THE ASSOCIATIONS OF ABNORMAL PROLACTIN SECRETION AND METABOLIC HEALTH IN ACYCLIC FEMALE AFRICAN ELEPHANTS (LOXODONTA AFRICANA)

by

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ABSTRACT

THE ASSOCIATIONS OF ABNORMAL PROLACTIN SECRETION AND METABOLIC HEALTH IN ACYCLIC FEMALE AFRICAN ELEPHANTS (LOXODONTA AFRICANA)

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Reproductive success is an increasing concern for captive African elephants (*Loxodonta africana*) in North America. Low fecundity rates indicate these populations are unsustainable. Chronic hyperprolactinemia (HPRL), elevated prolactin secretion, has been proven to have a strong association with ovarian dysfunction in African female elephants. Previous studies have identified metabolic effects correlated with acyclicity, such as higher concentrations of insulin and leptin and lower glucose-to- insulin (G: I) ratios. However, metabolic effects from chronic elevated prolactin have yet to be investigated. In humans, in addition to amenorrhea, hyperprolactinemic women have shown increased risks for accelerated atherosclerosis, hyperandrogenemia, reduced metabolism, and insulin resistance. The aim for the study was to explore possible associations between abnormal prolactin secretion (high and low) and metabolic disorders in elephants.

One year of serum samples, collected biweekly, were obtained from African female elephants (n=36) with varying status of prolactin secretion (high= 12, normal= 12, low= 12). Biomarkers were assessed to compare thyroid function, glucose and lipid metabolism and cardiovascular health amongst the high, normal, and low prolactin state groups. Generalized linear mixed models (GLMs) were performed in R.

Results determined that high prolactin secretion is associated with abnormal TSH and thyroid hormone production, elevated cortisol and cholesterol, and reduced fructosamine. Low prolactin individuals were found to have heightened levels of testosterone. Taken together, this study highlights several areas in need of further study to further advance our understanding of African elephant physiology and the etiology of hyperprolactinemia in female elephants. This study suggests that hyperprolactinemic females may have further hormone perturbations with associated changes in thyroid, cortisol and cholesterol levels, while hypoprolactinemic females have increased levels of serum testosterone. More studies are warranted to better understand the effects associated with acyclicity and abnormal prolactin secretion.

CHAPTER 1: LITERATURE REVIEW

History of Elephants in Managed Facilities

Egyptians began using ivory as an artistic media as early as 6000 BC (Pitman, 1953). Between 6000 BC and the 15th century, African elephants were still largely used for ivory artwork. However, hunting for sport became more detrimental to elephant populations than for food, ceremony, or art (Sikes, 1971c). Ancient kings were known to participate in elephant hunting expeditions for sport and collected individuals for personal living collections (Sikes, 1971c).

The Hellenistic Greek period (323-27 BC) featured a significant increase in exotic importation of elephants, but not for the purpose of animal collections (Kisling, 2000). Most elephants at this time were used as war animals, such as Hannibal's expedition through Spain with 37 war elephants used to infiltrate Trebia in the 2nd century (Kisling, 2000). Following the fall of the Carthaginian army, Romans collected the remaining war elephants and returned them to Rome (Kisling, 2000). Few elephants remained in military services thereafter, but others were repurposed to take part in ceremonial processions, gladiatorial events, and circus attractions (Kisling, 2000).

In 476 AD, the Roman Empire fell, marking the beginning of the medieval period (476-1453), where animal collections were abandoned (Kisling, 2000). Only the highest monasteries, monarchies, and municipalities kept animal collections (Kisling, 2000).

European Emperors and Kings were known to have personal animal collections, in which elephants were highly prized (Kisling, 2000). These animal collections were only accessible to the higher-class citizens; peasants rarely witnessed exotic animals from distant lands (Kisling, 2000).

As Europe grew in wealth and power, animal collections expanded in size and became known as menageries (Kisling, 2000). Menageries became an institution whereas when the Europeans explored new territories of Asia, Africa, and Europe, travelers would return with new exotic animals to exhibit (Kisling, 2000). Collecting animals quickly became an obsession (Kisling, 2000). To maximize profits, transporters would overload cargo carriers of the ship, disregarding the health and safety of their animal cargo (Kisling, 2000). In most cases, less than 50% of the animals arrived alive (Kisling, 2000).

