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Changes in plasma melanocyte stimulating hormone, ACTH, prolactin, GH, LH, FSH, and thyroid stimulating hormone in response to injection of sulpiride, thyrotropin releasing hormone, or vehicle in insulin sensitive and insensitive mares

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CHANGES IN PLASMA MELANOCYTE STIMULATING HORMONE, ACTH,
PROLACTIN, GH, LH, FSH, AND THYROID STIMULATING HORMONE IN RESPONSE
TO INJECTION OF SULPIRIDE, THYROTROPIN RELEASING HORMONE, OR VEHICLE
IN INSULIN SENSITIVE AND INSENSITIVE MARES

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
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in partial fulfillment of the
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Master of Science

in

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the School of Animal Sciences

by
Nicole Arana Valencia
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ABSTRACT

Six insulin sensitive and six insensitive mares were used in a replicated 3 x 3 Latin square design to determine the pituitary hormonal responses (compared to vehicle) to sulpiride and thyrotropin-releasing hormone (TRH), two compounds commonly used to diagnose pituitary pars intermedia dysfunction (PPID) in horses. Mares were classified as insulin sensitive or insensitive by their previous glucose responses to direct injection of human recombinant insulin. Treatment days were February 25 and March 10 and 24, 2012. Treatments were sulpiride (racemic mixture, 0.01 mg/kg BW), TRH (0.002 mg/kg BW), and vehicle (saline, 0.01 mL/kg BW) administered intravenously. Blood samples were collected via jugular catheters at -10, 0, 5, 10, 20, 30, 45, 60, 90, 120 min relative to treatment injection. Plasma ACTH concentrations were variable and were not affected by treatment or insulin sensitivity category. Plasma melanocyte stimulating hormone (MSH) concentrations responded ($P < 0.01$) to both sulpiride and TRH injection, and were greater ($P < 0.05$) in insulin insensitive mares than in sensitive mares. Plasma prolactin concentrations responded ($P < 0.01$) to both sulpiride and TRH injection, and the response was greater ($P < 0.05$) for sulpiride; there was no effect of insulin sensitivity. Plasma thyroid stimulating hormone (TSH) concentrations responded ($P < 0.01$) to TRH injection only, and were higher ($P < 0.05$) in insulin sensitive mares in almost all time periods. Plasma LH and FSH concentrations varied with time ($P < 0.05$), particularly in the first week of the experiment, but were not affected by treatment or insulin sensitivity category. Plasma GH concentrations were affected ($P < 0.05$) only by day of treatment. The greater MSH responses to sulpiride and TRH in insulin insensitive mares were similar to, but not as exaggerated as, those observed by others for PPID horses. Also, the reduced TSH concentrations in insulin insensitive mares are consistent with the previous observation of

elevated plasma triiodothyronine concentrations in hyperleptinemic horses (later shown to be insulin insensitive as well).

INTRODUCTION¹

Exaggerated α -melanocyte stimulating hormone (MSH) and adrenocorticotropin (ACTH) responses to an injection of thyrotropin releasing hormone (TRH) have been suggested as possible indicators of pituitary pars intermedia dysfunction (PPID) in horses. (McFarlane et al., 2006; Beech et al. 2007; Beech et al., 2011a,b). Similarly, the ACTH response to the dopamine antagonist, domperidone, was reported to be greater in horses affected with PPID than in normal horses (Sojka et al., 2006; Miller et al. 2008; Beech et al, 2011a). In addition to MSH and ACTH, TRH administration stimulates secretion of both thyroid-stimulating hormone (TSH) and prolactin (Thompson and Nett, 1984; Johnson, 1987; Johnson and Becker, 1987) in normal horses, and was reported to inhibit the secretagogue-induced secretion of growth hormone (GH) (Pruett et al., 2003). Domperidone, as well as sulpiride, another dopamine antagonist, also stimulates prolactin release in normal horses (Johnson and Becker, 1987; Brendemuehl and Cross, 2000; Kelley et al., 2006).

Gentry et al. (2002) first described a hyperleptinemic condition in obese mares housed on pasture side-by-side with other obese mares with normal plasma leptin concentrations. Cartmill et al. (2003) reported that these hyperleptinemic mares were also hyperinsulinemic and had elevated plasma triiodothyronine concentrations, but did not have altered cortisol concentrations. Subsequently, Caltabilota et al. (2010) confirmed that the hyperleptinemic mares were in fact relatively insulin insensitive compared to normal mares of similar body condition. Approximately 60% of horses diagnosed with PPID have been found to have elevated blood insulin concentrations, indicative of insulin resistance, and elevated plasma ACTH-like activity

¹This Introduction, as well as Chapters II, III, and IV, are reprinted from Domestic Animal Endocrinology, volume 44, pages 204-212, with permission from Elsevier, Ltd.

is one of the currently accepted diagnostic tests indicative of PPID (McFarlane, 2011). However, like the hyperleptinemic mares described by Cartmill et al. (2003), horses with PPID do not necessarily have elevated cortisol concentrations or signs of adrenal hyperactivity (McFarlane, 2011).

Some horses presenting with symptoms of PPID seem to have alterations in cortisol concentrations that are not obvious or are too subtle to detect (Beech et al., 2007, 2011b; Haritou et al, 2008); however, reduced insulin sensitivity seems to be a common symptom of horses with equine metabolic syndrome (Johnson et al., 2012) and in PPID horses that also display excessive hair growth (Klinkhamer et al., 2011).

The present experiment was conducted to determine whether the pituitary hormonal responses to sulpiride and TRH differed in normal mares relative to mares of known insulin insensitivity. The MSH and ACTH responses were monitored on the basis of their known responses in PPID horses. The remaining adenohypophyseal hormones were included 1) to confirm the biological activity of the injected compounds (e.g., prolactin and TSH after TRH administration) and 2) to assess any possible responsiveness not yet reported in the literature.

CHAPTER I REVIEW OF LITERATURE

The Hypothalamic-Pituitary-Adrenal Axis

The pituitary gland, also known as the hypophysis, is an endocrine organ comprised of two main parts, namely the adenohypophysis and the neurohypophysis, descending from two different embryological origins (Hadley and Livine, 2007). The adenohypophysis is comprised of endocrine cells originating from an inward invagination of the oral ectoderm of the primitive mouth cavity during development, known as Rathke's pouch (Hadley and Levine, 2007). The neurohypophysis, made up of the pars nervosa and the infundibulum, is mainly a storage organ derived from the downward growth of the neural ectoderm and thereby mainly consisting of modified glial cells and axonal terminals of magocellular neurosecretory cells extending from the supraoptic and paraventricular nuclei in the hypothalamus (Hadley and Livine, 2007). These axons produce, store, and release oxytocin and arginine vasopressin into the capillary plexus in the pars nervosa (Berne and Levy, 2008).

The adenohypophysis is anatomically subdivided into three sections: the pars tuberalis, pars distalis, and the pars intermedia. The pars tuberalis is a thin layer of endocrine tissue that surrounds the infundibular stalk or median eminence to provide an anatomical link between the pars distalis and the hypothalamus (Hadley and Livine, 2007). The pars distalis originates from the anterior wall of Rathke's pouch and makes up the majority of the adenophypophysis (Hadley and Levine, 2007). It is made up of a collection of endocrine cells that synthesize and release hormones in response to hypothalamic releasing or inhibiting hormones (hypophysiotropins) (Hadley and Levine, 2007; McFarlane, 2011). These hormones reach the pars distalis by means of the median eminence, which connects the hypothalamus with the pars distalis through the hypophyseal portal system (HPS; Hadley and Levine, 2007).

The pars intermedia results from contact between the infundibulum and the proximal wall of Rathke's pouch during embryogenesis (Berne and Levi, 2008). It is directly innervated by the periventricular hypothalamic dopaminergic neurons (PHDA) originating in the periventricular nucleus in the hypothalamus (Figure 1). Regulatory signals can also be delivered by direct systemic arterial supply and from the hypothalamic-hypophyseal portal veins (McFarlane, 2006). The development, anatomy, size and function of the pars intermedia differs species to species. In cetaceans, humans, and birds, for example, the pars intermedia is either absent or regresses

