticosteroid-binding globulin (pCBG) in plasma was measured by a direct ELISA as described by Roberts et al. (2003). Intra- and inter assay CV of a pooled pig plasma sample was 18 and 20%, respectively.

Free Cortisol Index. The free cortisol index was calculated using the ratio of plasma total cortisol to pCBG concentration (Le Roux et al., 2002, 2003).

Percent Free Cortisol. Percent free cortisol was determined using an ultrafiltration assay previously validated by Lewis et al. (2003). Radiolabeled cortisol (0.2 μ Ci of ^{3} H, NEN products, Boston MA) in ethanol was placed into tubes (12 x 75 mm) and allowed to dry. Unknown plasma samples (1 ml) were added to each tube, vortexed, and incubated in a 39°C water bath for 30 min. Duplicate fractions (40 μ l) of the mixture were pipetted into vials, and 4 ml of scintillation fluid (Ultima gold XP, Packard) was added for determination of total radioactivity. Two 400 μ l samples of the plasma containing the radiolabeled cortisol were placed in ultrafiltration devices (Ultrafree-MC 10,000 NMWL filter unit, Millipore, Billerica MA) preconditioned with assay buffer (0.05 mol/L of PBS, 0.1% Tween-20 and 0.1% gelatin), and centrifuged (16,110 x *g*, Eppendorf centrifuge 5415C) for 30 min at room temperature. Two 40 μ l aliquots of the ultrafiltrate were pipetted into vials, scintillation fluid added, and all vials transferred to a liquid scintillation counter (Packard tri-carb, 2900TR beta

counter) for measurement of radioactivity. Percent free cortisol was calculated as the ratio of ultrafiltrate to total radioactivity.

Free Cortisol. The concentration of free cortisol was calculated using the product of percent free cortisol and total cortisol (nmol/L). The concentration of free cortisol was expressed in nmol/L.

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (SAS Inc., v9.0 Cary, NC) for a completely randomized design. Repeated measures were utilized for indication of differences due to time. The fixed effects of treatment (ACTH, dexamethasone, and saline), time, and treatment x time interaction were used to analyze for differences in total plasma cortisol, pCBG, percent free cortisol, free cortisol, and FCI. Random effects were pig within treatment. The mean of samples taken prior to treatment administration was used as a baseline value when analyzing for differences over time. Data were represented 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.

CHAPTER IV

RESULTS

Experiment One

Blood samples were successfully collected from seven of the eight pigs at each of the designated sampling times. The catheter in one of the control pigs lost patency but was reinserted, resulting in missed samples from 2200 to 2330 h. Hematocrit values measured on samples collected at the beginning and end of the experiment were not different ($P > 0.10$).

Plasma Total Cortisol

The fluctuation in cortisol concentration for saline (control) and ACTH treated pigs over the 24 h sampling period is illustrated in Figure 1. Mean plasma total cortisol concentration prior to treatment was similar ($P > 0.10$) for saline (94.8 ± 19.4 nmol/L) and ACTH (116.3 ± 19.4 nmol/L) treated pigs. Cortisol values for saline treated pigs decreased ($P < 0.001$) to 51.2 ± 16.9 nmol/L at 2030 h and oscillated ($P < 0.001$) above and below this concentration throughout the remainder of the experiment. Plasma cortisol concentration in pigs administered ACTH was elevated ($P < 0.001$) from baseline at 1 h and returned to baseline within 4 h (Table 1). Cortisol values for ACTH treated pigs were lower ($P < 0.001$) than baseline beginning 5 h post injection and remained low for the remainder of the 24 h sampling period. Although statistically not different, total cortisol for saline treated pigs was higher than that measured for

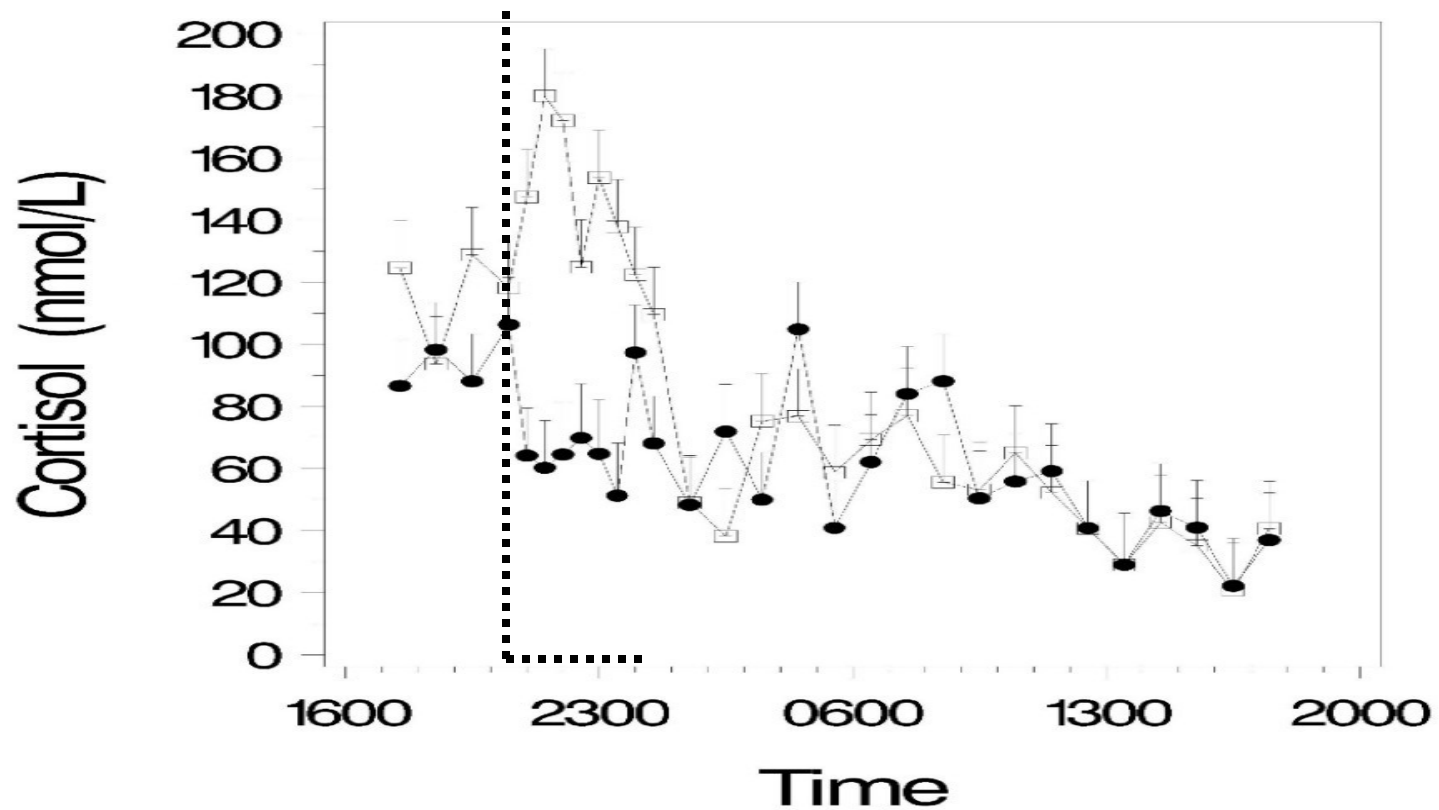


Figure 1. Changes in plasma total cortisol concentrations (nmol/L) in pigs sampled over 24 h following administration of adrenocorticotrophin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl). Each point represents the mean value ($\pm$ S.E., $n = 4$). Treatment was administered immediately following 2030 h sample (vertical dotted line). Horizontal dotted line indicates treatment differences ($P < 0.05$) during the first 4 h following administration of treatment. Statistically significant differences ($P < 0.001$) were also noted for time and treatment $\times$ time interaction.

Table 1. Least squares means $\pm$ S.E. for plasma total cortisol (nmol/L) in pigs prior to (1730 - 2030 h) and following (beginning 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples. The P-value column refers to treatment effects.

Time (h)	Saline	ACTH	P-value
1730 – 2030	98.6 $\pm$ 16.9 ^a	116.3 $\pm$ 16.9 ^{bc}	0.47
2100	64.2 $\pm$ 16.9 ^a	147.5 $\pm$ 16.9 ^{ab}	0.003
2130	60.3 $\pm$ 16.9 ^a	180.0 $\pm$ 16.9 ^a	0.001
2200	63.1 $\pm$ 18.4 ^a	172.1 $\pm$ 16.9 ^a	0.0004
2230	70.8 $\pm$ 18.4 ^a	124.8 $\pm$ 16.9 ^{bc}	0.04
2300	67.9 $\pm$ 18.4 ^a	153.7 $\pm$ 16.9 ^{ab}	0.003
2330	56.2 $\pm$ 18.4 ^a	137.9 $\pm$ 16.9 ^{abc}	0.004
2400	97.4 $\pm$ 16.9 ^a	122.5 $\pm$ 16.9 ^{bc}	0.31
0030	68.1 $\pm$ 16.9 ^a	102.1 $\pm$ 16.9 ^c	0.17
0130 - 1730	55.4 $\pm$ 16.9 ^a	51.7 $\pm$ 16.9 ^d	0.88

^{a,b,c,d} Means with uncommon superscripts within a column differ (P < 0.01).

ACTH treated pigs at 0430, 0730, and 0830 h. Cortisol concentration in both control and ACTH treated pigs exhibited a nadir at 1630 h.

Plasma pCBG Concentration

Mean plasma concentration of pCBG was similar ($P > 0.10$) among saline (6.7 ± 0.8 mg/L) and ACTH (8.7 ± 0.8 mg/L) treated pigs prior to treatment administration (Figure 2). For saline treated pigs, pCBG values were lower ($P < 0.01$) than baseline values at 0730 and 0830 h (4.6 and 4.9 ± 0.8 mg/L, respectively) and were similar to baseline for the remainder of the 24 h period. Pigs administered ACTH exhibited a lower ($P < 0.001$) pCBG concentration at 0330 h (6.7 ± 0.8 mg/L) that returned to baseline by 0930 h and remained unchanged for the remainder of the experiment. At 0230 h, pCBG for ACTH treated pigs (9.0 ± 0.8 mg/L) was greater ($P < 0.01$) than that measured for the control pigs (6.7 ± 0.8 mg/L). The pCBG for both saline and ACTH pigs decreased ($P < 0.01$) over the 24 h time period (Table 2). The lowest concentration of pCBG was observed at 0730 h in both control and ACTH animals.