Menageries were prevalent and popular throughout the 1800s, but as ownership transitioned from private collections to public institutions, menageries transitioned into zoological gardens, or zoos (Kisling, 2000). As knowledge and technology improved, zoos developed into scientific institutions, involving animal husbandry, veterinary medicine, education, and research (Kisling, 2000). This was the framework, which allowed zoos to evolve into modern day conservation parks. Modern zoos exhibit species that act as ambassadors to wild educate the general public on conservation issues and programs for species survival. Partnerships with colleges and universities allow for innovative research to investigate animal husbandry, nutrition, and reproduction (Kisling, 2000).

North American Zoological Populations

Current African elephant populations in North American zoos are not sustainable (Weise and Willis, 2006). Over a ten year period (2000- 2010), the zoo population experienced an average of 4.7 deaths and 3.5 births per-year, resulting in a population decline (Faust and Marti, 2011). This decline can be partially attributed to reproductive issues of captive elephants.

Behavioral incompatibility and sperm quality are two common reproductive challenges seen in African elephant males (Wiese, 2000; Olsen and Wiese, 2000; Hildebrandt, 2006). However, here onward, we discuss challenges specifically pertaining to female elephants. Such issues include birthing difficulties, such as dystocia, and infertility (Wiese, 2000; Olsen and Wiese, 2000; Hildebrandt, 2006).

Normal Female Endocrinology

Elephants are known to be non-seasonal, spontaneously ovulating, polyestrous breeders. Although substantial data are lacking, it is generally accepted that wild African elephants reach puberty between 10 and 12 years of age, where 7 years can be seen in captivity (Perry, 1953; Hildebrandt et al., 2011). Ovarian activity in females is characterized by a 13-17 week period known as the estrous cycle (Brown, 2006). Within this cycle, two phases are distinguished: a luteal, lasting 8-12 weeks, followed by a 4-6 week inter-luteal (follicular) phase (Knobil and Neill, 1998).

Determining the estrous cycle was not a particularly precise procedure until the development of progestogen assays allowed scientists to track luteal activity (Brown, 2000; Hess et al., 1983; Plotka et al., 1988). Progesterone is the major circulated progestogen in the estrous cycle in many mammals. However, elephants are distinctive. Concentrates of 5α- reduced pregnanes (5α pregnane-3, 20-dione, and 5α-pregnane-3-ol-20 one) circulate higher than progesterone (Brown, 2000; Heistermann et al., 1997; Schwarzenberger et al., 1997; Hodges, 1998). The cross-reactivity of antisera with circulating pregnanes allows the luteal functions in the elephant to be monitored appropriately (Brown, 2000). Elevated progestogen levels inhibit follicular development in the luteal phase, but when progestogens fall, follicular activity is induced, allowing for an anovulatory luteinizing hormone (LH) surge (anLH), and an ovulatory LH (ovLH) surge (Hildebrandt, 2006). The true purpose for the anLH surge remains unknown.

Around 19-21 days after the first LH surge, an ovLH surge peaks, resulting in ovulation and corpus luteum (CL) development (Brown, 2000; Hildebrandt et al., 2011).

In addition to the unique activity of LH, Follicle Stimulating hormone (FSH) also functions rather uniquely in the elephant. FSH is responsible for stimulating follicular growth in the ovary (Senger, 1999). In many mammals (e.g. humans and sheep), FSH slightly recedes during the follicular phase to recruit the dominant follicle(s) (Baird, 1983; Brown, 2000). In elephants, FSH concentrations peak near the beginning of the non- luteal phase, progressively declining until about 4 days prior to the ovLH surge (Ginther, 1992). Post ovLH surge, FSH concentrations rise in the early luteal phase and remain heightened for 7-8 weeks into the early follicular phase (Brown et al., 1999; Brown et al., 2004; Hildebrandt et al., 2011). One speculated reason for this prolonged FSH secretion is for the recruitment of functional follicles during the end of the non-luteal phase (Ginther, 1992).