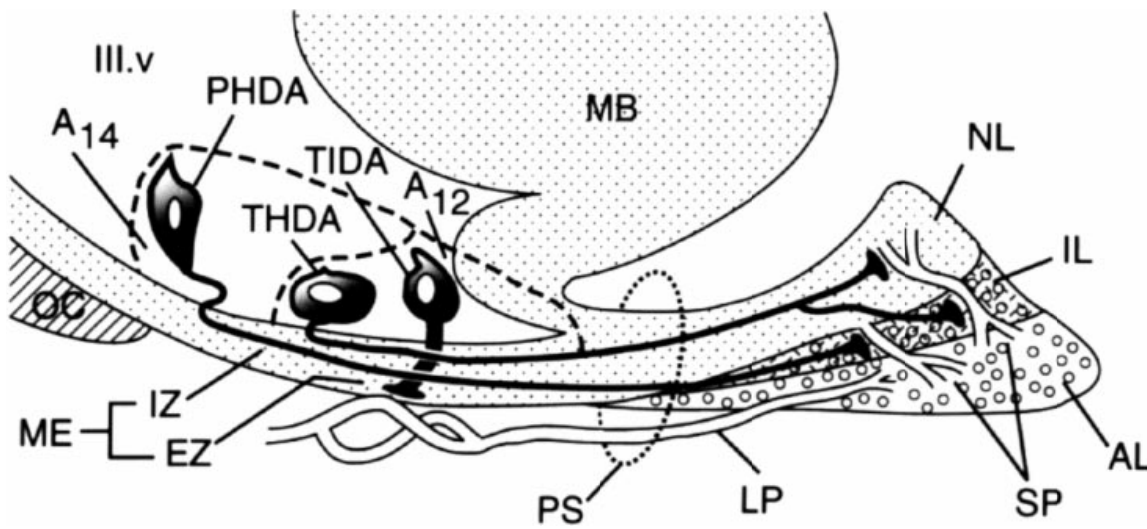


Figure 1. Neuroendocrine dopaminergic neuron populations in the rat hypothalamus. Perikarya of the periventricular hypothalamic dopaminergic (PHDA) neurons (A14 cell group) are located in the periventricular nucleus, and their axons terminate in the intermediate lobe of the pituitary gland (IL). The arcuate nucleus (A12 cell group) contains the perikarya of two distinct neuroendocrine dopaminergic neuron populations. The tuberohypophyseal dopaminergic (THDA) neurons project from the rostral arcuate nucleus both to the neural (NL) and intermediate (IL) lobes of the pituitary gland. From the dorsomedial part of the arcuate nucleus the tuberoinfundibular (TIDA) neurons project to the external zone (EZ) of the median eminence (ME). TIDA terminals release dopamine into the perivascular spaces of the fenestrated capillary loops of the EZ, giving rise to the long portal veins (LP). The long portal veins empty into the sinusoids of the anterior lobe (AL) of pituitary gland. Small short portal (SP) veins connect the fenestrated capillaries of the neural and intermediate lobes with the anterior lobe sinusoids. Thus dopamine of TIDA, THDA, and PHDA origin can reach lactotrophs, located in the anterior lobe of the pituitary gland. III.v, 3rd ventricle; OC, optic chiasma; MB, mammillary body; IZ, internal zone of the median eminence; PS, pituitary stalk. [From Lerant et al. (1996). Copyright The Endocrine Society.]

shortly after formation (Hadley and Levine, 2007). However, in amphibians, reptiles, and most mammals, including the horse, the pars intermedia is well-defined (Hadley and Levine, 2007)

The hypothalamus is the basal part of the diencephalon, which lies below the thalamus. It is found in all vertebrate nervous systems and is responsible for coordinating hormonal and behavioral rhythms (Hadley and Levine, 2007). It includes the optic chiasm, the median eminence, the infundibulum, and the mammillary bodies (Hadley and Levine, 2007). The parvocellular neurons of the periventricular nucleus secrete the hypophysiotropins, gonadotropin-releasing hormone (GnRH), TRH, corticotropin-releasing hormone (CRH), somatostatin (SST), dopamine, and growth hormone-releasing hormone (GHRH) into the primary capillary bed (plexus) on the median eminence according to systemic feedback or external stimuli (Hadley and Levine, 2007). The portal vein then carries the peptides from the primary plexus to a secondary plexus within the adenohypophysis. There they can then regulate the secretion of hormones into the systemic circulation. In contrast, the neurohypophysis is continuously connected with the media eminence via the infundibular stalk (Hadley and Levine, 2007). The main endocrine cells found in the pars distalis are the gonadotropes, somatotropes, thyrotropes, corticotropes, and lactotropes (Hadley and Levine, 2007; Berne and Levy, 2008).

The hypothalamus exerts control of reproduction by secreting GnRH, which regulates the synthesis and secretion of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH; Berne and Levi, 2008; McKinnon et al., 2011). In all species studied, including the horse, GnRH cells form a loosely connected groups scattered through the medial basal hypothalamus and called the GnRH pulse generator (Knobil, 1989). In horses, as in sheep, GnRH secretion is pulsatile, with pulse frequency varying with stage of cycle and season (McKinnon et al., 2011). Synthesis and secretion of GnRH are regulated by feedback from gonadal steroids

(Clarke and Pompolo, 2005). Neurotransmitters, such as noradrenaline, neuropeptide Y, dopamine, glutamate, and serotonin, can stimulate or inhibit GnRH neurons depending on the steroid milieu (McKinnon et al., 2011).

The somatotropes are acidophilic cells that synthesize GH. They are either inhibited by SST or stimulated by GHRH or the gastro-enteric hormone, ghrelin (Berne and Levy, 2008). Other factors, such as stress, exercise (Thompson et al., 1992), nutrition, sleep, and even GH, can also affect GH synthesis (Hadley and Levine, 2007). Growth hormone plays a major physiological role in growth and metabolism through direct and indirect pathways (Hadley and Levine, 2007). When secreted, GH stimulates the liver to produce insulin-like growth factor I (IGF-I; Hadley and Levine, 2007), which then affects growth through its action on skeletal, muscular, and other connective tissues (Hadley and Levine, 2007). Insulin-like growth factor-I can also negatively affect GH secretion through a long-loop feedback to the hypothalamus and induce SST secretion (Berne and Levy, 2008).

Thyrotropin releasing hormone is a naturally occurring hypothalamic tripeptide that is the main regulator of TSH production and secretion, which in turn controls the release of thyroxine and triiodothyronine in the thyroid (Hadley and Levine, 2007). Thyrotropin releasing hormone has also been shown to be an effective secretagogue of prolactin in many species (Hadley and Levine, 2007) as well as the horse (Thompson et al., 1984; Thompson et al., 1986; Johnson, 1987; Johnson et al., 1987). It has been shown to stimulate the equine pituitary gland and release proopiomelanocortin (POMC)-derived peptides, including MSH release from the pars intermedia and ACTH in the pars distalis (McFarlane et al., 2006). In contrast, TRH inhibits GH secretion induced by GHRH (Pruett et al., 2003). This is likely due to a direct effect on the somatotropes to block GH release or via stimulation of somatostatin (Pruett et al., 2003).

Corticotropes in the pars distalis are stimulated by CRH produced in the neurons in the paraventricular nucleus in the hypothalamus (Schott, 2002). Corticotropin releasing hormone is released in a pulsatile pattern into the hypothalamic-hypophyseal portal circulation and increases in frequency in the early morning hours, thereby influencing ACTH to peak 2 to 4 hours before awakening (Schott, 2002). Adrenocorticotropin is released in response to CRH, which subsequently acts on the cells in the zona fasciculata of the adrenal cortex to induce synthesis and release of cortisol (Schott, 2002; Hadley and Levine, 2007). Cortisol release provides negative feedback to inhibit release of CRH from the hypothalamic neurons and ACTH from the corticotropes (Schott, 2002). Other glucocorticoids, such as corticosterone and dexamethasone (a synthetic glucocorticoid) can also exert negative feedback on ACTH secretion on the hypothalamic-pituitary level (Hadley and Levine, 2007). Adrenocorticotropin in the corticotropes is produced from posttranslational cleavage of POMC by the enzyme prohormone convertase I into ACTH and β -lipotropin (Schott, 2002; McFarlane, 2011).

Of the hormones found in the pars distalis of the adenohypophysis, synthesis of prolactin from the lactotropes is unique in that it is predominantly under dopaminergic inhibitory control by the hypothalamus (McKinnon et al., 2011). Dopamine is secreted into the primary plexus of the hypothalamic-hypophyseal portal system by the tuberoinfundibular (TIDA) neurons (Figure 1; Lerant et al., 1996). Typically, prolactin secretion remains relatively constant with occasional surges occurring throughout the day (Roser et al., 1987; Thompson et al., 1994). Plasma concentrations of prolactin in most species is positively correlated with day length, with concentrations higher during the summer than in winter (Martinet et al., 1984; Johnson, 1987; Roser et al., 1987; Worthy et al., 1987). Exercise (Thompson et al., 1994), stress (Colborn et al.,

1991), prostaglandin- $F_{2\alpha}$ (Thompson et al., 2013), and TRH (Thompson et al., 1986) have also been shown to stimulate prolactin release independent of time of year.

The Equine Pars Intermedia

The equine pars intermedia is a long thin sheet of cells that separates the pars distalis from the pars nervosa of the neurohypophysis (McFarlane, 2011). It contains only one endocrine cell type, the melanotropes, which express and produce POMC (Saland, 2001), which is post-transcriptionally cleaved by pro-hormone convertase I (PCI) into ACTH, but unlike the corticotropes, the melanotropes contain pro-hormone convertase II (PCII), that further cleaves ACTH into α -MSH and corticotropin-like intermediate lobe peptide (CLIP), such that little immunoreactive ACTH is found in normal pars intermedia cells (Figure 2; Schott, 2002; McFarlane, 2011).

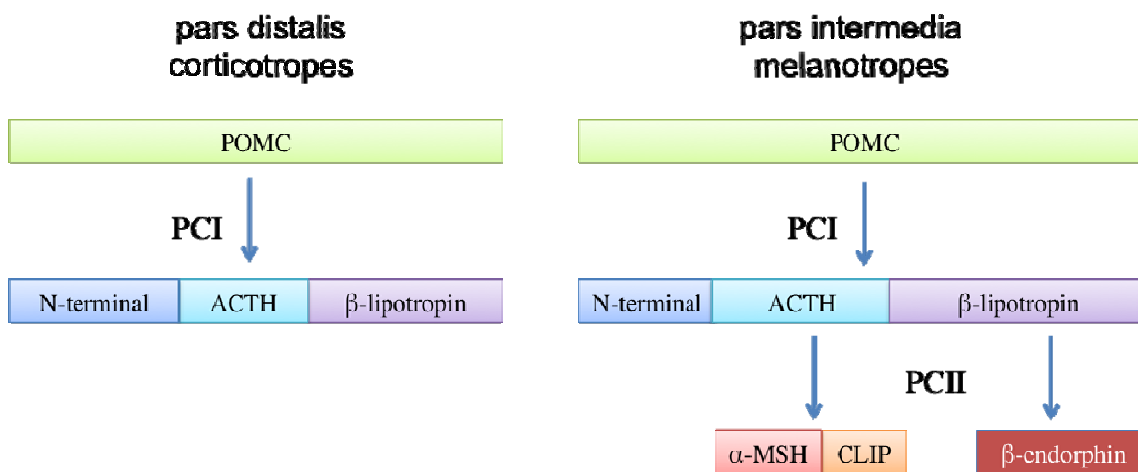


Figure 2. Pituitary POMC metabolism in the corticotropes in the pars distalis and the melanotropes in the pars intermedia (adapted from McFarlane, 2006).