Percent Free Cortisol

Mean percent free cortisol for saline (19.0 ± 0.7 %) and ACTH treated (19.2 ± 0.7 %) pigs were similar ($P > 0.10$) prior to treatment administration (Figure 3). No treatment or treatment by time ($P > 0.10$) effects were noted (Table 3). Regardless of treatment, percent free cortisol fluctuated ($P < 0.001$)

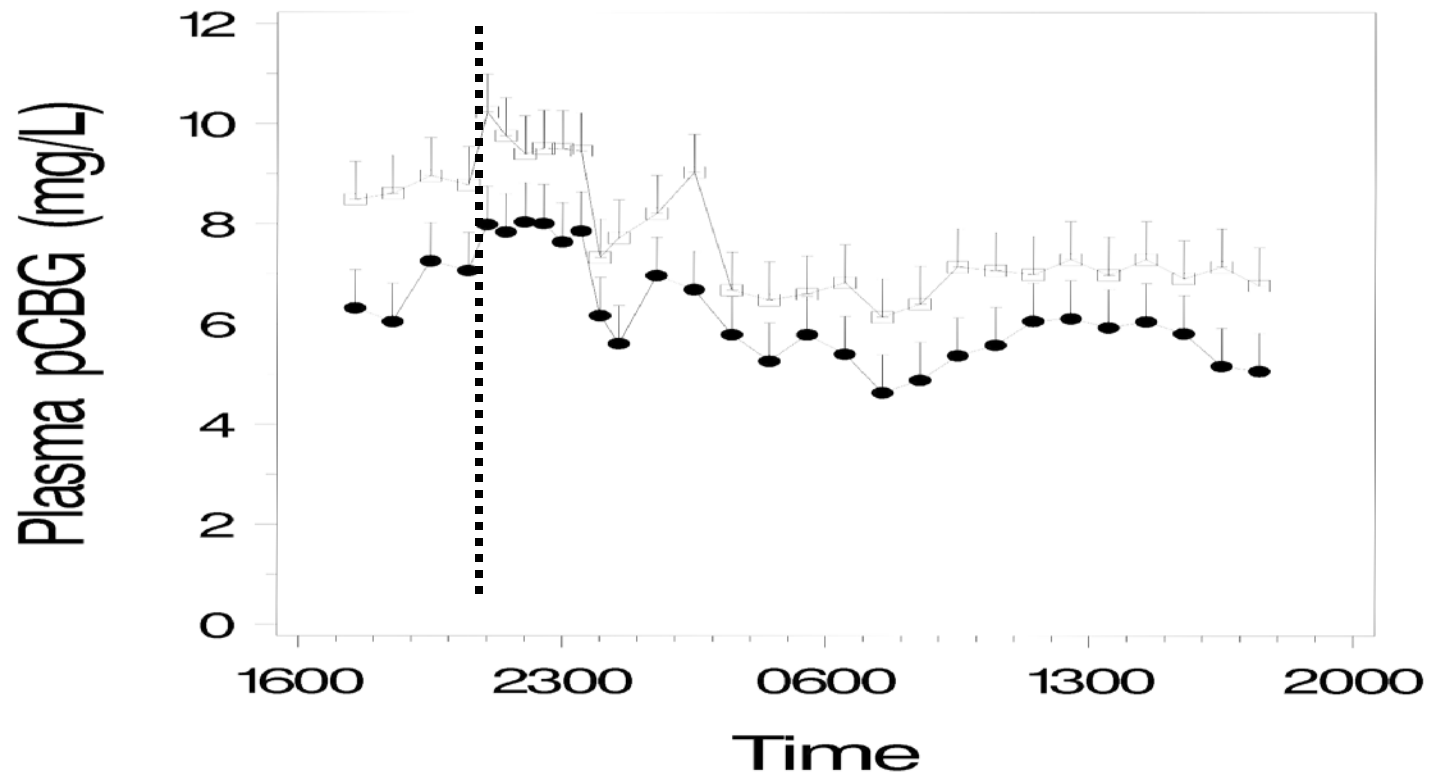


Figure 2. Changes in plasma pCBG (mg/L) in pigs sampled over 24 h following administration of adrenocorticotrophin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl). Each point represents the mean value ($\pm$ S.E., $n = 4$). Treatment was administered immediately following 2030 h sample (vertical dotted line). Statistically significant differences were noted for time ($P < 0.001$) and treatment $\times$ time ($P < 0.01$).

Table 2. Least squares means $\pm$ S.E. for plasma pCBG (mg/L) in pigs prior to (1730 – 2030 h) and following (beginning at 2030 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples. The P – value column refers to treatment effects.

Time (h)	Saline	ACTH	P-value
1730 – 2030	6.7 $\pm$ 0.8 ^{bc}	8.7 $\pm$ 0.8 ^{bc}	0.14
2100	8.0 $\pm$ 0.8 ^a	10.2 $\pm$ 0.8 ^a	0.10
2130	7.8 $\pm$ 0.8 ^a	9.8 $\pm$ 0.8 ^a	0.15
2200	8.1 $\pm$ 0.9 ^a	9.4 $\pm$ 0.8 ^{ab}	0.30
2230	8.0 $\pm$ 0.9 ^a	9.5 $\pm$ 0.8 ^{ab}	0.26
2300	7.7 $\pm$ 0.9 ^{ab}	9.5 $\pm$ 0.8 ^{ab}	0.17
2330	7.8 $\pm$ 0.9 ^{ab}	9.4 $\pm$ 0.8 ^{ab}	0.23
2400	6.2 $\pm$ 0.8 ^c	7.3 $\pm$ 0.8 ^{cd}	0.37
0030	5.6 $\pm$ 0.8 ^c	7.7 $\pm$ 0.8 ^{cd}	0.12
0130 - 1730	5.7 $\pm$ 0.8 ^c	7.0 $\pm$ 0.8 ^d	0.29

^{a,b,c,d} Means with uncommon superscripts within a column differ (P < 0.01).

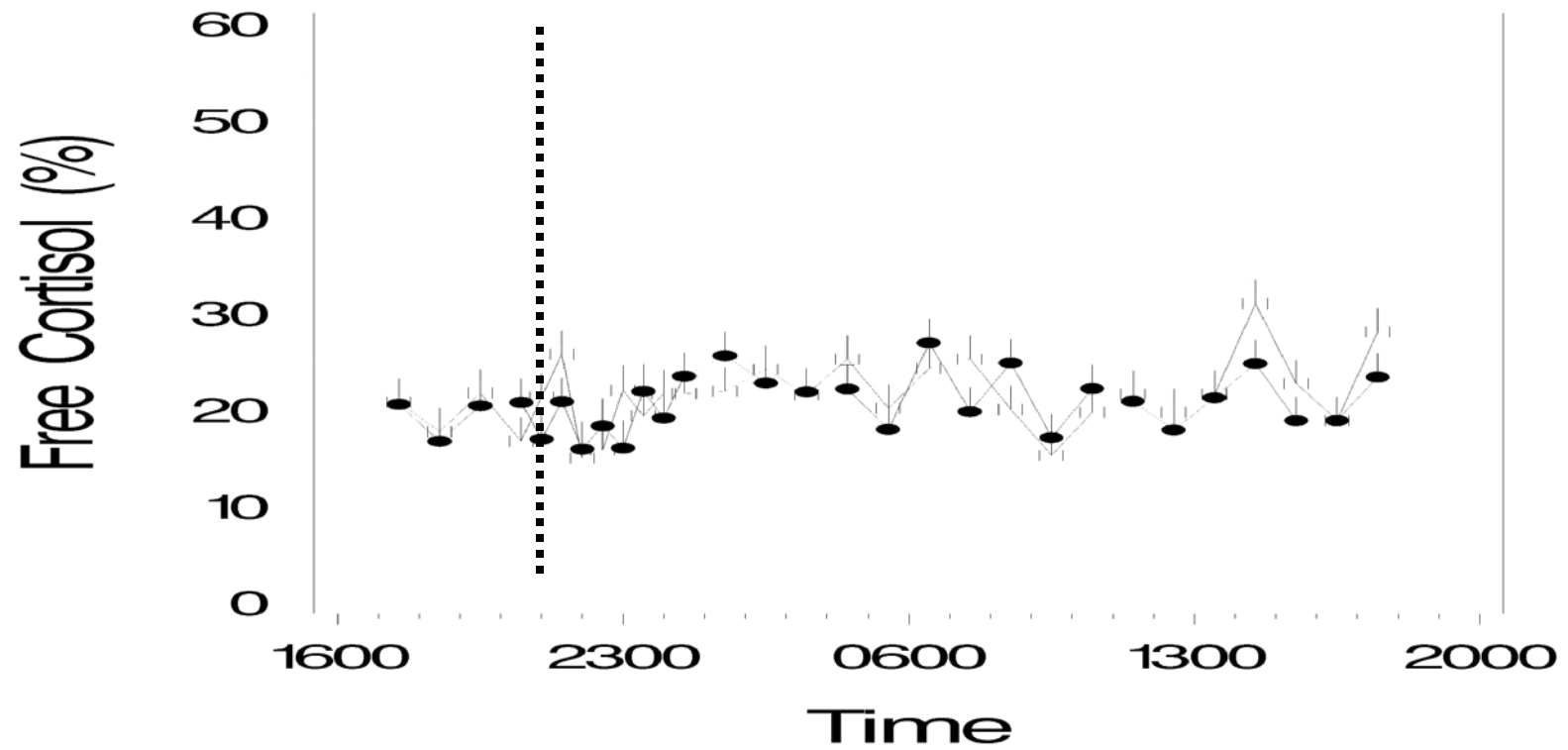


Figure 3. Changes in percent free cortisol (%) in pigs sampled over 24 h following administration of adrenocorticotrophin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl). Each point represents the mean value ($\pm$ S.E., $n = 4$). Treatment was administered immediately following 2030 h sample (vertical dotted line). Statistically significant differences ($P < 0.001$) were noted for time.

Table 3. Least squares means $\pm$ S.E. for percent free cortisol (%) in pigs prior to (1730 – 2030 h) and following (beginning at 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean hourly samples. The P – value column refers to treatment effects.

Time (h)	Saline	ACTH	P-value
1730 – 2030	19.0 $\pm$ 2.1 ^a	20.7 $\pm$ 2.1 ^{ab}	0.58
2100	16.9 $\pm$ 2.1 ^a	21.4 $\pm$ 2.1 ^{ab}	0.14
2130	20.8 $\pm$ 2.1 ^a	25.7 $\pm$ 2.1 ^a	0.11
2200	15.6 $\pm$ 2.4 ^a	15.0 $\pm$ 2.1 ^b	0.85
2230	18.0 $\pm$ 2.4 ^a	15.3 $\pm$ 2.1 ^b	0.41
2300	15.7 $\pm$ 2.4 ^a	22.0 $\pm$ 2.1 ^{ab}	0.06
2330	21.7 $\pm$ 2.4 ^a	19.4 $\pm$ 2.1 ^{ab}	0.26
2400	19.2 $\pm$ 2.1 ^a	21.7 $\pm$ 2.1 ^{ab}	0.39
0030	23.5 $\pm$ 2.1 ^a	21.7 $\pm$ 2.1 ^{ab}	0.54
0130 - 1730	21.6 $\pm$ 2.1 ^a	22.4 $\pm$ 2.1 ^{ab}	0.79

^{a,b} Means with uncommon superscripts within a column differ (P < 0.01).

over the 24 h period. Morning peak values (0630 and 0730 h) were observed in both saline and ACTH treated pigs. However, the actual percentage of free cortisol in saline pigs was higher than that in ACTH pigs during the morning peaks (26.9 vs. $19.8 \pm 2.5\%$).

Plasma Free Cortisol

Mean plasma free cortisol (total cortisol x percent free cortisol) was similar ($P > 0.10$) for the two groups of pigs prior to treatment (18.2 and 22.4 ± 3.1 nmol/L for control and ACTH pigs, respectively; Figure 4). Following administration of treatment, free cortisol for control pigs fluctuated within baseline with intermittent values that were lower ($P < 0.001$) than baseline over the 24 h. Free cortisol in ACTH treated pigs fluctuated in a similar fashion with higher ($P < 0.001$) values at 2100 and 2130 h, which subsequently fell below ($P < 0.001$) baseline values at 0130 h and remained low thereafter (Table 4). Both treatment groups exhibited their lowest ($P < 0.05$) concentrations of free cortisol at 1630 h.