Hyperprolactinemia and Infertility in Female Elephants

Infertility in African elephants is a major concern hindering reproductive success in zoos. It has been found that extended non-reproductive periods increase a female's susceptibility to reproductive tract pathologies and irregular or complete halt in cyclicity (Hildebrandt et al., 1997; Brown, Hildebrandt et al., 1999; Hildebrandt et al., 2000; Hildebrandt et al., 2003a; Hermes et al., 2004). Hildebrandt et al. (2006) documented vestibular cysts found in both African and Asian elephants, but vestibular polyps seem to be specific to African elephant cows. Nearly 70% of polyps occur in females over the age of 30 years. In older (> 30 years) nulliparous females, vaginal cysts and neoplastic formations can obstruct the vagina, inhibiting semen passage to the uterus, making mating uncomfortable (Hildebrandt et al., 2006; Hildebrandt et al., 2000). To produce a clear understanding of zoo demographics, Prado-Oviedo et al. (2016) found the

majority of female elephants in captive populations have already aged past 30 years, and only 25.7% of those elephants calved by the end of 2012.

In Humans, elevation in prolactin secretion, hyperprolactinemia, is the most common cause of infertility in women between 25-34 years of age (Sonigo et al. 2012; Molich, 2010). Natural prolactin (PRL) levels seasonally fluctuate within a range of 5 and 25 ng/ml (Majumdar and Mangal, 2013). This pituitary disorder was first discovered in elephants in 1997 and, as in humans, the occurrence of hyperprolactinemia has been consistently associated with acyclicity and hypogonadism (Brown and Lehnhardt, 1997; Serri et al., 2003). Currently, over half of acyclic African elephants are hyperprolactinemic, nearly 28% of the total North American population (Prado-Oviedo, 2016; Brown et al., 2016).

Majumdar and Mangal (2013) discuss how physiologic hypersecretion of PRL is typically due to common conditions such as pregnancy, lactation, chest wall trauma/surgery, exercise, sleep, and/or stress. If an underlying cause is not apparent, computerized axial tomography (CAT) or magnetic resonance imaging (MRI) of the pituitary region may determine if mild elevations are due to nonfunctioning adenomas pressing against the pituitary stalk (Majumdar and Mangal, 2013; Serri et al., 2003). Pressure against the stalk may lead to an interruption of the dopamine pathway from the hypothalamus to the pituitary, decreasing the inhibitory effect on PRL secretion, also known as the "stalk effect" (Serri et al., 2003). PRL secreting prolactinomas are the most likely cause if prolactin levels exceed 250 ng/ml and a macroprolactinoma will produce levels of 500 ng/ml or greater. Cases where adenomas are not present after imaging, the observed hyperprolactinemia is then considered idiopathic (Majumdar and Mangal, 2013). There is yet to be data reported on the incidence of prolactin-secreting tumors in African elephants, as imaging of the pituitary is not feasible and pituitary histopathology is rarely performed at necropsy; however this remains a possibility.

Prolactin

Discovered by Oscar Riddle in the 1930s, prolactin was described as a unique protein produced by lactotrophic cells found in the anterior pituitary gland (Stricker and Grueter, 1928; Riddle, 1933). This hormone serves a particular role in reproduction by assisting mammary gland development, maturation, and milk production (Freeman et al., 2000). Ormandy et al. (1997) described the dependency of mammary on prolactin activity to stimulate secretory epithelial tissue proliferation. Prolactin is now understood to be a pleiotropic neurohormone, regulating more than 300 biological processes, a few of which will be discussed later (Freeman et al., 2000).

Human prolactin is encoded on a single gene within the genome, located on the sixth chromosome (Freeman et al., 2000). The gene contains five coding exons, transcribed from a primary, known as the pituitary promoter, and the secondary, or extra-pituitary, promoter (Freeman et al., 2000). Prolactin is constructed of a single chain of 199 amino acids with three disulfide bonds between six cysteine residues (Senger, 1999; Freeman et al., 2000; Li et al., 1987), which are thought to protect prolactin from possible short-term degradation during transport to target tissues (Senger, 1999). The major form of prolactin is a 23 kDa monomeric molecule found in the pituitary and makes up about 85- 95% of circulating prolactin (Freeman et al., 2000). Prolactin also has variant isoforms because of post-transitional modifications of the amino acids within the lactotrophs (Shelly et al., 2012; Freeman et al., 2000). Each isoform has specific binding receptors and biological functions. A few post-translational modifications of prolactin include enzymatic cleavage, dimerization, phosphorylation, and glycosylation. Phosphorylation of prolactin occurs within the lactotroph where a phosphate group is covalently bonded to a serine or threonine residue (Goffin et al., 2002. Freeman et al., 2000). Phosphorylated prolactin has much reduced biological activity than the non-phosphorylated form (Freeman et al., 2000; Wang and Walker, 1993).