Alpha-MSH was initially known for its role in controlling skin pigmentation in amphibians (Hadley, 1972). It has also shown to have potent anti-inflammatory actions by decreasing pro-inflammatory cytokines release in response to endotoxin administration in some species (Catania and Lipton, 1993) as well as playing an integral role in the leptin-melanocortin pathway, functioning in appetite-satiety balance and fat metabolism (Cone, 2005).

The pars intermedia is directly innervated by the periventricular hypothalamic dopaminergic neurons originating in the periventricular nucleus in the hypothalamus (Saland, 2001). Regulatory signals can also be delivered by direct systemic arterial supply and from the hypothalamic-hypophyseal portal veins (McFarlane, 2006). Dopamine is secreted by the nerve terminals and binds to the dopamine D2 receptor (D2R) thereby inhibiting transcription of POMC and release of POMC-derived peptides (McFarlane, 2006) and prohormone convertase activity in mice (Saiardi and Borrelli, 1998). On the other hand, TRH has been shown to stimulate the pars intermedia via TRH receptors on the melanotropes (McFarlane et al., 2006; Beech et al., 2011a,b).

Seasonality of Prolactin, MSH, and ACTH

Serum concentrations of many metabolic hormones in the horse vary relative to season. For prolactin, serum concentrations are highest from June through August and lowest from November through February (Johnson, 1986). Johnson (1986) also determined that seasonal changes in concentrations of serum prolactin were directly correlated to both photoperiod and temperature, which parallels the loss (spring) and acquisition (autumn) of the winter hair coat (Kooistra and Ginther, 1975; Martinet et al., 1984; Thompson et al., 1997). Plasma concentrations of the melanocortins, including MSH and ACTH, have been shown to have a seasonal rhythm in the horse with concentrations peaking during the fall months (McFarlane et

al., 2004; Donaldson et al., 2005; Schreiber et al., 2012). Seasonal biorhythms of hormones are typically regulated by changes in photoperiod, mediated by nighttime production of melatonin from the pineal gland (McFarlane et al., 2011). It is possible that horses and ponies have a seasonal increase in POMC-derived peptides and a decrease in prolactin to metabolically prepare them for winter weather and a decrease in forage (McFarlane et al., 2004), but the mechanism that regulates the seasonal rhythm in the pars intermedia is still unknown.

Apart from photoperiod, age has also been shown to be an important factor in affecting seasonal secretion of POMC peptides. Schreiber et al. (2012) reported a moderate but significant positive correlation between age and endogenous ACTH concentrations, but minimal age influence, on MSH concentration. Latitude has been shown to affect ACTH secretion in the autumn, with concentrations markedly higher in horses residing in Florida, modestly increased in horses in Massachusetts, and not increased in horses in Finland (McFarlane et al., 2011); MSH was apparently not affected by latitude (McFarlane et al., 2011).

Insulin Resistance and Leptin

Insulin resistance is defined as a condition in which normal concentrations of insulin produce a subnormal physiologic response (Kahn, 1978). In humans, it is caused by a failure of glucose uptake into muscle and adipose tissue by way of the insulin-sensitive glucose transporter-4 transport protein (Cline et al., 1999). Although the exact mechanism in horses is still unknown, horses develop a reduced sensitivity to insulin, which causes the pancreas to increase insulin production in order to maintain glucose homeostasis (Klinkhamer et al., 2011). Obesity in horses [as defined by Henneke et al. (1983) of having a body condition score of 7 or more] has been linked with insulin resistance (Frank et al., 2010). While not all insulin resistant horses are obese and not all obese horses are insulin resistant, obese horses are at most risk for developing

metabolic disorders and laminitis (Frank et al., 2006). In humans, obesity and lack of exercise are primary risk factors for insulin resistance, and the risk of developing type 2 diabetes increases with severity of obesity (Frank et al., 2006).

Leptin, a 16-kDa protein hormone secreted by white adipocytes, been found to play a regulatory role in insulin secretion by β cells of the pancreas and in insulin action and metabolism (Houseknecht et al., 1998). It has also been implicated in the regulation of food intake, energy expenditure, and energy balance in rodents and humans (Houseknecht et al., 1998). Leptin acts in the hypothalamus to suppress appetite in normal individuals and thereby regulate the accumulation of adipose tissue in the body (Johnson, 2002). Leptin concentrations have been shown to vary directly with body mass index and percentage body fat in some species (Houseknecht et al., 1998; Prolo et al., 1998). This correlates with several studies that have determined an association between hyperleptinemia and insulin resistance in obese horses (Cartmill et al., 2003; Frank et al., 2006; Caltabilota et al., 2010). Cartmill et al. (2003, 2005) reported that hyperleptinemic horses had increased resting insulin concentrations. Since leptin can be directly stimulated by administration of insulin, while maintaining glucose within normal limits (Cartmill et al., 2003), Caltabilota et al. (2010) suggested that the hyperleptinemic condition is a result of reduced insulin insensitivity, which equates to long-term increases in insulin concentrations and hence a long-term stimulation of adipose tissue output of leptin.

Increases in leptin secretion have been observed in one study (Buff et al., 2002) to increase with age, with the greatest concentrations of leptin occurring in horses greater than 12 years of age.

Equine Metabolic Syndrome

Metabolic syndrome in humans, also referred to as “peripheral Cushing’s Syndrome”, insulin-resistance syndrome, and Syndrome X, is a collection of metabolic disorders, which include glucose intolerance, insulin resistance, central obesity, dyslipidemia, and hypertension (Eckel et al., 2005). When grouped together, they are associated with increased risk of coronary artery disease and type 2 diabetes (Eckel et al., 2005; Frank et al., 2010). In 2002, Johnson first wrote the term equine metabolic syndrome (EMS) when he proposed that obesity, insulin resistance, and laminitis were all clinical signs of a syndrome recognized in horses and ponies. The term EMS was adopted to describe the condition in equids, as it bears similarities with the metabolic syndrome in humans (Eckel et al., 2005).

Equine metabolic syndrome affects horses that are characteristically obese and tend to be between the ages of 8 to 18 (Johnson, 2002). Established symptoms of EMS include increased regional or general adiposity, insulin resistance characterized by hyperinsulinemia, and predisposition towards laminitis (Frank et al., 2010). Other factors that might be present are hypertriglyceridemia or dyslipidemia, hyperleptinemia, arterial hypertension, and loss of cyclicity in mares (Frank et al., 2010).

Pituitary Pars Intermedia Dysfunction

Pituitary pars intermedia dysfunction, formerly known as equine Cushing’s disease, is a progressive neurodegenerative disorder resulting from neurodegeneration of the dopaminergic periventricular neurons innervating the melanotropes of the pars intermedia (McFarlane et al., 2005), that is characterized by hypertrophy and hyperplasia of the melanotropes resulting in an increased expression of POMC derived peptides (Dybdal et al., 1994; McFarlane et al., 2003). It is common in aged equids, and all breeds of horses and ponies can be affected (Schott, 2002).

Pars intermedia tissue from affected horses were reported to have an 8-fold decrease in dopamine concentration relative to tissue from age matched controls (Millington et al., 1988), and hypothalamic and pituitary tissues from horses with PPID were found to have a 6-fold reduction in levels of dopaminergic nerve terminals in the pars intermedia and 50% reduction levels of dopaminergic cell bodies in the periventricular nucleus (McFarlane et al., 2005). Similar histological findings were reported for D2R-deficient mice (Saiardi and Borrelli, 1998). Loss of D2R expression leads to increased POMC expression, hyperplasia of the pars distalis due to aberrant increase in prolactin levels from lactotropes, and hypertrophy of the pars intermedia (Saiardi and Borrelli, 1998). In these mice, the PCI activity increased 4 to 5-fold while the PCII activity increased only 2 to 3-fold (Saiardi and Borrelli, 1998). The relative difference in magnitude is thought to explain why horses with PPID produce pars intermedia-derived ACTH, as PCII activity cannot keep up with the relatively more abundant PCI activity (McFarlane, 2011).

The increased expression of POMC derived peptides such as ACTH, MSH, β -endorphin, and CLIP is thought to lead to the clinical symptoms seen in PPID (McFarlane, 2011). The most unique and specific clinical sign associated with PPID is hirsutism, a delayed or incomplete coat shedding (McFarlane, 2011; McGowan et al., 2013). Other clinical signs include muscle atrophy, laminitis, polyuria/polydipsia, excessive sweating, susceptibility to infections, and lethargy or depression (Dybdal et al., 1994; Schott, 2002; McFarlane, 2011).

The diagnosis of PPID is difficult, due to the slow progression of the disease. Currently available tests can yield false-negative results early in the course of the disease (Cordero et al., 2012). Season also tends to confound tests if taken during autumn when ACTH and MSH levels are naturally elevated. Although horses with PPID maintain a seasonal regulation of the

hypothalamic-pituitary axis compared to normal horses, horses suspected of PPID have a more exaggerated seasonal hormone increase than normal horses (McFarlane et al., 2011). This suggests the seasonal hormonal rhythm is not regulated by dopamine, given that loss of functional dopaminergic neuronal input to the PI is expected (McFarlane et al., 2011).