Free Cortisol Index (FCI)

Mean FCI (total cortisol/pCBG) values were similar ($P > 0.10$) for control (15.1 ± 2.6 nmol/mg) and ACTH (13.7 ± 2.6 nmol/mg) pigs prior to treatment administration (Figure 5). FCI for control pigs fluctuated from 4.5 to 21.9 ± 2.5 nmol/g over the 24 h, with lower ($P < 0.001$) values persisting for three hours following the administration of saline and again during the late afternoon and evening hours. The FCI for ACTH treated pigs increased ($P < 0.001$) by 175%

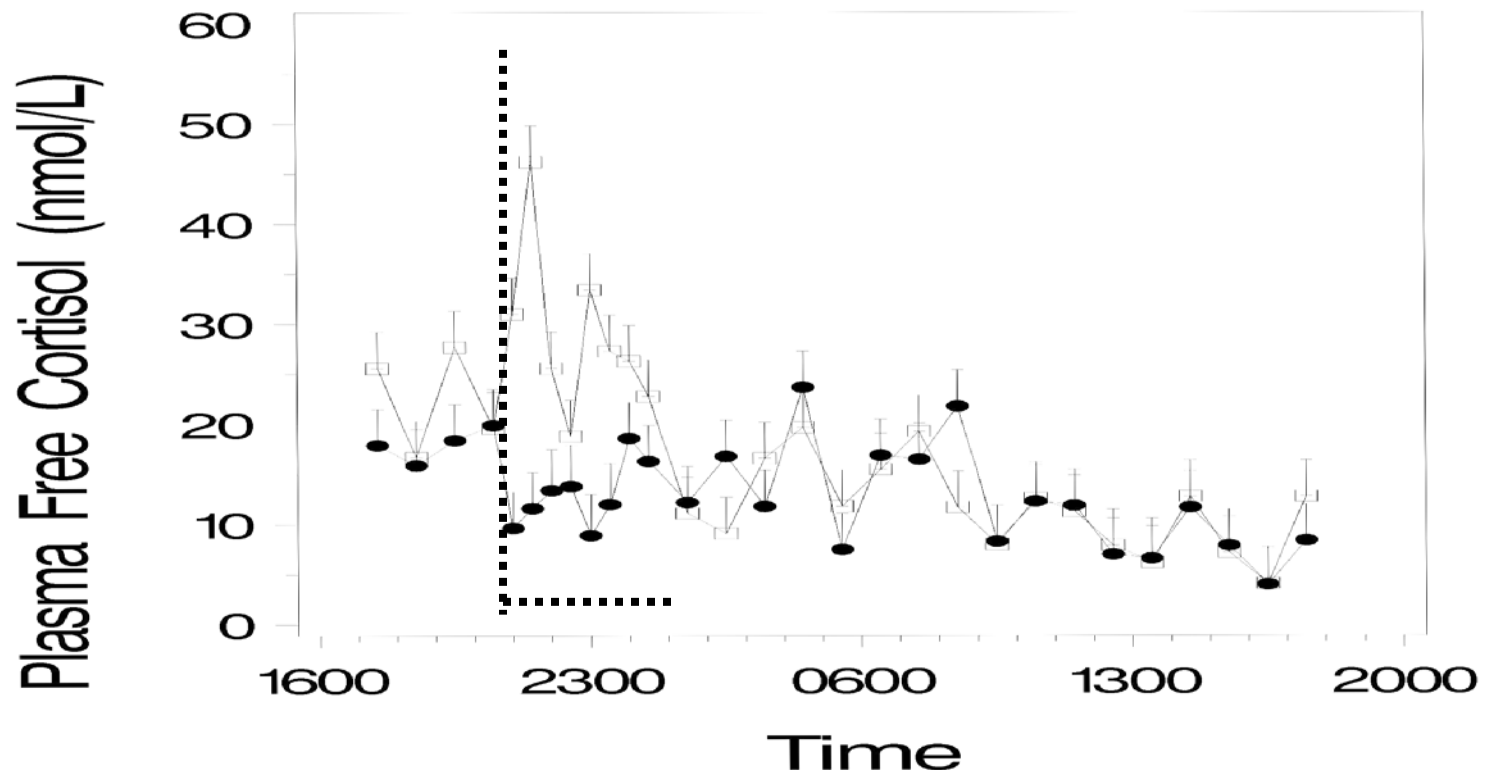


Figure 4. Changes in plasma free cortisol (nmol/L) in pigs sampled over 24 h following administration of adrenocorticotrophin ($\square$; 1 IU/kg BW) or saline ($\bullet$; 0.9% NaCl). Each point represents the mean value ($\pm$ S.E., $n=4$). Treatment was administered immediately following 2030 h sample (vertical dotted line). Horizontal dotted line indicates differences ($P < 0.05$) between treatment groups for the first 4 h following administration of treatment. Statistically significant differences ($P < 0.001$) were noted for time and treatment $\times$ time

Table 4. Least squares means $\pm$ S.E. for plasma free cortisol (nmol/L) in pigs prior to (1730 – 2030 h) and following (beginning at 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples. The P-value column refers to treatment effects.

Time (h)	Saline	ACTH	P-value
1730 – 2030	18.1 $\pm$ 3.8 ^a	21.7 $\pm$ 3.8 ^{cde}	0.49
2100	9.6 $\pm$ 3.8 ^a	31.0 $\pm$ 3.8 ^{bc}	< 0.001
2130	11.6 $\pm$ 3.8 ^a	46.2 $\pm$ 3.8 ^a	< 0.001
2200	13.6 $\pm$ 4.2 ^a	25.6 $\pm$ 3.8 ^{bcd}	0.04
2230	14.1 $\pm$ 4.2 ^a	18.8 $\pm$ 3.8 ^{de}	0.42
2300	9.3 $\pm$ 4.2 ^a	33.4 $\pm$ 3.8 ^b	0.0002
2330	12.4 $\pm$ 4.2 ^a	27.3 $\pm$ 3.8 ^{bcd}	0.14
2400	18.6 $\pm$ 3.8 ^a	26.3 $\pm$ 3.8 ^{bcd}	0.17
0030	16.3 $\pm$ 3.8 ^a	22.8 $\pm$ 3.8 ^{bcd}	0.24
0130 - 1730	12.2 $\pm$ 3.8 ^a	11.6 $\pm$ 3.8 ^e	0.91

^{a,b,c,d,e} Means with uncommon superscripts within a column differ (P < 0.01).

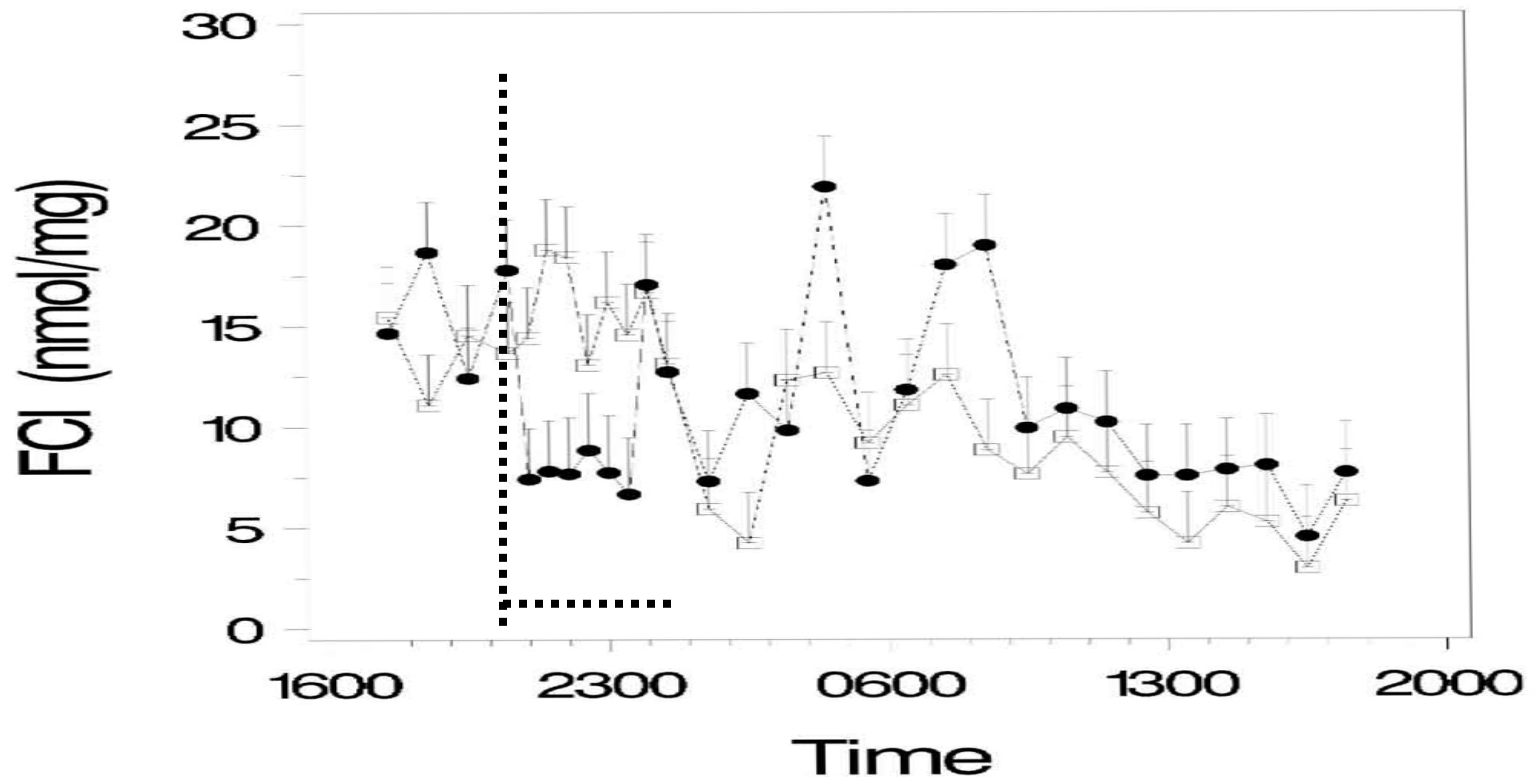


Figure 5. Changes in FCI (nmol/mg) in pigs sampled over 24 h following administration of adrenocorticotrophin (□; 1 IU/kg BW) or saline (●; 0.9% NaCl). Each point represents the mean value ($\pm$ S.E., $n = 4$). Treatment was administered immediately following 2030 h sample (vertical dotted line). Horizontal dotted line indicates differences ($P < 0.01$) among treatment groups 4 h following treatment administration. Statistically significant differences ($P < 0.001$) were noted for time.

compared to that for saline treated pigs over the 4 h period following injections (Table 5). The FCI in pigs given ACTH was lower ($P < 0.01$) than baseline from 0130 to 0230 h and from 1130 to 1730 h. Saline treated pigs exhibited peaks in FCI at 0430 h and 0730 to 0830 h, while the FCI for ACTH treated pig's remained low during these same time periods.

Relationships Among Total Cortisol, Free Cortisol, pCBG and FCI

A high correlation ($P < 0.001$) was found between FCI and free cortisol in saline ($r = 0.78$; Figure 6) and ACTH ($r = 0.83$; Figure 7) treated pigs. Plasma total cortisol was not correlated ($P > 0.10$) with pCBG concentration in either saline or ACTH pigs during the pre-treatment period (1730 – 2030 h). During the 4 h following injections (2100 – 0030 h), a positive correlation ($r = 0.67$; $P < 0.001$) existed between plasma total cortisol and pCBG in pigs administered ACTH, while no such relationship was found in saline treated pigs. During the early morning through afternoon hours (0130 – 1730 h) a negative correlation ($r = -0.48$; $P < 0.001$) between cortisol and pCBG was evident in ACTH treated pigs but not in saline treated pigs. A similar relationship ($r = -0.38$; $P < 0.001$) was found during this same period between plasma free cortisol and pCBG in ACTH treated pigs due to plasma total cortisol values being lower than baseline values and pCBG slightly increasing during this same time.