Glycosylated prolactin has an N-glycosylated chain bonded to the asparagine residue in the Asn-Leu-Ser sequence (Lewis et al., 1985). This results in a higher molecular weight of approximately 25 kDa and reduced biological activity to the non-glycosylated monomeric prolactin (Freeman et al., 2000; Pellegrini et al., 1988). Glycosylated prolactin is also known to be immuno-reactive and cross-reactive to the monomeric non-glycosylated form (Pellegrini et al., 1988).

There is an isoform of PRL that is approximately 40-60 kDa, known as bigPRL. Little is known about this form, bit it is thought that some bigPRL is a dimer form of monomeric prolactin and some may be a breakdown product of the macroprolactin form (Fahie-Wilson et al., 2005; Garneir et al., 1978). Big-big PRL, also known as macroprolactin, is a general term for a polymer group of high molecular weight prolactin complexes. These variant forms of prolactin have a typical molecular weight of between 150 and 160 kDA (Freeman et al., 2000). The macroprolactin most frequently identified complex is composed of an immunoglobulin G (IgG) and a monomeric prolactin molecule (Kavanagh-Wright et al., 2009; Leite et al., 1992). Macroprolactin is present in circulating serum, but has little biological activity (Freeman et al., 2000).

Sites of Synthesis and Secretion of Prolactin

Prolactin can be regulated via influence of the central nervous system, intra-pituitary factors, and peripheral organs. In the central nervous system, dopamine is the primary inhibiting factor for the control of prolactin secretion (Freeman et al., 2000; Ben-Jonathan and Hnasko, 2001). It was found that environmental and reproductive stimuli, such as suckling, pregnancy, and stress, can cause withdrawal of dopamine release, which incites spontaneously high levels of prolactin secretion from the inner zone of the anterior pituitary (Freeman et al., 2000). Oxytocin is a neurohormone that stimulates prolactin secretion (Freeman et al., 2000). In turn, prolactin

then will inhibit the oxytocinergic neurons in the hypothalamic paraventricular nucleus, thus maintaining an inhibitory feedback loop relationship with oxytocin (Sirzen-Zelenskaya et al., 2011). Conversely, serotonin is an inhibitory factor on prolactin secretion, acting as a neurotransmitter from hypothalamic neurons that emanate from the raphe nucleus (Freeman et al., 2000). For example, in response to suckling serotonin levels rapidly decline, allowing increasing levels of its precursor, 5-hydroxyindoleacetic acid, and prolactin (Freeman et al. 2000).

Local regulators in the anterior pituitary also influence prolactin secretion via paracrine and autocrine signaling. Vasoactive intestinal peptide and galanin are local hormones shown to influence prolactin secretion in an autocrine manner (Freeman et al. 2000). Paracrine regulators of prolactin secretion include hormones such as calcitonin, gonadotropin-releasing hormone (GnRH), and acetycholine (Freeman et al. 2000). Lastly, extra-pituitary tissues, such as the ovaries, can have significant influence on prolactin production in lactotrophs. Freeman et al. (2000) described ovarian estradiol as a prolactin regulator in two manners. Estradiol can act directly on the pituitary lactotroph, controlling prolactin's gene expression, and increases lactotroph sensitivity to physiological stimuli. At the hypothalamus, estradiol can inhibit an enzyme, tyrosine hydroxylase that promotes dopamine synthesis (Freeman et al. 2000; Villegas-Gabutti et al., 2016). Additionally, leptin, found in adipose tissue, has been found to stimulate prolactin secretion from lactotroph cells (Yu et al., 1997; Accorsi et al., 2007).

Prolactin and Thyroid Hormones

In adults, the thyroid has a significant role in regulating biological processes such as thermogenesis, protein synthesis, and overall metabolic activity (Chen et al., 2009; John et al., 2013). Triiodothyronine (T_3) and Thyroxine (T_4), a prohormone for T_3, are hormones located in the thyroid follicles, which are stimulated from the release of Thyroid- stimulating hormone (TSH) by the anterior pituitary (John et al., 2013). A deficiency in regular thyroid hormone

concentrations may be caused by a dysfunctional connection between the hypothalamus and thyroid. Thyrotropin- releasing hormone (TRH), a hypothalamic neurohormone, is the primary stimulus for TSH release (Harris et al., 1978; Kanasaki et al., 2015). In humans, TSH is known to have a stimulatory effect on serum prolactin, with a higher effect in women than men (Noel et al., 1974). Hypothyroidism is a condition of an underactive thyroid that may elevate serum prolactin concentrations through an increased stimulation of TRH (Freeman et al. 2000).