Rationale for Present Experiment

Pituitary pars intermedia dysfunction is a very difficult disease to diagnose especially in its early stages. Several experiments (McFarlane et al 2006; Beech et al., 2007, 2011) using TRH have been promising as an ambulatory method of differentiating PPID horses from regular horses, although season has to be taken into consideration when performing such tests (McFarlane et al., 2006; Beech et al., 2011). The use of oral domperidone administration to diagnose PPID proved not to be superior to TRH stimulation (Beech et al., 2011). The use of an injectable dopamine antagonist such as sulpiride to diagnose PPID has not been tested.

As some horses age, they begin to lose their ability to regulate proper glucose levels, resulting often times in development of insulin resistance. Since insulin resistance appears to be present in 60% of horses with PPID (McFarlane, 2011), the current study focused on comparing various pituitary hormonal responses after administration of TRH, sulpiride, or vehicle in healthy and insulin resistant mares to see how these results compared to PPID horses reported by other researchers. As stated earlier in the Introduction (page 2), the remaining adenohypophyseal hormones were included 1) to confirm the biological activity of the injected compounds (e.g., prolactin and TSH after TRH administration) and 2) to assess any possible responsiveness not yet reported in the literature.

CHAPTER II

MATERIALS AND METHODS

Animals and Treatments

All procedures described herein were approved by the Institutional Animal Care and Use Committee of the LSU Agricultural Center. The mares used were of light horse breeds and were long-term residents of the Louisiana State University Agricultural Center horse farm in Baton Rouge, Louisiana. They were kept on native grass pasture most of the year and on winter ryegrass pasture when native grasses were dormant; grass hay was provided in transitional periods when grasses were insufficient to maintain body conditions. They remained on pasture except when experimental procedures were being performed.

Twelve light horse mares were used that ranged in age from 6 to 21 yr, weighed between 480 and 616 kg, and had body condition scores (Henneke et al., 1983) between 5 and 8. The mares were selected from the larger herd based on their relative insulin sensitivity, determined multiple times with consistent results, in previous experiments (Caltabilota et al., 2010; Earl et al., 2012). Six mares with low insulin sensitivity and six mares with normal sensitivity were then used in a replicated 3 x 3 Latin square design experiment to assess the hormonal responses to the two secretagogues reported to be useful in diagnosing PPID in horses (TRH and sulpiride (McFarlane et al., 2006; Sojka et al., 2006; Beech et al., 2007; Miller et al., 2008; Beech et al., 2011a,b).

Treatments in the experiment were as follows: 1) vehicle, 0.155 M NaCl in sterile water, 0.01 mL/kg of body weight (BW), administered intravenously (i.v.), 2) TRH (obtained from Sigma Chem. Co., St. Louis, MO), 0.002 mg/kg BW in saline, administered i.v., and 3) sulpiride (from Sigma), 0.01 mg/kg BW of the racemic mixture in saline, administered i.v. Days of the experiment were February 25, 2012 and March 10 and 24, 2012. Treatments were predetermined

such that on each day of treatment, two mares of each insulin sensitivity category were administered each treatment.

Mares were brought in from pasture the evening before each treatment day and were held in a dry lot with no feed or grass but free access to water. At approximately 07:00 the next morning, the mares were walked to an outdoor chute and were restrained to minimize contact with each other. Each mare then received a 14-gauge jugular catheter inserted on her left side that was held in place with cyanoacrylate glue. As long-term residents of the farm, the mares were routinely handled (moved into the lot, restrained, and catheterized) in this manner; thus, it posed no novel stimulation, and the mares displayed no signs of discomfort or anxiety.

The mares were allowed to acclimate to the catheters and after approximately 1 hour, two samples of jugular blood were collected via the catheter (-10 and 0 samples) of each mare, and then the treatment for that day was injected through the catheter. Post-treatment blood samples were collected at 5, 10, 20, 30, 45, 60, 90, 120 min relative to time of treatment. In each case, the blood sample was split between two tubes for processing: one 7-mL tube containing 140 units sodium heparin (Sigma) and one 3-mL tube containing disodium EDTA as the anticoagulant (Sigma; 2 mg/mL of blood) and aprotinin (Sigma; 600 kallikrein inactivator units/mL of blood) as a protease inhibitor. All tubes with blood were placed on ice and were centrifuged within 15 min at 1200 x g for 15 min. Plasmas were harvested from the two tubes and stored at -15°C. After all blood samples had been collected for that day, the mares were returned to pasture.

In addition to the three treatment days, mares were also sampled between treatment days 1 and 2 (March 3, 2012) to assess their 24-hour patterns of cortisol in plasma. For this sampling, the mares were kept in a dry lot that abutted the chute used on treatment days and were provided water and grass hay for ad libitum consumption. Blood samples (5 mL) were collected from the

left jugular vein by venipuncture (21-gauge needles) every 4 hours from 08:00 the first day until 08:00 the next morning, for a total of 7 samples per horse. Mares were moved into the chute for each blood sampling and then returned to the dry-lot. Blood sampling was conducted under minimal lighting shielded from the mares' eyes. All blood samples from this procedure were placed in heparinized tubes and processed as described for the three treatment days.

Radioimmunoassay Procedures

The 10 samples of plasma for each mare on each treatment day were assessed for concentrations of LH (Thompson et al., 1983a), FSH (Thompson et al., 1983b), TSH (Thompson et al., 1984), prolactin (Colborn et al., 1991), and GH (Thompson et al., 1992) with previously validated radioimmunoassays developed in our laboratory. Commercially available reagents were used to measure concentrations of MSH (Euria α -MSH RIA, Immuno-Biological Laboratories, Minneapolis, MN) and ACTH (MP Biomedicals, Santa Ana, CA) in assays previously described by others (Karpac et al., 2008; Wade et al., 2010; Beech et al., 2011); only the 0-, 5-, 10-, 20-, and 30-min samples were assayed. Cortisol concentrations were also measured using commercially available reagents (Pantex, Santa Monica, CA) in duplicate 50- μ L samples of plasma after extraction with 1.5 mL acetone; the extracts were air dried, reconstituted in assay buffer, and assayed directly.

To confirm the hyperleptinemic status of the mares in the two insulin categories, the -10 blood sample for each horse and each week were assessed for plasma leptin concentration with a radioimmunoassay previously validated in our laboratory (Cartmill et al., 2003). Retrospectively, plasma concentrations of thyroxine and triiodothyronine were assessed in the same samples by radioimmunoassay with kits obtained commercially (MP Biomedicals).

Intra- and interassay coefficients of variation and limit of detection (concentration of hormone equivalent to the mean number of counts per minute of the assay zero standard tubes minus two standard deviations of those counts from the mean) for the assays averaged, respectively, 6%, 9%, and 0.2 ng/mL for LH; 7%, 11%, and 1.4 ng/mL for FSH; 5%, 8%, and 0.02 ng/mL for TSH; 7%, 12%, and 0.2 ng/mL for prolactin; and 8%, 11%, and 0.5 ng/mL for GH. The cortisol, MSH, and ACTH samples were assayed in one batch each, and the intra-assay coefficient of variation and limit of detection were 6.0% and 0.1 µg/dL for cortisol, 5.5% and 1.2 pmol/L for MSH, and 6.4% and 1.0 pg/mL for ACTH. The intra-assay coefficient of variation and limit of detection were 6.1% and 0.2 ng/mL for leptin, 2.7% and 1.0 µg/dL for thyroxine and 2.3% and 8.0 ng/dL for triiodothyronine.

Statistical Analyses

Data for the hormonal responses over time after TRH, sulpiride, or vehicle injection were analyzed with SAS statistical software (SAS Institute, Cary, NC) by analysis of variance (ANOVA) that accounted for the repetitive nature of the sampling (repeated measures, Steel et al., 1997). Factors in the main analyses included insulin category, horse within category, treatment, category-treatment interaction, and day; these were tested with the mean square of the residual horse by category by treatment by day interaction term. The effects of time and the treatment by time and category by time interactions were tested with the residual error mean square. Data for leptin, thyroxine, triiodothyronine, and cortisol were analyzed in a repeated measure ANOVA with category tested by mare within category and time and the treatment by time interaction tested with residual error. For all analyses, differences between means were assessed by the least significant difference test (Steel et al., 1997).

Analysis of the LH and FSH data revealed that surges in one or both hormones occurred on several occasions in some mares. Although not preplanned, the frequency of these surges were analyzed by assigning a subjective intensity code (0 = no surge, 1 = small surge, and 2 = large surge) to all day by mare combinations. Small surges were considered as rapid increases in hormonal concentration of 0.5 to 2 ng/mL for LH and 5 to 20 ng/mL for FSH; corresponding large surges were >2 ng/mL and >20 ng/mL for LH and FSH, respectively. The effect of treatment, day of treatment, insulin sensitivity category, and the category-treatment interaction were analyzed with ANOVA and were tested with residual error.

CHAPTER III RESULTS

The mean responses of plasma MSH concentrations to TRH, sulpiride, and vehicle injection are presented in Figure 3. A category by treatment by time interaction in the ANOVA ($P < 0.0001$) was observed for MSH concentrations, which did not change after vehicle injection, but increased ($P < 0.001$) within 5 min after TRH or sulpiride injection. The greatest responses were after sulpiride injection, and the responses in insulin-insensitive mares were greater ($P < 0.05$) than in sensitive mares after both TRH and sulpiride injections. Mean areas under the response curves were greater in insensitive mares than in sensitive mares after both TRH ($P = 0.0013$) and sulpiride ($P = 0.0004$) injection.