Table 5. Least squares means $\pm$ S.E. for free cortisol index (nmol/mg) in pigs prior to (1730 – 2030 h) and following (beginning at 2100 h) administration of saline (n = 4) or ACTH (n = 4) over a 24 h period. Time presented as a range represents the mean of hourly samples. The P-value column refers to treatment effects.

Time (h)	Saline	ACTH	P-value
1730 – 2030	15.1 $\pm$ 2.2 ^{ab}	13.7 $\pm$ 2.2 ^{ab}	0.64
2100	7.4 $\pm$ 2.2 ^c	14.4 $\pm$ 2.2 ^a	0.03
2130	7.8 $\pm$ 2.2 ^c	18.8 $\pm$ 2.2 ^a	0.002
2200	7.5 $\pm$ 2.4 ^c	18.4 $\pm$ 2.2 ^a	0.002
2230	8.8 $\pm$ 2.4 ^{bc}	13.1 $\pm$ 2.2 ^{ab}	0.20
2300	7.9 $\pm$ 2.4 ^c	16.2 $\pm$ 2.2 ^a	0.02
2330	7.1 $\pm$ 2.4 ^c	14.6 $\pm$ 2.2 ^a	0.03
2400	17.1 $\pm$ 2.2 ^a	16.7 $\pm$ 2.2 ^a	0.90
0030	12.7 $\pm$ 2.2 ^{abc}	13.1 $\pm$ 2.2 ^{ab}	0.89
0130 - 1730	10.6 $\pm$ 2.2 ^{bc}	7.8 $\pm$ 2.2 ^b	0.36

^{a,b,c} Means with uncommon superscripts within a column differ (P < 0.01).

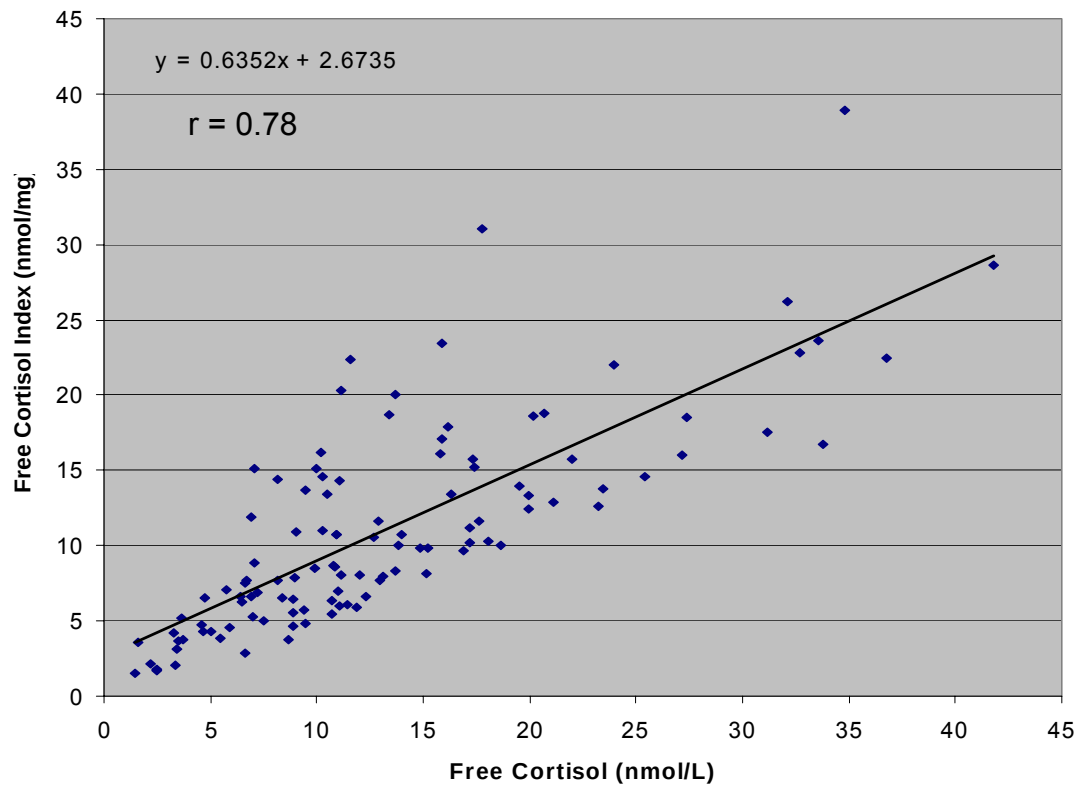


Figure 6. Relationship between the calculated free cortisol index (total cortisol/pCBG) and free cortisol for saline (0.9% NaCl) treated pigs (n = 4).

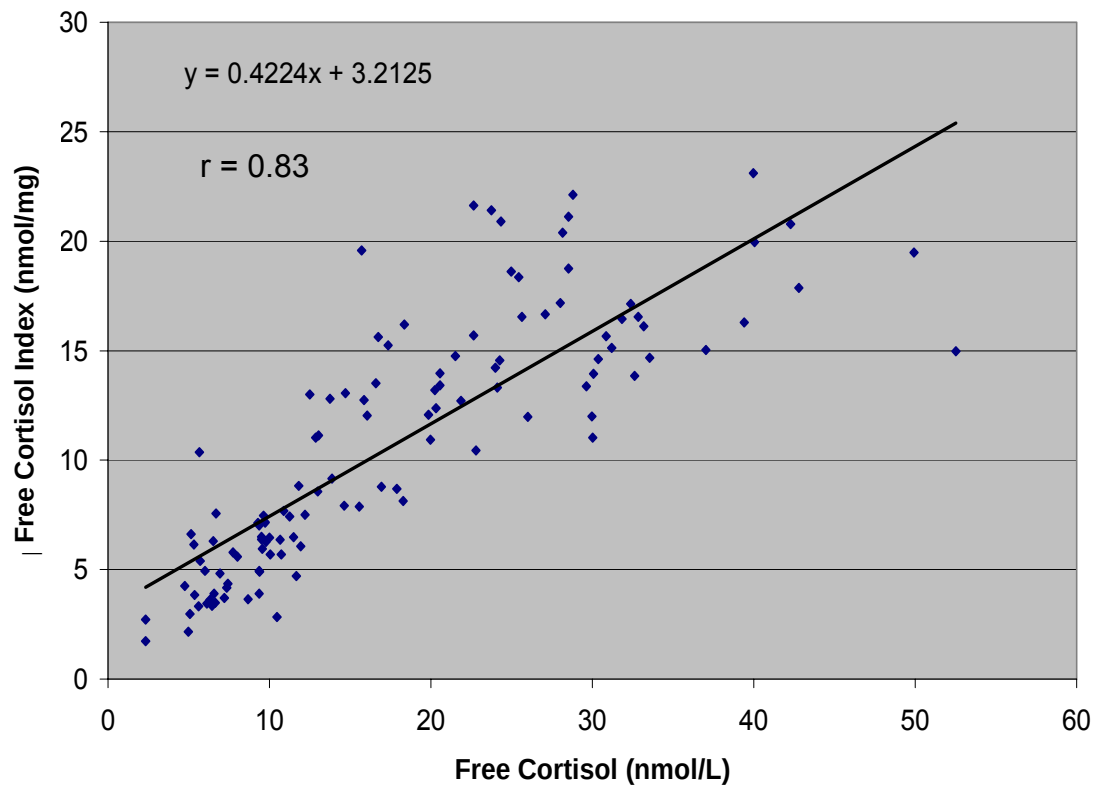


Figure 7. Relationship between the calculated free cortisol index (total cortisol/pCBG) and free cortisol for adrenocorticotropin (1IU/kg BW) treated pigs (n = 4).

Experiment Two

One saline treated pig in the first replicate died during recovery following catheterization. During the second replication, the catheter of a control pig lost patency one hour after sampling began. Preliminary analysis indicated there was no effect due to replicate for any of the variables measured; hence, data from both replicates were combined. Hematocrit values for all treatment groups were similar among pigs as well as over the sampling period.

Plasma Total Cortisol

Mean plasma total cortisol concentrations were similar ($P > 0.10$) between saline (77.3 ± 11.0 nmol/L), ACTH (77.6 ± 9.5 nmol/L), and dexamethasone (93.5 ± 9.5 nmol/L) treated animals prior to treatment administration (Figure 8). Plasma total cortisol in saline treated pigs decreased ($P < 0.001$) from baseline values beginning at 0.5 h (52.7 ± 12.5 nmol/L) and remained low for the remainder of the experiment. Pigs administered ACTH exhibited a greater ($P < 0.001$) cortisol concentration compared to baseline by 0.25 h (213.8 ± 10.9 nmol/L), which continued until 2.75 h (101.67 ± 10.9 nmol/L). At 3 h following ACTH injection, plasma total cortisol values returned to baseline and were lower ($P < 0.001$) than baseline by 3.75 h (45.7 ± 10.9 nmol/L) where they persisted for the remainder of the experiment. In the dexamethasone treated group, plasma cortisol values were lower ($P < 0.001$) than baseline at 0.5 h (54.5 ± 10.9 nmol/L) and remained suppressed for the duration of the experiment.