Prolactin and Glucose Metabolism

Previous studies have conflicting results indicating that prolactin levels have a positive correlation with weight gain (Greenman et al., 1998; Atmaca et al., 2013). However, there is evidence that circulating prolactin has a significant role in glucose metabolism by β cell proliferation, promoting insulin production, and enhanced glucose-stimulated insulin secretion with a lower glucose threshold (Cejkova et al., 2009; Weinhaus et al., 1996; Wang et al., 2013).

In a 2009 study, Tuzcu et al. associated high circulating prolactin levels with insulin resistance. Using a euglycemic hyperinsulinemic clamp technique, they found that hyperprolactinemic patients have higher HOMA- β (β- cell function) and lower HOMA- S (insulin sensitivity) index scores than healthy patients without hyperprolactinemia.

Prolactin and Lipid Metabolism

Adipose tissue serves an important role in energy balance via lipid metabolism (Ahima, 2006, Ailhaud, 2006, Ben-Jonathan and Hugo, 2013). Lipids can be created via lipogenesis in the form of triglycerides and free fatty acids (FFA). They are stored within the cytoplasmic droplets of adipocytes, and released via lipolysis (Ben-Jonathan and Hugo, 2013). Insulin secretion by the pancreas stimulates lipogenesis and inhibits lipolysis in adipose tissue and the liver (Ben-Jonathan and Hugo, 2013). Although mainly thought of as only a pituitary hormone, prolactin is also produced by adipose tissue. It is secreted as a paracrine hormone, which plays a role in lipid

metabolism modulation on adipose tissue, and therefore on energy homeostasis (ling et al., 2000; Zinger et al., 2003; Ben-Jonathan and Hugo, 2013; Carrè and Binart, 2014). During gestation in humans, elevated PRL decreased lipoprotein lipase (LPL) and the concentration of malonyl-CoA, a factor of lipogenesis. However, in mice, PRL increased LPL, causing an accumulation of triglycerides (Brandebourg et al., 2006; Nilsson et al., 2009; Carrè and Binart, 2014).

Prolactin and Cardiovascular Health

Prolactin has a complex role in cardiovascular function. Previous studies have conflicting results suggesting that PRL acts on endothelial cells to stimulate and inhibit angiogenesis, vasodilation, and vasopermeability (Clapp et al., 2013; Rosas-Hernandez et al., 2013; De Spiegelaere et al., 2012). However, these results may be due to the presence of vasoinhibins. Vasoinhibins are proteolytic cleaved PRL fragments, ranging from 14-18 kDa, that act on endothelial cell receptors, which are entirely separate from typical PRL receptors (Clapp et al., 2013; Clapp et al., 1993; Faupel-Badger et al., 2010; Ishida et al., 2014). Inhibins were found to inhibit angiogenesis, vasodilation, and vasopermeability (Clapp et al., 2013). In humans, women with elevated PRL levels were shown to have higher levels of low-density lipoprotein (LDL), lower high-density lipoprotein (HDL), thickened carotid intima media, and increased arterial stiffness, (Medic-Stojanoska et al., 2015; Jiang et al., 2014; Georgiopoulos et al., 2009).

Androgens have a role in cardiovascular function; however, a complete understanding of that effect remains unknown. In human and animal studies, testosterone has polarizing vasoconstriction and vasodilation effects on blood vessels (Tambo et al., 2016). Studies in women have shown testosterone levels at the higher end of the normal range have lower carotid intima-media thickness, suggesting potential cardio-protective effects. Yet, elevated levels in women with PCOS have increased CVD risk, although this is not the case in postmenopausal women (Akishita and Yu, 2012; Bernini et al., 1999).

Testosterone in women is produced primarily in the Graafian follicles of the ovaries (25%), adrenal cortex (25%), and peripheral conversion of androgen precursor hormones (androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S)) (50%) (Maturana et al., 2008; Kumar et al., 2005; Tambo et al., 2016). Testosterone circulates in the blood either as free testosterone or bound to albumin and sex hormone-binding globulin (SHBG) (Burger, 2002; Brand and van der Schouw, 2010). Previous studies revealed that high levels of testosterone with low levels of SHBG increased risk of CVD (Brand and van der Schouw, 2010).