Concentrations of ACTH (Figure 4) were variable and were not affected ($P > 0.1$) by category, treatment, time, or any interaction of these terms. Inspection of the data of individual mares indicated that one mare had an increase in ACTH concentrations from 1 to 600 pg/mL when treated with vehicle; most of the mares ($n = 7$) had no apparent changes after vehicle injection, whereas half ($n = 6$) the mares appeared to have responses after TRH or sulpiride.

The responses of TSH to vehicle, TRH, and sulpiride injection are presented in Figure 5. A category by treatment by time interaction in the ANOVA ($P = 0.0448$) was observed for TSH concentrations, which did not change after injection of vehicle or sulpiride, but increased ($P < 0.05$) within 20 min after TRH injection and remained elevated through 120 min after injection. Moreover, TSH concentrations were lower ($P < 0.05$) in insulin-insensitive mares compared to sensitive mares for most of the time periods during sampling except for 120 min after TRH injection and at 10, 20, 30, and 90 min relative to sulpiride injection.

A treatment by time interaction was observed for prolactin concentrations (Figure 6) in the ANOVA ($P < 0.0001$), but no effect of insulin-sensitivity category. Prolactin concentrations

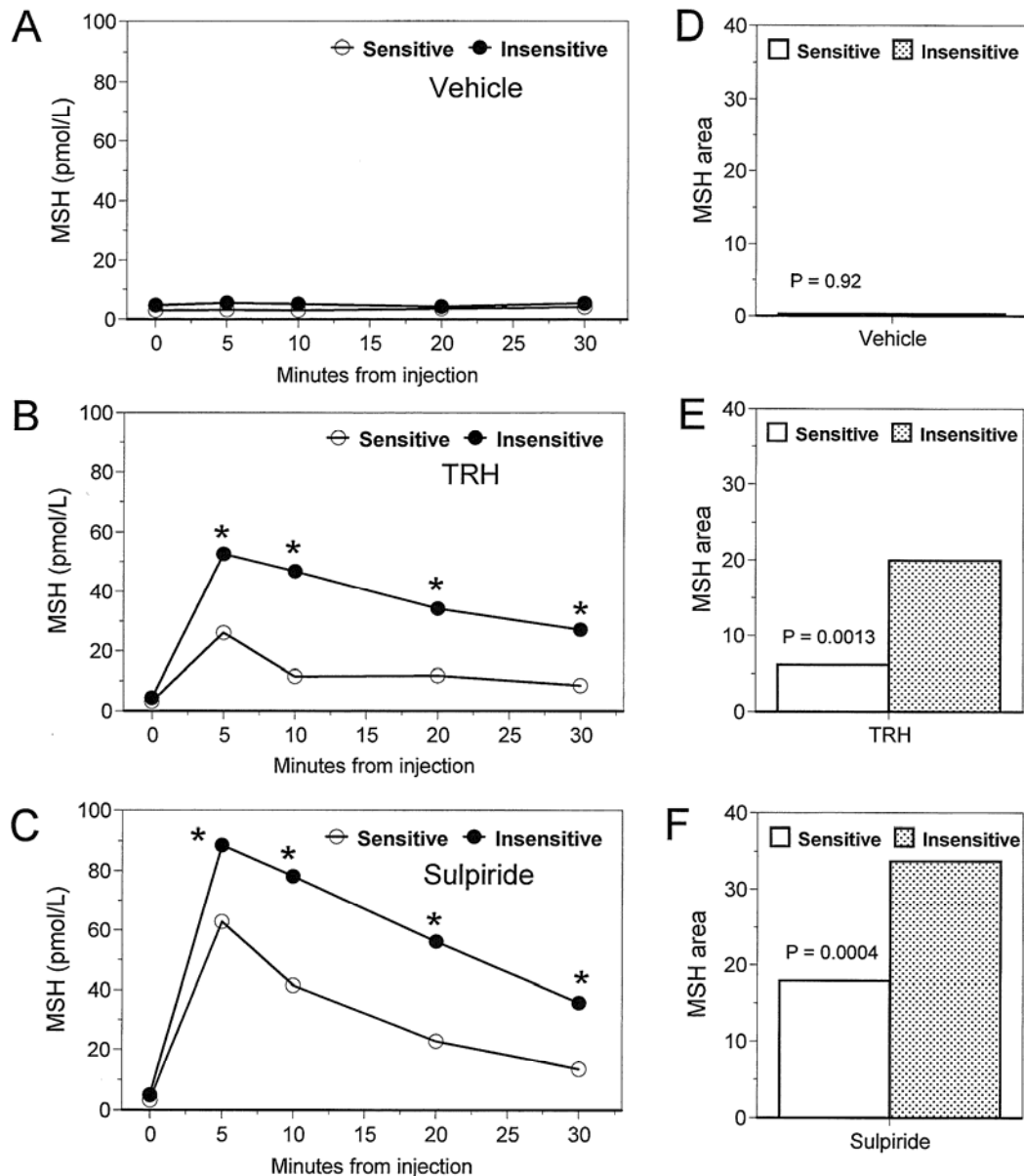


Figure 3. Mean plasma MSH concentrations (A, B, and C) and MSH areas under the response curves (D, E, and F) for insulin-sensitive vs insulin-insensitive mares after administration of vehicle, TRH, or sulpiride. *Differences ($P < 0.05$) between sensitive and insensitive mares for the time points marked; actual P values for the differences in areas between sensitive and insensitive mares are provided. Pooled SEM for plasma concentrations and areas were 4.9 pmol/L and 3.6 pmol/mL x hour, respectively. MSH, α -melanocyte-stimulating hormone; TRH, thyrotropin-releasing hormone.

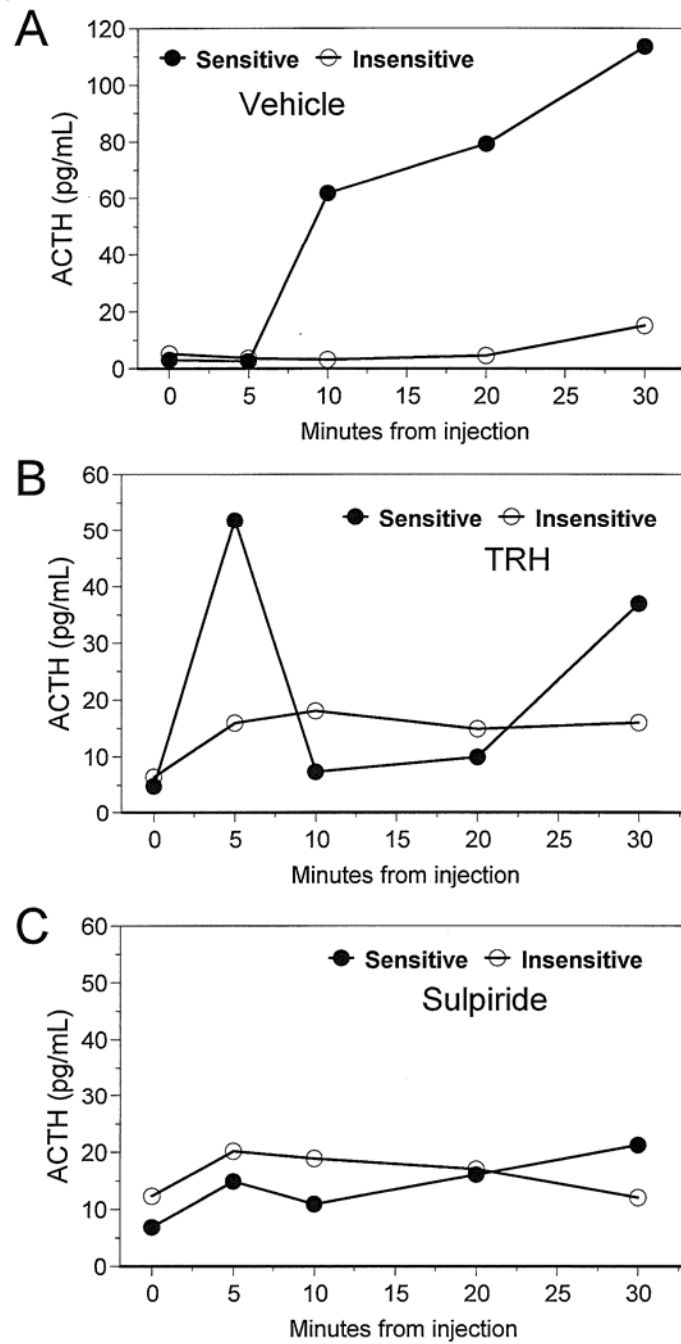


Figure 4. Mean plasma ACTH concentrations for insulin-sensitive vs insulin insensitive mares after administration of vehicle (A), TRH (B), or sulpiride (C). No difference ($P > 0.1$) was observed because of treatment or between sensitive and insensitive mares. Pooled SEM was 29 pg/mL. TRH, thyrotropin-releasing hormone.