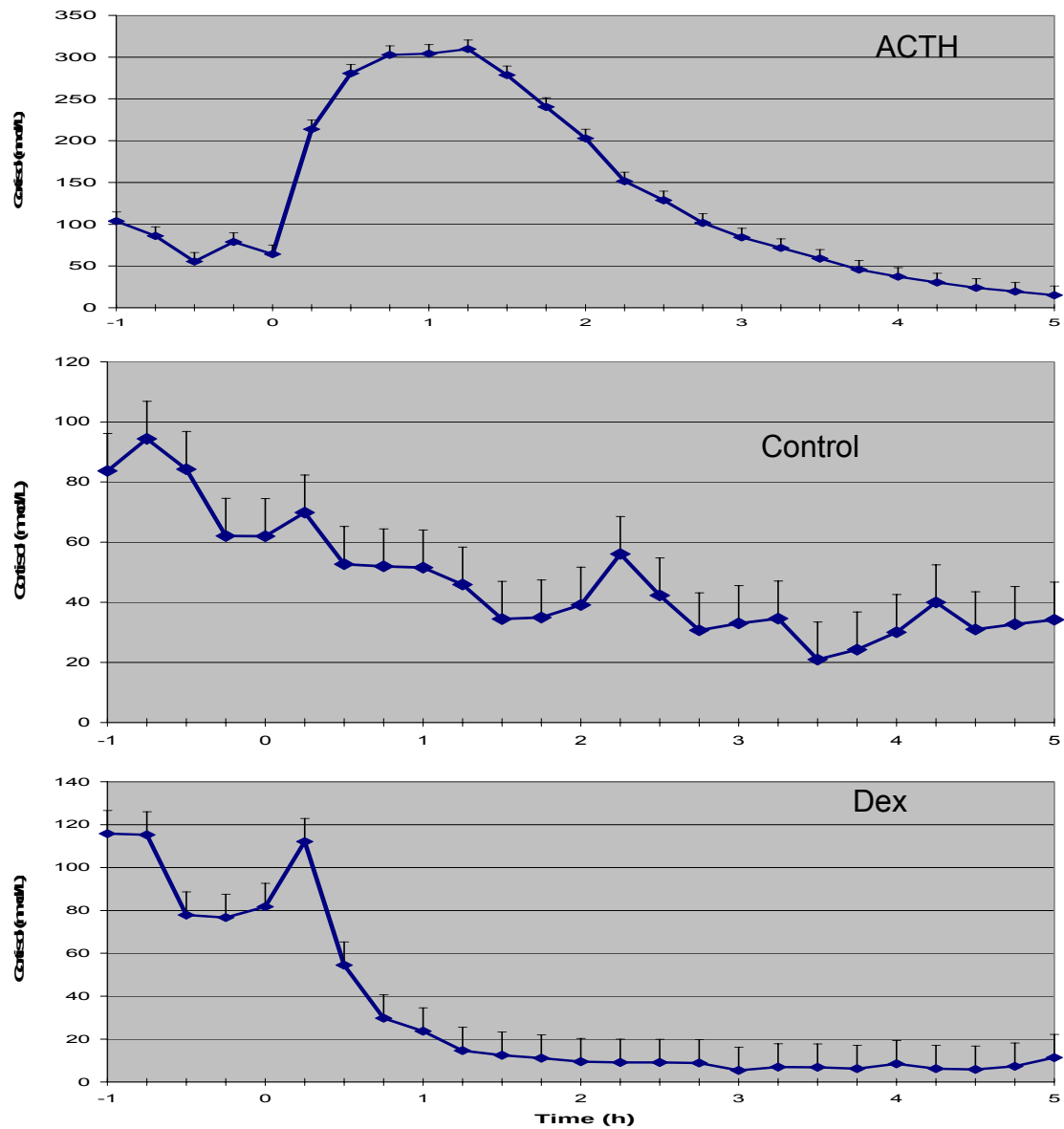


Figure 8. Changes in plasma total cortisol (nmol/L) in pigs following administration of adrenocorticotrophin (ACTH; 1 IU/kg BW), saline (control; 0.9% NaCl), or dexamethasone (Dex; 40 μ g/kg BW). Treatment was administered immediately following 0 h sample. Each point represents the mean value ($\pm$ SE, $n = 7 - 8$). Statistically significant differences ($P < 0.001$) were observed due to time, treatment, and time $\times$ treatment.

Plasma pCBG Concentration

Mean plasma pCBG concentrations were similar ($P > 0.10$) among treatment groups prior to administration of saline (9.9 ± 1.6 mg/L), ACTH (13.0 ± 1.3 mg/L) or dexamethasone (9.3 ± 1.3 mg/L) (Figure 9). No differences ($P > 0.05$) were observed due to treatment or time. The pCBG concentrations for saline treated pigs remained similar to baseline values throughout the course of the experiment. In ACTH pigs, pCBG concentrations were lower ($P < 0.001$) than the mean baseline value from 1.75 to 3.25 h post-treatment, and were similar to the baseline value for the rest of the experiment. The concentration of pCBG in dexamethasone treated pigs was elevated ($P < 0.05$) by 1 h post-injection (13.6 ± 1.5 mg/L) and remained elevated until 4.5 h (13.0 ± 1.5 mg/L), before returning to baseline by 4.75 h. At the end of the experiment (5 h) pCBG for ACTH treated pigs was greater ($P < 0.05$) than that of the dexamethasone or saline treatment groups.

Free Cortisol Index

Dexamethasone treated pigs had a higher ($P < 0.05$) FCI baseline value (10.9 ± 1.1 nmol/mg) compared to the saline and ACTH treatment groups (9.5 and 6.7 ± 1.1 nmol/mg, respectively) (Figure 10). FCI decreased ($P < 0.001$) in saline treated pigs at 0.5 h (6.0 ± 1.8 nmol/mg) and remained low for the remainder of the experiment. Following administration of ACTH, FCI increased ($P < 0.001$) at 0.25 h to 20.8 ± 1.9 nmol/mg and remained elevated until 2.75 h. At 3 h post ACTH injection, FCI returned to baseline and was lower ($P < 0.001$)

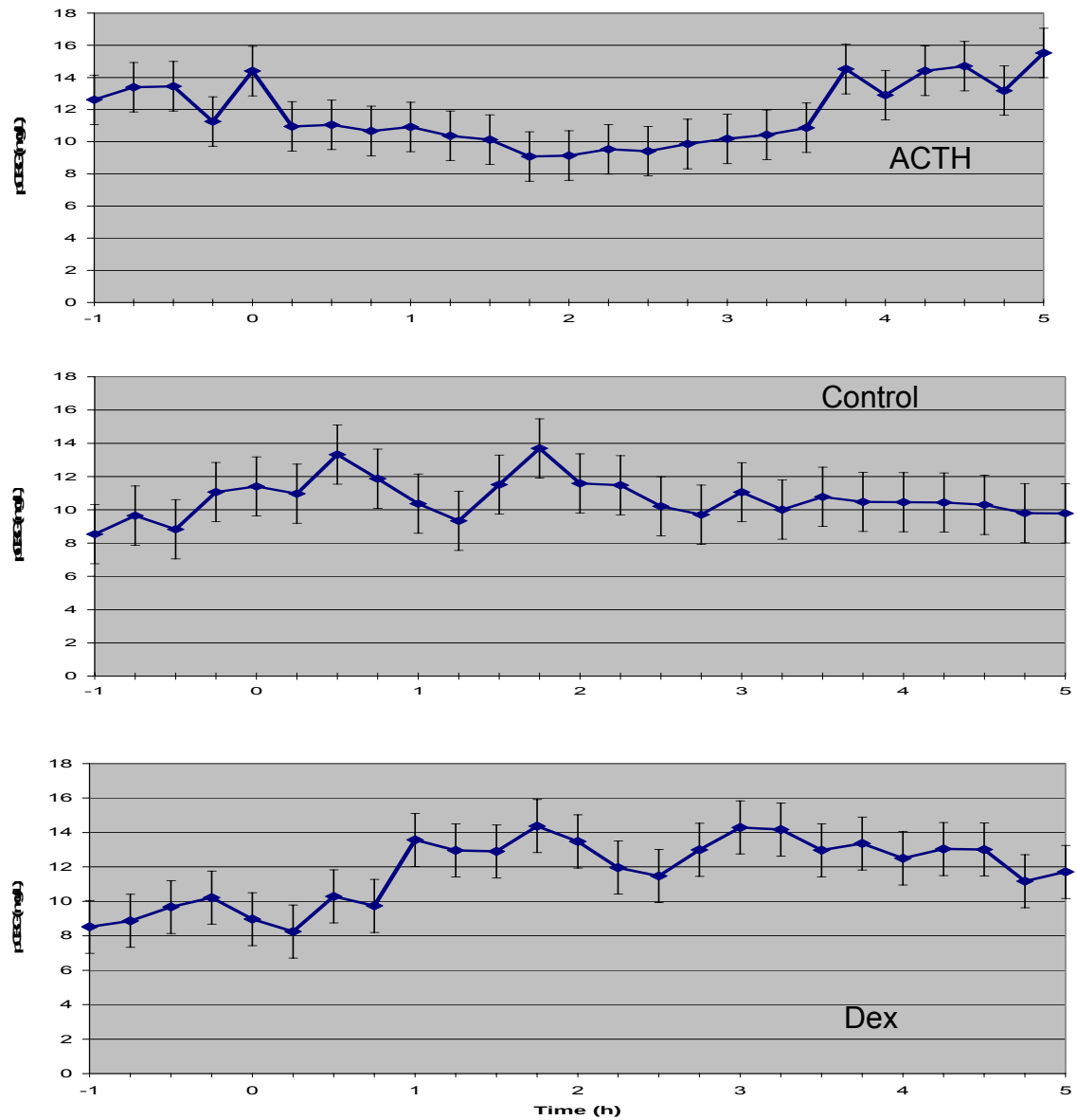


Figure 9. Changes in pCBG concentrations (mg/L) in pigs following administration of adrenocorticotrophin (ACTH; 1 IU/kg BW), saline (control; 0.9% NaCl), or dexamethasone (Dex; 40 µg/kg BW). Treatment was administered immediately following 0 h sample. Each point represents the mean value ($\pm$ SE, $n = 7 - 8$). Statistically significant differences ($P < 0.05$) were observed due to time x treatment.

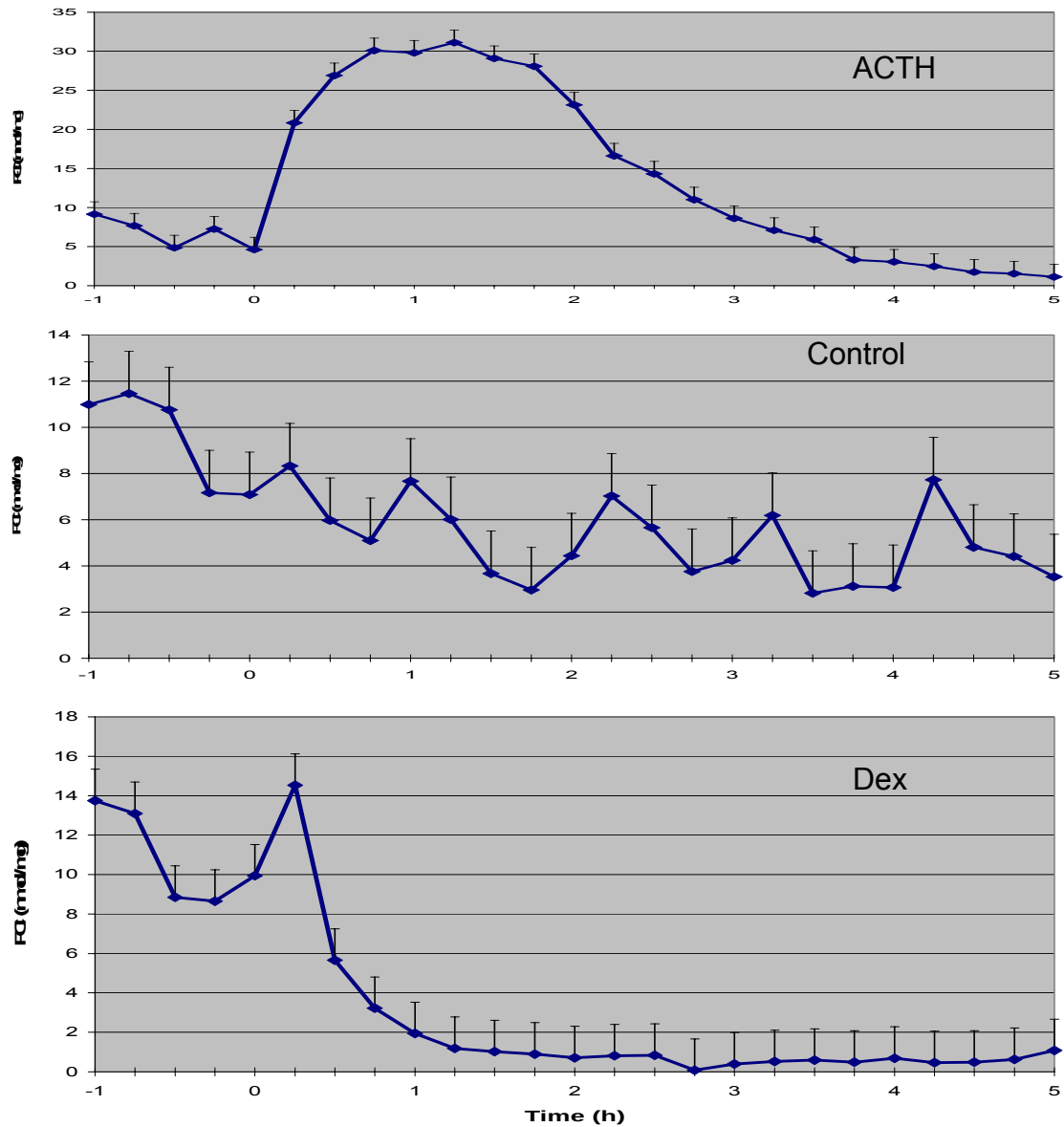


Figure 10. Changes in FCI (nmol/mg) in pigs following administration of adrenocorticotrophin (ACTH; 1 IU/kg BW), saline (control; 0.9% NaCl), or dexamethasone (Dex; 40 μ g/kg BW). Treatment was administered immediately following 0 h sample. Each point represents the mean value ($\pm$ SE, $n = 7 - 8$). Statistically significant differences ($P < 0.001$) were observed for treatment, time and treatment x time.

at 0.25 h to 20.8 ± 1.9 nmol/mg and remained elevated until 2.75 h. At 3 h post ACTH injection, FCI returned to baseline and was lower ($P < 0.001$) from 3.75 to 5 h compared to baseline values. In pigs administered dexamethasone, FCI increased ($P < 0.001$) to 14.5 ± 1.6 nmol/mg within 15 min of treatment administration. However, by 0.5 h the FCI decreased ($P < 0.001$) to 5.5 ± 1.6 nmol/mg and remained lower ($P < 0.001$) than baseline as well as the saline or ACTH treatment group values at similar sampling times for the remainder of the experiment.

Relationships Among Plasma Total Cortisol and Plasma pCBG

No relationship was found between plasma total cortisol and pCBG for any of the groups, prior to treatment administration. A positive relationship ($r = 0.27$) was observed between plasma cortisol and pCBG for the ACTH treated pigs for samples collected from 0.25 to 3.0 h. An inverse ($P < 0.05$) relationship was observed between plasma cortisol and pCBG for saline ($r = -0.36$) and dexamethasone ($r = -0.19$) treated pigs for all samples collected following treatment administration.

CHAPTER V

DISCUSSION AND IMPLICATIONS

The results from the present studies affirm our contention that FCI is a suitable estimate of free cortisol in swine. A better illustration of the temporal relationships between plasma total cortisol and pCBG may be assessed by the measurement of both along with the calculation of the FCI.

Initial plasma total cortisol values for the 24 h study were elevated and may be a result of the pigs not receiving an acclimation period from being moved from the group pen into their individual pens, as was not the case for pigs in experiment 2. The diurnal pattern for plasma cortisol was evident in the present study with a morning peak occurring at 0730 - 0830 h and an afternoon trough at 1630 h. Whipp et al. (1970) reported a morning peak in 12 - 24 wk old pigs at 0800 h and a trough at 1600 h, while Bottoms et al. (1972) observed a morning peak at 1000 h and a trough at 1400 h in adult pigs. Higher cortisol values in the morning have been associated with the awakening response in diurnal animals (Edwards et al., 2001). Values for the afternoon trough were similar to those reported by Bottoms et al. (1972) and Whipp et al. (1970). The concentration of cortisol is typically in humans lower in the afternoon in response to the onset of the sleep cycle (Czeisler, 1995).

Plasma pCBG concentrations for pigs in the present study were similar to those reported previously (Roberts et al., 2003). Hematocrit values did not

change over the 24 h period suggesting that the decrease in pCBG concentration, as well as that of total cortisol, was not a result of changes in plasma volume but rather actual physiological changes in total cortisol and pCBG concentration within the circulation. A diurnal pattern was observed for pCBG over the 24 h period with higher concentrations observed during 2100 to 2330 h and lower values evident through the rest of the morning and afternoon. Compared to previous studies, pCBG values in the present study did not parallel oscillations of cortisol as observed in female rats (Hsu et al., 1988), nor was there a lag time detected between the two plasma constituents similar to that observed in humans (Angeli et al., 1978).

The percentage of free cortisol as determined by a ligand-binding assay did follow a circadian pattern like that of cortisol, increasing through the night hours before a gradual decrease through the afternoon hours. This is consistent with studies by Edwards et al. (2001) who observed an increase in free cortisol within 30 min of awakening in humans. In our study, the afternoon trough (1630 h) that was observed for plasma total cortisol was also present for percent free cortisol. Actual free cortisol in circulation, that which is biologically available for uptake by cells (Hammond, 1990), is the product of plasma total cortisol and percent free cortisol. The diurnal pattern of plasma free cortisol in our study is similar to that measured in saliva of 8 d old pigs, as documented by Gallagher et al. (2002), where a gradual decrease was observed from morning to afternoon and evening. Ruis et al. (1997) measured salivary cortisol every 4 h over a 24 h

period in 12 to 25 wk old pigs. Their results indicated that the circadian rhythm of salivary cortisol oscillated in a bell shape with peak values present shortly after 1200 h. The amplitude of the curve decreased as pigs aged from 12 wks to 24 wks. Results from our study illustrated an oscillating pattern. Free cortisol values oscillated during the morning hours to reach a peak value and then began to decrease through the late morning and early afternoon, but not at a constant decline.

The FCI, a ratio of circulating total cortisol to CBG, has been demonstrated to be a reliable and easy to use estimate of plasma free cortisol in humans (Lewis et al., 2003). A circadian pattern was observed in FCI, with elevated values observed during 0730 to 0830 h when pCBG was low and total cortisol concentration was high. While in the afternoon, when both plasma pCBG and cortisol were reduced, the FCI was at its lowest value. The inability to detect a relationship between plasma total cortisol and pCBG concentrations for control pigs may be due to the fact that FCI does not take into consideration the amount of cortisol that is loosely bound to albumin. Previous research in our laboratory has indicated that concentrations of pCBG do not peak until 12 weeks of age in swine and remain constant over the next 3 months (Roberts et al., 2003). The pigs used in the present study were 8 weeks old and therefore may not have reached the peak of pCBG concentrations observed previously.

Injection of ACTH temporarily interrupted the circadian rhythm of plasma cortisol for 4 h before returning to values similar to control pigs. A previous study

in 8 wk old pigs showed that following an ACTH injection, plasma cortisol reached peak concentrations at 1 h and returned to baseline values within 2 h post injection (Manteuffel et al., 2002). While in calves, plasma cortisol values reached peak activity at 40 min post ACTH injection and remained elevated for 5 h (Nagrão et al., 2004). 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 the control pigs. Gilts that were tether housed had an increase in plasma cortisol in the evening but did not have a morning peak, resulting in a suppressed diurnal rhythm (de Jong et al., 2000). Afternoon concentrations of plasma total cortisol and percent free cortisol were elevated in gilts following restraint (Kattesh et al., 1995). This restraint temporarily interrupted the diurnal rhythm of total cortisol for four days before returning to a pre-restraint rhythm.

In the 24 h study, pCBG values for ACTH treated pigs fluctuated over time in a similar fashion as that of the control pigs. Armario et al. (1994) reported a similar finding that CBG values did not change in rats given a single injection of ACTH. The positive correlation between plasma total cortisol and pCBG observed following ACTH administration in our study was probably a result of increasing plasma total cortisol values and stable pCBG concentrations. The negative correlation in the ACTH treated pigs between total cortisol and pCBG over the 5 h following treatment, suggests a delayed effect of the elevated cortisol immediately following treatment administration on circulating pCBG

concentrations. Fleshner et al. (1995) reported that corticosterone concentrations were elevated immediately following inescapable tail shock, while CBG values were not decreased until 6.5 h following tail shock. However, the authors concluded that the reduced CBG values were due to an increase in CBG clearance and not by corticosterone itself.

Following injection of ACTH, percent free cortisol remained similar to that in control pigs. However, actual free cortisol (percent free x total cortisol) was elevated for 4 h following ACTH injection. This was due to plasma total cortisol being elevated, while pCBG concentrations remained constant during the 4 h following injection. 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, resulting in a low FCI. Percent free cortisol remained stable over the 24 h relative to the amount of total and actual free cortisol in circulation. Excess cortisol may have bound to albumin and then shifted to CBG later, or been excreted. This shift of cortisol from unbound and albumin-bound to CBG bound has been documented in pigs from time of birth to 6 wks of age (Kattesh et al., 1990).

The FCI was elevated for 4 h following administration of ACTH due to elevated plasma total cortisol and stable pCBG values. Once the FCI peaked in pigs administered ACTH, it then fell below baseline concentrations for 2 h before returning to values similar to the saline treated pigs. However, the FCI in ACTH treated pigs did not increase similar to that of the control pigs during the morning

hours. Our laboratory proposed that a decrease in CBG synthesis, corresponding to increasing cortisol levels, would effectively increase the clearance rate and thereby lower circulating cortisol concentrations (Behrens, et al., 1993). This is evident in the ACTH treated pigs where the concentration of plasma total cortisol, pCBG, and free cortisol fell below baseline values after increasing in response to ACTH. In a recent study, the FCI in pigs following 7 d of heat and social stress was not different compared to d 0 due to a simultaneous decrease in plasma total cortisol and pCBG concentrations (Heo et al., 2005). 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.

A closer assessment of the relationship between plasma total cortisol, pCBG and FCI was performed over a shorter time period in the second experiment. The plasma total cortisol pattern for saline treated pigs in the 6 h study decreased in a fashion similar those in the first study with respect to the time of day that they were sampled. In the second experiment, plasma total cortisol was elevated from the baseline value within 15 min of ACTH injection and returned to pre-injection levels 3 h post injection. This response was similar to that observed for ACTH-treated pigs in the 24 h study and previous studies in pre-pubertal pigs (Parrott et al., 1989) and 3 month-old Holsteins (Negrão et al., 2004). However, in the second experiment, the magnitude of total cortisol concentration following ACTH was greater. In the first experiment the ACTH was filtered, which may have decreased its actual concentration. Parrott et al. (1989)

concluded there was no difference in the plasma cortisol response as a result of differing doses of ACTH administered to pigs ranging in weight from 30 to 40 kg. The maximum value of 65 nmol/L was observed at 75 min following injection of 200 IU of ACTH. However, the 50, 100, and 200 IU concentrations of ACTH in their study were much greater than that used in our two experiments. The authors concluded that the failure to show a differential response to various ACTH doses may be due to adrenal insensitivity in 100 to 160 day old pigs.

In pigs administered dexamethasone, plasma total cortisol was suppressed for a longer period of time compared to the elevated cortisol levels following the administration of ACTH. This extended effect of dexamethasone on circulating cortisol concentration has been attributed to its antagonistic effect on glucocorticoid receptors within the pituitary gland (Cole et al., 2000). The spike in total cortisol immediately following the administration of dexamethasone was unexpected. No such spike was noted in previous studies examining the effects of dexamethasone on total cortisol within the circulation since samples were collected hours or days after dexamethasone treatment (Smith and Hammond, 1992; Zhao et al., 1997; and Lopes et al., 2004). A possible explanation for the transient increase of cortisol in our study may be as a result of the alcohol that was used to dissolve the dexamethasone. Jenkins and coworkers (1968) observed an increase in plasma cortisol within 30 min of an intravenous infusion of ethanol.

Concentrations of pCBG in the second experiment were similar to those previously reported by Roberts et al. (2003), as well as those measured in the first experiment. As a corticosteroid agonist, dexamethasone has less than 0.1 % binding affinity to CBG compared with cortisol and therefore dexamethasone should not interfere with cortisol binding to CBG (De Kloet et al., 1975). The increase in pCBG concentrations observed following dexamethasone treatment was most likely a result of decreasing cortisol concentration (Feldman et al., 1979).

In the second study pCBG decreased 1.75 to 3.25 h following ACTH injection. While the effects of ACTH on plasma total cortisol were almost immediate, pCBG levels did not reach their lowest values until 2 h post injection before rising to pre-injection levels. The delay between the onset of changes for plasma total cortisol and pCBG in the present study could reflect the affect of glucocorticoids have on CBG production rates as well as circulating CBG concentrations (Feldman et al., 1979). Behrens et al. (1993) observed a decrease in CBG binding capacity in gilts administered hydrocortisone acetate twice daily over a 10 d period. The authors suggested that the decrease in CBG binding capacity, an indirect measure of CBG concentration, may aid in maintaining stable free cortisol levels within the circulation.