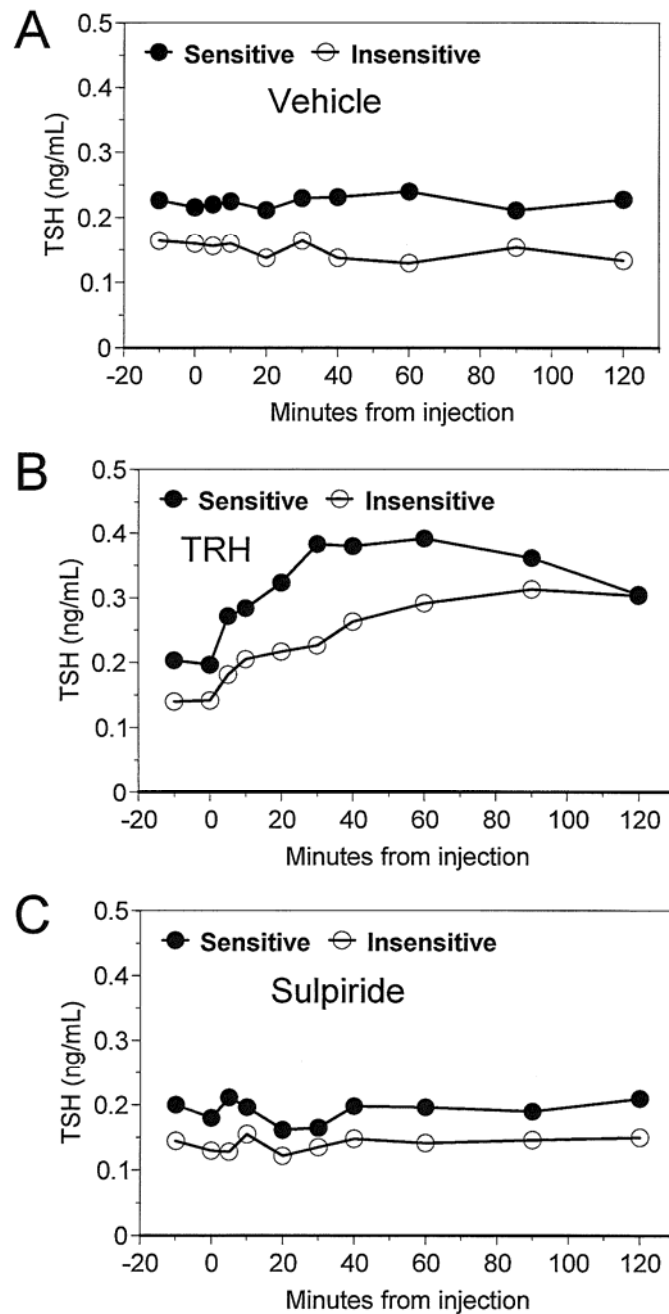


Figure 5. Mean plasma TSH concentrations for insulin-sensitive vs insulin-insensitive mares after administration of vehicle (A), TRH (B), or sulpiride (C). An interaction ($P = 0.045$) was observed between insulin-sensitivity category and time after injection. Means in the 3 graphs differ ($P < 0.05$) between sensitive and insensitive mares for all time periods except for time 120 after TRH and times 10, 20, 30, and 90 for sulpiride. Pooled SEM was 0.02 ng/mL. TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

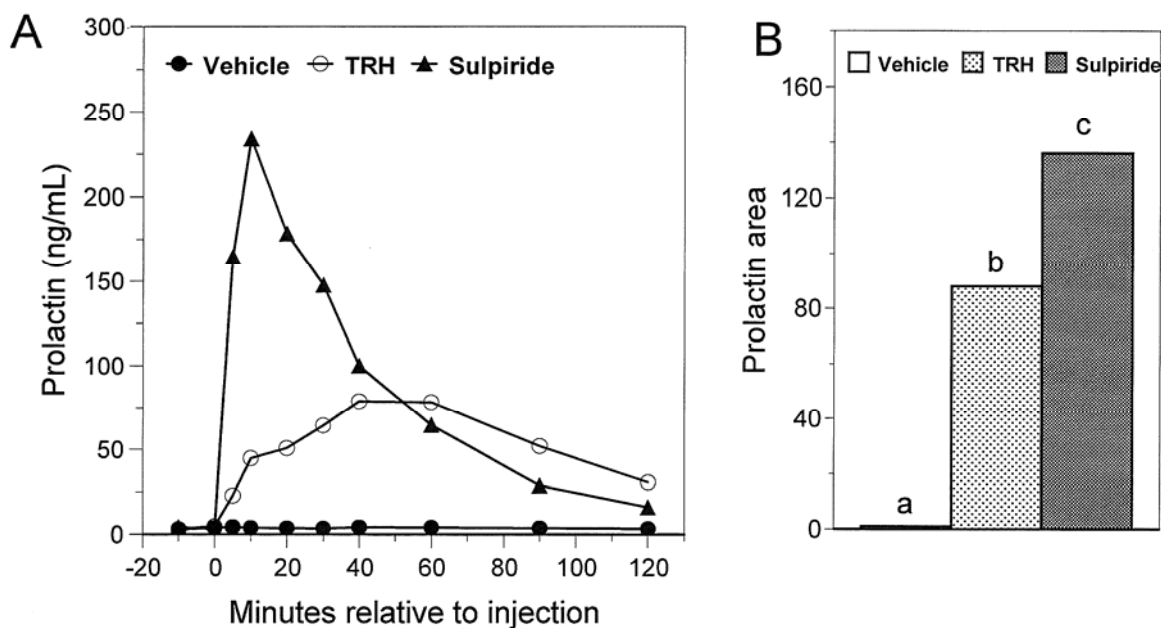


Figure 6. Mean plasma prolactin concentrations (A) and prolactin areas under the response curves (B) for all mares after administration of vehicle, TRH, or sulpiride. Insulin sensitivity category did not affect ($P > 0.1$) prolactin responses. Prolactin responses to sulpiride and TRH were both greater ($P < 0.003$) than that after vehicle injection (b or c vs a), and the sulpiride response was greater ($P = 0.073$) than that after TRH (b vs c). Pooled SEM for plasma concentrations and areas were 22 ng/mL and 37 ng/mL x hour respectively. TRH, thyrotropin-releasing hormone.

did not change after vehicle injection, but increased ($P < 0.01$) after TRH or sulpiride injection. Based on areas under the curve, the prolactin response to sulpiride was greater ($P = 0.079$) than the response to TRH.

Concentrations of GH averaged 3.5 ng/mL in all samples (Figure 7), and were affected only by day in the ANOVA ($P = 0.018$). Means for the three treatment days, in chronological order, were 3.9, 3.6, and 3.1 ng/mL. Concentrations of LH and FSH (Figure 7) were affected by the time factor (minutes from injection) in the ANOVA ($P < 0.002$). Concentrations of FSH were

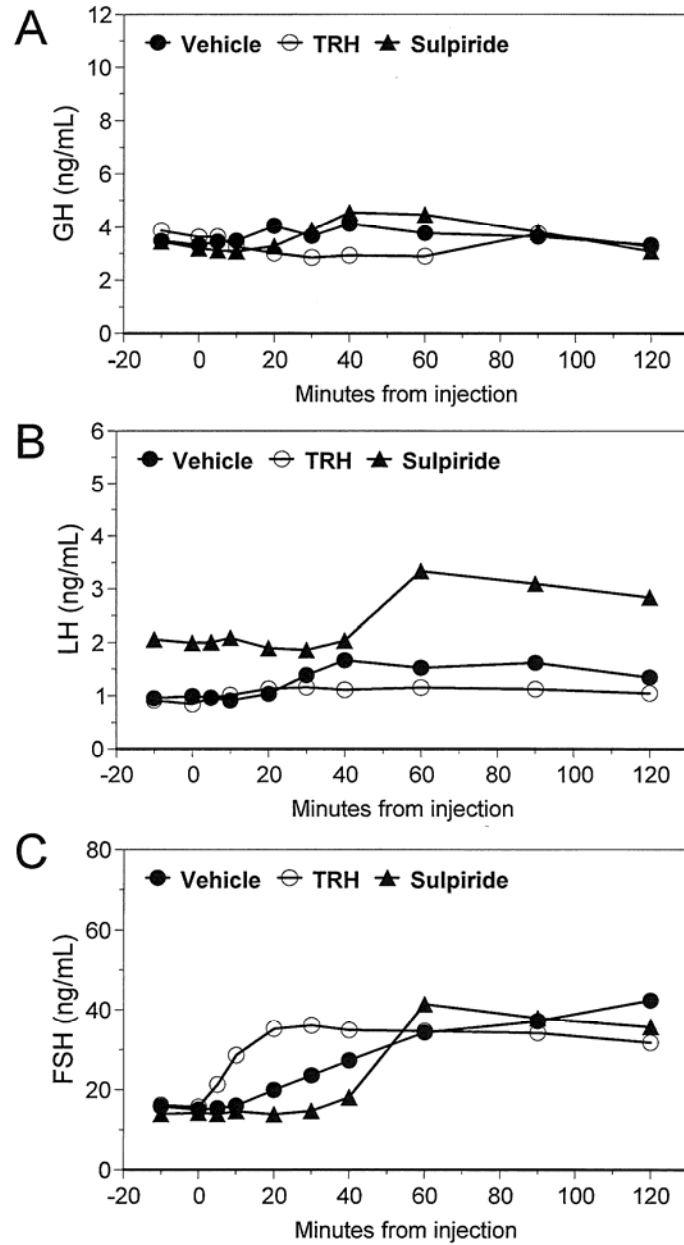


Figure 7. Mean plasma concentrations of GH (A), LH (B), and FSH (C) for all mares after administration of vehicle, TRH, or sulpiride. Concentrations of GH were affected only by day of injection ($P = 0.018$), as were FSH concentrations ($P = 0.0012$). Concentrations of LH and FSH were affected by time relative to injection ($P < 0.002$). Pooled SEMs were 0.7, 0.5, and 12 ng/mL for GH, LH and FSH concentrations, respectively. TRH, thyrotropin-releasing hormone.

also affected by day ($P = 0.0012$). The time effect was due primarily to increases that occurred on the first treatment day, on which several mares had surges in LH ($n = 7$) and FSH ($n = 11$) that occurred at some time after treatment injections (generally beginning at approximately 20 min). On the second treatment day, 4 mares had surges in LH concentrations and 4 mares had surges in FSH concentrations. Oddly, no LH or FSH surges were observed on the third treatment day. The observed surges were not associated with treatment ($P > 0.2$). Analysis of the surge intensities in the post hoc analyses confirmed an effect of day of treatment ($P = 0.0029$ for LH and $P < 0.0001$ for FSH) but no effect of insulin sensitivity category or treatment.