Pigs administered dexamethasone exhibited elevated plasma pCBG, while corresponding plasma total cortisol concentration was reduced. Gilts administered dexamethasone every 12 h from d 13 to 22 of pregnancy had a

similar CBG binding capacity to that of saline injected gilts (Madej et al., 1997). The authors concluded that the dose of dexamethasone may have been too low to elicit a response. Dexamethasone treatment has been reported to increase plasma CBG concentrations within the ovine fetus (Berdusco et al., 1993) and in liver and kidney of the immature mouse (Zhao et al., 1997). Conversely, adult rats (Kawai et al., 1972), sheep (Berdusco et al., 1993), and adult mouse (Zhao et al., 1997) had decreased levels of CBG when challenged with dexamethasone. It was concluded that glucocorticoids' effect on CBG may be specific to the stage of development within a particular animal (Zhao et al., 1997). No relationship between total or free cortisol and CBG was observed in adult guinea pigs (Fujieda et al., 1982). The authors concluded CBG may have a minor role in the distribution of plasma cortisol in guinea pigs due to a low affinity for cortisol relative to humans or pigs. In our study, the effect of dexamethasone on pCBG concentration was a gradual increase compared to the immediate suppression of plasma cortisol. In that CBG helps determine plasma cortisol concentrations relative to its production (Bright and Darmaun, 1995; Bright, 1995), a decrease in circulating cortisol would prompt an increase in CBG to maintain physiological concentrations of glucocorticoids.

The significance of the observed plasma cortisol-CBG relationship reported here and in previous studies may reside in the calculation of the FCI. In the second experiment, the FCI increased following the administration of ACTH resulting from an increase in total cortisol and a decrease in pCBG

concentrations. In the dexamethasone treatment group, the FCI decreased as a result of a suppression of total cortisol and an increase in pCBG. An increase in CBG synthesis corresponding with decreased cortisol levels may increase cortisol conservation by binding as much cortisol as possible (Kawai et al., 1972). Since 60% of total cortisol is bound to pCBG in the pig (Kattesh et al., 1990), the amount of cortisol that is available to cells may be misrepresented if the concentration of pCBG is not taken into consideration along with that of total cortisol. The FCI can provide a better understanding of assessing temporal changes in the adrenal response in animals than by measuring total plasma cortisol alone.

Implications

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. It is also the first experiment to indicate the relationship between plasma cortisol and pCBG in swine immediately following acute stimulation or suppression of the adrenal gland. By factoring in CBG values through the application of the FCI, a more accurate portrayal of the availability of circulating cortisol is possible. The calculation of the FCI illustrates the amount of free cortisol available to cells without employing time consuming and expensive analytical procedures for the actual measure of free cortisol.

LITERATURE CITED

LITERATURE CITED

- Ali, S., and Bassett, J.R. 1995. Studies on the role of glycosylation in the origin of the electrophoretic variants for rat corticosteroid-binding globulin. *Steroids*. 60:743-752.
- Angeli, A., R. Frajria, R. DePaoli, D. Fonzo, and F. Ceresa. 1978. Diurnal variation of prednisolone binding to serum corticosteroid-binding globulin in man. *Clin. Pharmacol. Ther.* 23:47-53.
- Armario, A., M. Giralt, O. Marti, A. Gavalda, J. Hidalgo, B. R-S. Hsu, and R.W. Kuhn. 1994. The effect of acute and chronic ACTH administration on pituitary-adrenal response to acute immobilization stress. Relationship to changes in corticosteroid-binding globulin. *Endocrine Res.* 20: 139-149.
- Ballard, P.L. 1979. Delivery and transport of glucocorticoids to target cells; in Baxter and Rosseau, *Monographs on endocrinology: glucocorticoid hormone action* 12:25-48.
- Barnett, J.L., G.M. Cronin, and C.G. Winfield. 1981a. The effects of individual and group penning of pigs on total and free plasma corticosteroids and the maximum corticosteroid binding capacity. *Gen. Comp. Endocrinol.* 44:219-225.
- Barnett, J.L., C.G. Winfield, G.M. Cronin, and A.M. Makin. 1981b. Effects of photoperiod and feeding on plasma corticosteroid concentrations and maximum corticosteroid-binding capacity in pigs. *Aust. J. Biol. Sci.* 34:577-85.
- Bartalena, L., G.L. Hammond, A. Farsetti, I.L. Flink, and J. Robbins. 1993. Interleukin-6 inhibits Corticosteroid-binding globulin synthesis by human hepatoblastoma-derived (Hep G2) cells. *Endocrinology* 133:291-296.
- Baxter, J.D., and G.G. Rousseau. 1979. Glucocorticoid hormone action: an overview; in Baxter, Rousseau, *Monographs on endocrinology: glucocorticoid hormone action* 12:1-24.
- Becker, B.A., J.A. Nienaber, J.A. DeShazer, and G.L. Hahn. 1985. Effect of transportation on cortisol concentrations and on the circadian rhythm of cortisol in gilts. *Am. J. Vet. Res.* 46: 1457-1459.

- Behrens, M.J., H.G. Kattesh, and G.A. Baumbach. 1993. Plasma and uterine cortisol, progesterone and protein changes in pseudopregnant gilts treated with hydrocortisone acetate. *Theriogenology* 40:1231-1241.
- Berdusco, E.T.M., G.L. Hammond, R.A. Jacobs, A. Grolla, K. Akagi, D. Langlois, and J.R.G. Challis. 1993. Glucocorticoid-induced increase in plasma corticosteroid-binding globulin levels in fetal sheep associated with increased biosynthesis and alterations of glycosylation. *Endocrinol.* 132:2001-2008.
- Berdusco, E.T.M., K. Yang, G.L. Hammond, and J.R.G. Challis. 1995. Corticosteroid-binding globulin (CBG) production by hepatic and extra-hepatic sites in the ovine fetus; effects of CBG on glucocorticoid negative feedback on pituitary cells in vitro. *J. Endocrin.* 146:121-130.
- Bertherat J., L. Billaud, and B. Guilhaume. 2002. Cushing's syndrome and adrenal insufficiency in pregnancy. *Annales D Endocrinologie* 63:452-456.
- Bonte, H.A., R.J. van den Hoven, G. van der Sluijs Veer, and I. Vermes. 1999. The use of free cortisol index for laboratory assessment of pituitary-adrenal function. *Clin. Chem. Lab. Med.* 37:127-132.
- von Borell E. 1995. Neuroendocrine integration of stress and significance of stress for the performance of farm animals. *Appl. Anim. Behav. Sci.* 44:219-227.
- Bottoms, G.D., O.F. Roesel, F.D. Rausch, and E.L. Akins. 1972. Circadian variation in plasma cortisol and corticosterone in pigs and mares. *Am. J. Res.* 33:785-790.
- Breuner, C.W. and M. Orchinik. 2002. Beyond carrier proteins: Plasma binding proteins as mediators of corticosteroid action in vertebrates. *J. Endocrinol.* 175:99-112.
- Bright, G.M., and D. Darmaun. 1995. Corticosteroid-binding globulin modulates cortisol concentration responses to a given production rate. *J. Clin. Endocrinol. Metab.* 80:764-769.
- Bright, G.M. 1995. Corticosteroid-binding globulin influences kinetic parameters of plasma cortisol transport and clearance. *J. Clin. Endocrinol. Metab.* 80:770-775.

- Buckingham, J.C., A.M. Cowell, G.E. Gillies, A.E. Herbison, and J.H. Steel. 1997. The neuroendocrine system: anatomy, physiology and response to stress; in Buckingham, Gillies and Cowell, Stress, Stress hormones and the immune system 1:10-47.
- Calvano, S.E., and R.W. Reynolds. 1984. Circadian fluctuations in plasma corticosterone, corticosterone-binding activity and total protein in male rats: possible disruption by serial blood sampling. *Endocr. Res.* 10:11-23.
- Carroll, J.A., J.A. Daniel, D.H. Keisler, and R.L. Matteri. 1998. Non-surgical catheterization of the jugular vein in young pigs. *Labor. Anim.* 33:129-134.
- Cole, M.A., P.J. Kim, B.A. Kalman, and R.L. Spencer. 2000. Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. *Psychoneuroendocrinology* 25:151-167.
- Coolens, J.L., H. Van Baelen, and W. Heyns. 1987. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J. Steroid Biochem.* 26:197-202.
- Czeisler, C.A. 1995. The effect of light on the human circadian pacemaker. In: Chadwick, D.J., Ackrill, K. (Eds.), *Circadian clocks and their adjustments*. John Wiley & Sons, New York, Ciba Foundation Symposium 183, pp. 254-302.
- Dhillon, W., W.M. Kong, C.W. Le Roux, J. Alaghband-Zadeh, J. Jones, G. Carter, N. Mendoza, K. Meeran, and D. O'Shea. 2002. Cortisol-binding globulin is important in the interpretation of dynamic tests of the hypothalamic-pituitary-adrenal axis. *Eur. J. Endocrinol.* 146:231-235.
- Edwards, S., A. Clow, P. Evans, and F. Hucklebridge. 2001. Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sci.* 68:2093-2103.
- Elenkov, I. J., and G. P. Chrousos. 2002. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Ann. N.Y. Acad. Sci.* 966: 290-303.
- Evans, F.D., R.J. Christopherson, and F.X. Aherne. 1988. Development of the circadian rhythm of cortisol in the gilt from weaning until puberty. *Can. J. Anim. Sci.* 68:1105-1111.

- Exton, J.H. 1979. Regulation of gluconeogenesis by glucocorticoids; in Baxter and Rousseau, Monographs on endocrinology: glucocorticoid hormone action 28:535-546.
- Facui, A.S. 1979. Immunosuppressive and Anti-inflammatory effects of glucocorticoids; in Baxter and Rousseau, Monographs on endocrinology: glucocorticoid hormone action 24 449-465.
- Feldman, D., J.W. Funder, and I.S. Edelman. 1973. Evidence for a new class of corticosterone receptors in the rat kidney. *Endocrinology* 92:1429-1441.
- Feldman, D., C.E. Mondon, J.A. Horner, and J.N. Weiser. 1979. Glucocorticoid and estrogen regulation of corticosteroid-binding globulin production by rat liver. *Amer. J. Physiol.* 237:E493-E499.
- First, N.L., and M.J. Bosc. 1979. Proposed mechanisms controlling parturition and the induction of parturition in swine. *J. Anim. Sci.* 48:1407-1421.
- Fleshner, M., T. Deak, R.L. Spencer, M.L. Laudenslager, L.R. Watkins, and S.F. Maller. 1995. A long term increase in basal levels of corticosterone and a decrease in corticosteroid-binding globulin after acute stressor exposure. *Endocrinology* 136:5336-5342.
- Fujieda, K., A.K. Goff, M. Pugeat, and C.A. Strott. 1982. Regulation of the pituitary-adrenal axis and corticosteroid-binding globulin-cortisol interaction in the guinea pig. *Endocrinol.* 111:1944-1950.
- Gallagher, N.L., L. R. Giles, and P.C. Wynn. 2002. The development of a circadian pattern of salivary cortisol secretion in the neonatal piglet. *Biol Neonate* 81:113-118.
- Gayrard, V., M. Alvinerie, and P.L. Toutain. 1996. Interspecies variations of corticosteroid-binding globulin parameters. *Domest. Anim. Endocrinol.* 13:35-45.
- Giannopoulos, G. 1976. A comparative study of receptors for natural and synthetic glucocorticoids in fetal rabbit lung. *J. Steroid Biochem. Mol. Bio.* 7:553-559.
- Goulding, N.J., and R.J. Flower. 1997. Glucocorticoids and the immune system. In *Stress, stress hormones, and the immune system*. J.C. Buckingham, G.E. Gillies, and A.M. Cowell, ed. John Wiley and Sons Ltd., New York, NY.