Concentrations of leptin, thyroxine, and triiodothyronine on treatment days 1, 2, and 3 are presented in Figure 8. Leptin concentrations were several-fold higher ($P < 0.05$) in insulin-insensitive mares on treatment days 2 and 3 relative to sensitive mares. Insulin concentrations were higher ($P > 0.001$) in insulin-insensitive mares than in sensitive mares for all three days of treatment. Thyroxine concentrations were not affected ($P > 0.1$) by insulin sensitivity category or day of treatment, whereas triiodothyronine concentrations were higher ($P < 0.01$) in insulin-insensitive mares than in sensitive mares on the first day of treatment, but not on subsequent days.

Cortisol concentrations in the samples collected every 4 hours over 24 hours are presented in Figure 9. Cortisol concentrations varied with time of day ($P = 0.0002$), being lowest in the afternoon and evening (15:00 through 23:00), but were not affected ($P > 0.1$) by insulin sensitivity category.

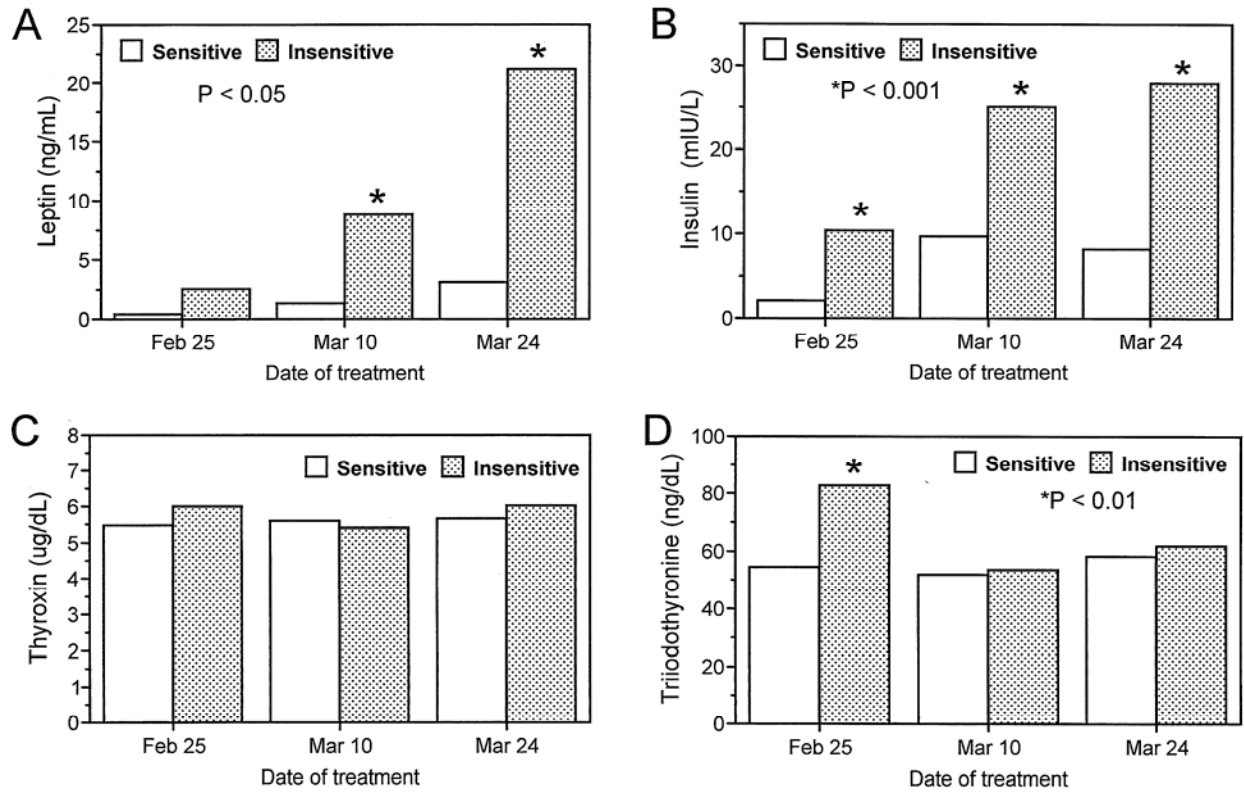


Figure 8. Mean plasma concentrations of leptin (A), insulin (B), thyroxin (C), and triiodothyronine (D) in insulin-sensitive and insulin-insensitive mares on the 3 days of treatment. Leptin ($*P < 0.05$), insulin ($*P < 0.001$), and triiodothyronine ($*P < 0.01$) concentrations differed between insulin sensitivity categories across days. Pooled SEMs were 2.6 ng/mL, 3.3 mIU/L, 0.5 mg/dL, and 9.6 ng/dL for leptin, insulin, thyroxin, and triiodothyronine concentrations, respectively.

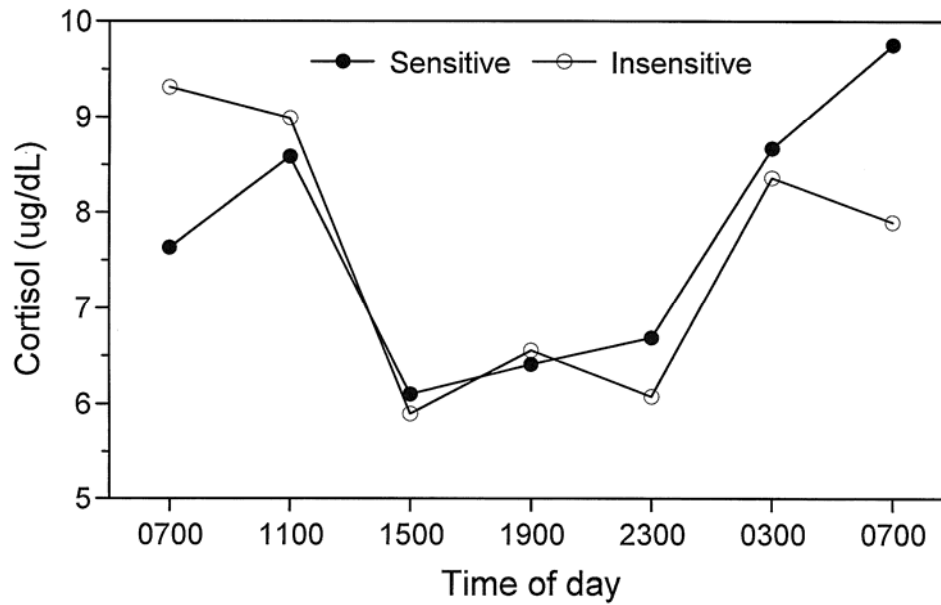


Figure 9. Mean plasma cortisol concentrations in insulin-sensitive and insulin-insensitive mares over 24 h of blood sampling on March 3, 2012. Cortisol concentrations varied throughout the day ($P = 0.0002$) but were not affected by insulin sensitivity category. Pooled SEM was 1.1 mg/dL.

CHAPTER IV DISCUSSION

Gentry et al. (2002) first described a hyperleptinemic condition in mares of good body condition (scores ranging from 5 to 7; Henneke et al., 1983) that persisted even after the body conditions were reduced by limited grazing. Cartmill et al. (2003) subsequently showed that the hyperleptinemic condition was accompanied by hyperinsulinemia and an exaggerated insulin response to glucose infusion, as well as a greater plasma concentration of triiodothyronine. In 2010, Caltabilota et al. confirmed that these hyperleptinemic mares were indeed insulin insensitive relative to mares with normal leptin concentration. The mares used herein had been consistently determined to be insulin sensitive or insensitive by the method of Caltabilota et al. (2010) in previous studies in our laboratory (Caltabilota et al., 2010; Earl et al., 2012; Lestelle, 2012), and were chosen according to that history. The elevated plasma leptin concentrations in Figure 8 are thus consistent with our previous observations that hyperleptinemic mares are relatively insulin insensitive (i.e., insulin resistant).

Sulpiride and TRH were selected for use as secretagogues in the present experiment according to their use in previous studies for the diagnosis of PPID. Several reports have confirmed that ACTH and MSH respond to TRH injection in horses (McFarlane et al., 2006; Beech et al. 2007; Beech et al., 2011a,b), and a few studies have confirmed an ACTH response to domperidone (Sojka et al., 2006; Miller et al., 2008; Beech et al., 2011a). Although no direct assessment of sulpiride has been reported for ACTH or MSH secretion, the known effect of domperidone and the fact that intermediate lobe dopamine receptors are of the D2 type (Munemura et al., 1980) in rats would indicate that these two hormones should respond to sulpiride as well. Not only did MSH concentrations increase after sulpiride injection in the present experiment, but the response was greater than the response to the standard TRH dose

used in previous trials (0.002 mg/kg, or 1 mg for a 500 kg horse). The dose of sulpiride used herein (0.01 mg/kg, or 5 mg of the racemic mixture for a 500 kg horse) was calculated to be approximately 50% of that dose producing maximal prolactin response in mares in March, as described by Clavier et al. (2012). For comparison, we have reported previously that the prolactin and TSH responses to TRH were similar for doses of 0.4, 2, and 10 mg (Thompson et al., 1984); thus the approximate 1-mg dose used herein could be considered saturating. Whether the MSH response to a saturating (maximal) dose of sulpiride differs from that to a saturating dose of TRH will have to be determined in future experiments.