- Griffith, M.K., and J.E. Minton. 1991. Free-running rhythms of adrenocorticotrophic hormone (ACTH), cortisol and melatonin in pigs. *Domest. Anim. Endocrinol.* 8:201-208.
- Hammond, G.L. 1990. Molecular properties of corticosteroid binding globulin and the sex-steroid binding properties. *Endocr. Rev.* 11:65-79.
- Hammond, G.L., C.L. Smith, I.S. Goping, D.A. Underhill, M.J. Harvey, and J. Reventos. 1987. Primary structure of human corticosteroid binding globulin, deduced from hepatic and pulmonary cDNAs, exhibits homology with serine protease inhibitors. *Proc. Natl. Acad. Sci. USA* 84:5153-5157.
- Hammond, G.L., C.L. Smith, N.A.M. Paterson, and W.J. Sibbald. 1990. A role for corticosteroid-binding globulin in delivery of cortisol to activated neutrophils. *J. Clin. Endocrin. Metab.* 71:34-39.
- Hammond, G.L., C.L. Smith, and D.A. Underhill. 1991. Molecular studies of corticosteroid binding globulin structure, biosynthesis and function. *J. Steroid Biochem.* 40:755-762.
- Hauger R.L., M. Lorang, M. Irwin, and G. Aguilera. 1990. CRF receptor regulation and sensitization of ACTH responses to acute ether stress during chronic intermittent immobilization stress. *Brain Res.* 532:34-40.
- Heo, J., H.G. Kattesh, M.P. Roberts, and J.F. Schneider. 2003. Plasma levels of cortisol and corticosteroid-binding globulin (CBG) and hepatic CBG mRNA expression in pre- and postnatal pigs. *Domest. Anim. Endocrinol.* 23:263-273.
- Heo, J., H.G. Kattesh, M.P. Roberts, J.L. Morrow, J.W. Dailey, and A.M. Saxton. 2005. Hepatic corticosteroid-binding globulin (CBG) messenger RNA expression and plasma CBG concentrations in young pigs in response to heat and social stress. *J. Anim. Sci.* 83:208-215.
- Hsu, B.R.S., and R.W. Kuhn. 1988. The role of adrenal in generating the diurnal variation in circulating levels of corticosteroid-binding globulin in the rat. *Endocrinology* 122:421-426.
- Janssens, C.J.J.G., F.A. Helmond, and V.W. Wiegant. 1995. The effect of chronic stress on plasma cortisol concentrations in cyclic female pigs depends on the time of day. *Domest. Anim. Endocrinol.* 12:167-177.
- Jenkins, J.S., and J. Connolly. 1968. Adrenocortical response to ethanol in man. *J. Brit. Med.* 2:804-805.

- de Jong, I.C., I.T. Prella, J.A. van de Burgwal, E. Lambooij, S. M. Korte, H.J. Blokhuis, and J. M. Koolhaas. 2000. Effects of environmental enrichment on behavioral responses to novelty, learning, and memory, and the circadian rhythm in cortisol in growing pigs. *Physiol. Behav.* 68:571-578.
- Kattesh, H.G., G.A. Baumbach, B.B. Gillespie, J.F. Schneider, and J.T. Murai. 1997. Distribution between protein-bound and free forms of plasma cortisol in the gilt and fetal pig near term. *Biol. Neonate* 72:192-200.
- Kattesh, H.G., M.E. Brown-Crutchfield, J.L. Wolf II, and J.F. Schneider. 1995. Effect of restricted movement on the physiology and behavior of swine. *TN Agri Science* 176:31-35.
- Kattesh, H.G., S.F. Charles, G.A. Baumbach, and B.E. Gillespie. 1990. Plasma cortisol distribution in the pig from birth to six weeks of age. *Biol. Neonate* 58:220-226.
- Kawai, A., and N. Kuzuya. 1972. Studies on the role of the pituitary gland in biosynthesis of corticosteroid-binding globulin in the male rat. *Endocrinol. Japan* 19:19-26.
- Klemcke, H.G., J.A. Nienaber, and G.L. Hahn. 1989. Plasma Adrenocorticotrophic hormone and cortisol in pigs: effects of time of day on basal and stressor-altered concentrations. *Proc. Soc. Exper. bio. Med.* 190:42-53.
- De Kloet, E.R., G. Wallach, and B.S. McEwen. 1975. Differences in corticosterone and dexamethasone binding to rat brain and pituitary. *Endocrinology* 96:598-609.
- Koch, B., B. Lutz, B. Briaud, and C. Mialhe. 1976. Sex difference in glucocorticoid binding to the adenyhypophysis. *Horm. Metab. Res.* 8:402.
- Kuhn, R.W. 1988. Corticosteroid-binding globulin interactions with target cells and plasma membranes. *Ann. N.Y. Acad. Sci.* 538:146-158.
- Lewis, J.G., M.G., Lewis, and P.A. Elder. 2003. An enzyme-linked immunosorbent assay for corticosteroid-binding globulin using monoclonal and polyclonal antibodies: decline in CBG following synthetic ACTH. *Clinica. Chimica. Acta.* 328:121-128.
- Madej, A., K. Romanowicz, S. Einarsson, M. Forsberg, and B. Barcikowski. 1997. Effect of glucocorticoid treatment on biochemical and hormonal blood parameters in early pregnant gilts. *Acta Vet. Scand.* 38:263-273.

- Manteuffel, G., E. Kanitz, O. Bellmann, and B. Stabenow. 2002. Temporal relationship of hippocampal liquor- and plasma-cortisol-concentrations in the domestic pig challenged with synthetical adreno-corticotrophic hormone. *Neuroscience Let.* 333:119-122.
- Mayer, M., N. Kaiser, R.J. Milholland, and F. Rosen. 1975. Cortisol binding in rat skeletal muscle. *J. Biol. Chem.* 250:1207-1211.
- Mendel, C.M. 1992. The free hormone hypothesis, Distinction from the free hormone transport hypothesis. *J. Andrology.* 13:107-116.
- Mickelson K.E., Harding G.B., Forsthoefel M., and Westphal U. 1982. Steroid-Protein Interactions. Human Corticosteroid-Binding Globulin: characterization of dimer and electrophoretic variants. *Biochemistry.* 21:654-660.
- Moberg, GP. 2000. The biology of animal stress; Basic principles and implications for animal welfare. CABI Publishing, New York. 1:1-22.
- Munck, A., P.M. Guyre, and N.J. Holbrook. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr. Rev.* 5:25-41.
- Negrão, J.A., M.A. Porcionato, A.M. de Passille', and J. Rushen. 2004. Cortisol in saliva and plasma of cattle after ACTH administration and milking. *J. Dairy Sc.* 87:1713-1718.
- Paris, A.L., and J.A. Ramaley. 1974. Adrenal-gonadal relations and fertility: The effects of repeated stress upon the adrenal rhythm. *Neuroendocrinology* 15:126-136.
- Parrott, R.F., B.H. Misson, and B.A. Baldwin. 1989. Salivary cortisol in pigs following adrenocorticotrophic hormone stimulation: comparison with plasma levels. *J. Br. Vet.* 145:362-366.
- Rafai, P., and E. Fodor. 1980. Studies on porcine adrenocortical function. *Acta Vet. Acad. Sci. Hung.* 28:433-441.
- Randall, G.C.B. 1983. Changes in the concentrations of corticosteroids in the blood of fetal pigs and their dams during late gestation and labor. *Biol. Reprod.* 29:1077-1084.

- Roberts, M.P., H.G. Kattesh, G.A. Baumbach, B.E. Gillespie, J.D. Godkin, J.F. Schneider, and A.M. Saxton. 2003. Age-related changes in porcine corticosteroid-binding globulin (pCBG) as determined by an enzyme-linked immunosorbent assay. *Domest. Anim. Endocrinol.* 24:323-339.
- le Roux, C.W., S. Sivakumaran, J. Alaghband-Zadeh, W. Dhillon, W.M. Kong, and M.J. Wheeler. 2002. Free cortisol index as a surrogate marker for serum free cortisol. *Ann. Clin. Biochem.* 39:406-408.
- le Roux, C.W., G.A. Chapman, W.M. Kong, W.S. Dhillon, J. Jones, and J. Alaghband-Zadeh. 2003. Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. *J. Clin. Endocrinol. Metab.* 88:2045-2048.
- Ruis, M.A.W., J.H.A. Te Brake, B. Engel, E.D. Ekkel, W.G. Buist, H.J. Blokhuis, and J.M. Koolhaas. 1997. The circadian rhythm of salivary cortisol in growing pigs: effects of age, gender, and stress. *Physiol. Behav.* 62:623-630.
- Scroggs, L.V., H.G. Kattesh, J.L. Morrow, K.J. Stalder, J.W. Dailey, M.P. Roberts, J.F. Schneider, and A.M. Saxton. 2002. The effects of split marketing on the physiology, behavior, and performance of finishing swine. *J. Anim. Sci.* 80:338-345.
- Seralini, G.E., C.L. Smith, and G.L. Hammond. 1990. Rabbit corticosteroid-binding globulin: primary structure and biosynthesis during pregnancy. *Mol. Endocrinol.* 4:1166-1172.
- Selye, H. 1950. Stress and the general adaptation syndrome. *Br. Med. J.* 4667:1383-92.
- Siiteri, P.K., J.T. Murai, G.L. Hammond, J.A. Nisker, W.J. Raymore, and R.W. Kuhn. 1982. The serum transport of steroid hormones. *Recent prog. Horm. Res.* 38:457-510.
- Takahashi K., K. Hanada, K. Kobayashi, C. Hayafuji, S. Otani, and Y. Takahashi. 1979. Development of the circadian adrenocortical rhythm in rats: Studied by determination of 24- or 48- hour patterns of blood corticosterone levels in individual pups. *Endocrinology* 104:954-961.
- Viau, V., S. Sharma, and M.J. Meaney. 1996. Changes in plasma adrenocorticotropin, corticosterone, corticosteroid-binding globulin, and hippocampal glucocorticoid receptor occupancy translocation in rat pups in response to stress. *J. Neuroendocrinology* 8:1-8.

- Weitzman, E.D., D. Fukushima, C. Roffwarg, H. Gallagher, and T.F. Hellman. 1971. Maturation of the circadian rhythm of the adrenocortical functions in human neonates and infants. *Horm. Res.* 12:237-244.
- Westphal, U. 1983. Corticosteroid-binding globulin. A review of some recent aspects. *Mol. Cell. Biochem.* 55:145-157.
- Whipp, S.C., R.L. Wood, and N.C. Lyon. 1970. Diurnal variation in concentrations of hydrocortisone in plasma of swine. *Am. J. Vet. Res.* 31: 2105-2107.
- Zhao, X., L.A. Scrocchi, and G.L. Hammond. 1997. Glucocorticoids induce corticosteroid-binding globulin biosynthesis by immature mouse liver and kidney. *J. Steroid Biochem.* 60:163-169.

VITA

Rebecca J. Adcock, daughter of Joey and Genie Jackson, was born February 17, 1980 and raised in Blountville, Tennessee. In August 1998, she entered Montreat College, Montreat, North Carolina and received a Bachelor of Science in Environmental Studies in May 2002. In August of 2002 she entered University of Tennessee, Knoxville in the Department of Animal Science and in December of 2005 was awarded a Master of Science degree in Animal Science.