Although chosen for their low insulin sensitivity, the insensitive mares in the present experiment showed no overt signs normally ascribed to PPID (Schott, 2002; McFarlane, 2011). It is interesting, therefore, that these insulin-insensitive mares displayed a greater MSH response to TRH injection, similar to mares known to have PPID (McFarlane et al., 2006; Beech et al., 2011a), as well as a greater MSH response to sulpiride. It should be noted that the difference in magnitude of the increase in MSH response between insulin-insensitive and sensitive mares (approximately 50 to 100%) in the present experiment is nothing near the 10-fold increase in response in mares confirmed with PPID (Beech et al., 2011a) relative to normal mares. Regardless, insulin insensitivity has been reported as a symptom of advanced PPID (Johnson et al., 2012), although more recent studies indicate a lesser (approximately 40%) prevalence in all diagnosed PPID cases (McGowan et al., 2013). Moore et al. (1979), was perhaps the first to describe symptoms in horses consistent with advanced PPID as a pituitary adenoma that resulted in hyperactivity of the adrenal gland. More recently, the peptide products of the hyperactive intermediate lobe themselves (ACTH, MSH, β -endorphin, etc.) have been indicated as the cause of some of the symptoms of PPID (McFarlane, 2011). Hyperadrenalism is typically associated

with hyperglycemia, hyperinsulinemia, and insulin resistance in various mammalian species (Hadley and Levine, 2007). In 1995, van der Kolk et al. reported that of twelve horses presented to the clinic with hirsutism and confirmed to have pituitary pars intermedia adenomas, 11 had elevated plasma insulin concentrations. One test that did not reliably predict PPID in the study of Dybdal et al. (1994) was diurnal cortisol patterns or levels. Similarly, Cordero et al. (2012) reported that normal mares often have blunted diurnal rhythms of cortisol, minimizing its usefulness for diagnosing PPID. Mean cortisol concentrations and cortisol diurnal rhythms in the insensitive mares in the present experiment were the same as those in sensitive mares. Thus, we would conclude that the underlying cause of their insulin insensitivity is not hyperadrenalism, unless the perturbation were so small as to be undetectable with normal cortisol assays.

Unlike others (McFarlane et al., 2006; Beech et al. 2007; Beech et al., 2011a,b), we observed no significant changes in plasma ACTH concentrations in response to either sulpiride or TRH injection. As stated in Chapter III, approximately one-half of the mares, when their data were viewed individually, had apparent ACTH responses to one secretagogue or the other. More specifically, four mares had apparent responses to both sulpiride and TRH (but not saline), two mares had responses to sulpiride only, one mare had a response to TRH only, and two mares had large increases in ACTH after saline injection. Speculating that these latter two mares may have experienced some acute stressor to cause the rise in plasma ACTH, we examined the simultaneous concentrations of prolactin and GH, both of which respond to stressful situations within minutes in horses (Colborn et al., 1991; Thompson et al., 1992), but found little to no change in plasma concentrations associated with the surges of ACTH. Season has been shown to affect basal ACTH concentrations (McFarlane et al., 2011) as well as the ACTH response to TRH (Funk et al., 2011) in both normal horses and horses with PPID. The highest concentrations

occur in late summer and fall (Funk et al., 2011; McFarlane et al., 2011), and the lowest responses to TRH were found in February (Funk et al., 2011). Thus, season may partially account for the poor response of these mares to TRH and sulpiride in the present experiment (conducted in February and March). Age is another factor that correlates to plasma ACTH concentrations (Schreiber et al., 2012), particularly in the fall months. However, no apparent effect of age on the ACTH response to TRH or sulpiride was observed in this experiment.

As stated earlier, the pituitary hormones other than MSH and ACTH were measured to confirm the biological activities of the injected sulpiride and TRH, from known effects reported previously, and to potentially detect any yet-to-be-determined effects on the other pituitary hormones. Although no new, unknown responses to sulpiride or TRH were observed, the expected effects on TSH and prolactin were in fact confirmed. Injection of TRH resulted in a somewhat gradual TSH response in all twelve mares, similar to the responses reported earlier by Thompson and Nett (1984). A novel observation was that TSH concentrations were consistently lower in insulin-insensitive mares relative to sensitive mares. Considering the known model for the hypothalamic-hypophyseal-thyroid axis (Hadley and Levine, 2007), these lower circulating TSH concentrations are likely because of elevated plasma triiodothyronine concentrations, as detected in the first week of the present experiment, and also reported by Cartmill et al. (2003) for hyperleptinemic mares and geldings. If this were the case, it could be that the constantly elevated insulin concentrations occurring in insulin-insensitive horses in some way affect the thyroid gland to secrete more triiodothyronine than normal or affect the peripheral conversion of thyroxin to triiodothyronine. Such an effect would be analogous to the hyperinsulinemic effect on leptin concentrations, causing hyperleptinemia in horses (Cartmill et al., 2005).

The prolactin responses to sulpiride and TRH were as expected, from previous reports (Johnson, 1987; Johnson and Becker, 1987; Kelley et al., 2006), and were produced consistently by the two secretagogues. Although prolactin concentrations produced after injection differed in their timing of increase (rapid after sulpiride and more slowly after TRH), areas under the response curves were not different when calculated over the entire 2-hour blood sampling period. Spurious increases in prolactin secretion have been reported (Roser et al., 1987; Thompson et al., 1992; Funk et al., 2011), and occurred occasionally in the present experiment after vehicle injection. However, the relative increase in prolactin concentrations after such occurrences were small (e.g., 3 to 5 ng/mL) compared to the responses to sulpiride and TRH and had little influence on the overall mean of the twelve mares.

Secretion of GH in horses, as in many species, is episodic (Thompson et al., 1992), with increases in plasma GH occurring every few hours. The episodes of GH secretion in the present experiment were minimal, as seen by the relatively constant mean GH concentrations in Figure 7. The significant variation of GH concentrations because of day of the experiment (3.9 ng/mL on the first day, dropping to 3.1 ng/mL on the last day) did not seem to have any physiological relevance.

Spurious increases in plasma concentrations of LH and FSH were observed in the present experiment, but the cause of these increases is unknown. As stated in Chapter III, most of these spurious increases occurred approximately 20 min or more after injection, and most occurred on the first day of treatments, with none occurring on the last day of treatments. Thompson et al. (1983c, 1984) reported surges in plasma LH and FSH concentrations in response to insertion of intravaginal sponges in mares in winter, and later reported episodes of secretion in seasonally anovulatory mares not touched other than for blood sampling through a jugular catheter

(Thompson et al., 1987). Retrospective measurement of progesterone in the first sample on each treatment day in the present experiment, coupled with the average concentration of LH for that day, indicated that 8 of the 12 mares were still seasonally anovulatory (low LH and no progesterone) on the first day of treatments (February 25), and 5 mares remained that way through March 24, the last treatment day. The majority of surges in LH (64%) and FSH (73%) across all mares occurred on days when both LH and progesterone were low (i.e., likely anovulatory), which is consistent with our previous observations (Thompson et al., 1983c; Thompson et al., 1984).

In conclusion, insulin-insensitive mares displayed an exaggerated MSH response to both TRH injection and sulpiride injection relative to sensitive mares. Insulin-insensitive mares also had reduced plasma TSH concentrations and higher insulin, leptin and triiodothyronine concentrations in general. In contrast, mean cortisol concentrations and the pattern of cortisol over a 24-hour period were similar in both groups. Although the differences in MSH characteristics were analogous to those observed with PPID, their magnitude was relatively small. Possible cause-effect relations that involve hyperleptinemia, hyperinsulinemia, elevated triiodothyronine concentrations, and elevated MSH responses to secretagogues are not obvious from the current literature and deserve further study in horses.

SUMMARY AND CONCLUSIONS

In this experiment, insulin-insensitive and sensitive mares were administered TRH or sulpiride, a dopamine antagonist, to evaluate the response of the pars distalis and pars intermedia hormones. Cortisol was monitored as well, although several reports show horses with PPID do not necessarily have elevated cortisol concentrations or signs of adrenal hyperactivity (McFarlane, 2011; Cordero et al., 2012).

Results showed no significant difference in plasma ACTH concentration to TRH or sulpiride in both insulin-insensitive and sensitive mares. In contrast, insulin-insensitive mares had a significant MSH response to TRH, similar to mares known to have PPID (McFarlane et al., 2006; Beech et al., 2011a), and an even greater response to sulpiride, although not near the magnitude seen in PPID (Beech et al., 2011a). Prolactin responses to TRH and sulpiride were as expected from previous reports (Johnson and Becker, 1987; Johnson, 1987; Kelley et al., 2006) and there were no noticeable changes in thyroxine, triiodothyronine, GH, LH, and FSH. Plasma insulin and leptin were higher in insulin-insensitive mare with levels rising as mares transitioned from winter into spring. Mean cortisol concentration and cortisol diurnal rhythm were the same in both groups.

In conclusion, insulin-insensitive mares were easily distinguishable from sensitive mares from their increased MSH response to TRH and even greater response to sulpiride, which parallels the characteristics observed for PPID horses, though to a lesser extent. Sulpiride could potentially be effective in diagnosing PPID, and research using sulpiride to distinguish between PPID horses and normal horses is warranted. The relationship between insulin insensitivity and MSH secretion in response to TRH or sulpiride also deserves further investigation.

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Nicole Arana Valencia, daughter of Norberto Arana Soto and Yolanda Valencia Lucena, was born in 1987 in San Juan, Puerto Rico. Nicole attended Wesleyan Academy and Colegio Rosa Bell, graduating with honors from Rosa Bell in Guaynabo, Puerto Rico, in May of 2005. During her undergraduate time at Louisiana State University, Nicole met and married Allan James McIlwain in April of 2011. She received her Bachelor of Science degree in the School of Animal Sciences at Louisiana State University in May of 2012. She began her Master of Science degree at Louisiana State University in the summer of 2012 with an interest in equine endocrinology.