Diagnosis and Treatment of Hyperprolactinemia in Humans

Hyperprolactinemia is a normal occurrence during pregnancy, as it assures proper nutrients are delivered to the fetus for optimal development. Outside of this physiological state, prolonged prolactin levels can suppress GnRH secretion, resulting in reduced estradiol secretion. This extended state of hyperprolactinemia may eventually lead to irregular estrus cycles and amenorrhea (Koike et al., 1991).

To treat hyperprolactinemia, Majumdar and Mangal (2013) categorized three groups of patients. Group 1 included patients with hyperprolactinemia caused by microadenomas or diagnosed as idiopathic. For patients in this group, bromocriptine medication is a common treatment. Bromocriptine is a strong dopamine agonist that will bind to dopamine receptors. It directly inhibits prolactin secretion by reducing prolactin synthesis, cell proliferation, and if present, a reduction in prolactinoma size. Results showed prolactin levels to decrease over a span of one week. Ovulation and menstruation resumed 4-8 weeks later. If adenomas are asymptomatic/nonfunctioning and grow slowly, no treatment is necessary. In other cases, medical treatment of bromocriptine or cabergoline (also a dopamine agonist) can be implemented for a time period of 18 months to 6+ years (Majumdar and Mangal, 2013).

Patients categorized into Group 2 have diagnosed macroadenomas, causing hyperprolactinemia. Treatments in this group are aimed to reduce the size of the adenoma and restore fertility. First, a dopamine agonist is used as an initial attempt to reduce the size of the macroadenoma. If reduction is not seen within a few weeks, a trans-nasal, trans-sphenoidal, microsurgical excision of the prolactinoma is performed. Risks of surgery include regrowth of the prolactinoma and/or hypopituitarism. Radiation therapy is a last resort in an attempt to treat persistent tumors that return after surgery (Majumdar and Mangal, 2013).

Group 3 consisted of patients that have mildly elevated prolactin levels due to primary hypothyroidism. An under active thyroid may cause decreased concentrations of serum thyroid hormones (thyroxin [T4] and triiodothyronine [T3]). This can disrupt the thyroxin negative feedback loop and cause hyper-secretion of thyroid releasing hormone (TRH) from the hypothalamus (Koller et al., 1987). TRH primarily stimulates the release of thyroid stimulating hormone (TSH) from the anterior pituitary, but also has a mild prolactin releasing effect on pituitary lactotroph cells (Yen, 2001). Some case studies have shown that a thyroxin hormone replacement has been successful in controlling primary hypothyroidism and normalizing prolactin levels (Ahmed et al., 1989).

Treatment of Hyperprolactinemia in Elephants

Various degrees of stress have been correlated with cases of hyperprolactinemia in humans (Freeman et al., 2000). It is possible that similar situations may be the cause for hyperprolactinemia in other social animals such as elephants (Prado-Oviedo, 2015). Elephants naturally form tight social bonds with herd mates, and rely on those individuals during environmental and social stress events. Social instability, such as an individual alternating between two or more social groups can be stressful for the individual and amongst group members (Brown et al., 2016). Brown et al. (2016) suggest, “[In relation to] female African

elephants, not being in a stable social group may be a stressor that elicits an increased prolactin response." Prado-Oviedo (2015) found that elephants with hyperprolactinemia were more likely to exhibit nurturing and filial behaviors compared to normal cycling prolactin individuals. Hyperprolactinemic elephants may potentially over-compensate for the lack of reproductive ability by becoming peacekeepers to maintain social bonds within the herd (Prado-Oviedo, 2015).

A yearlong study of cabergoline treatments, a dopamine agonist, tested if it could suppress prolactin secretion in hyperprolactinemic elephants (Morfeld et al., 2104). The goal of these trials was to stimulate the dopamine-prolactin negative feedback loop (Morfeld et al., 2014). The treatment successfully reduced serum prolactin; however, females did not resume normal cycling (Morfeld et al., 2014). Although no treatment has yet to be identified, Prado-Oviedo (2015) reported a correlation between ovarian activity and prolactin secretion with parturition age. The birth of one offspring at a reproductively young age can reduce the risk of irregular ovarian activity and abnormal prolactin secretion by 60% and 80%, respectively (Prado-Oviedo, 2015). Estrogens are another factor that can influence the dopamine-prolactin feedback loop. Estrogens are steroid hormones that can both increase lactotroph sensitivity to release prolactin and inhibit tyrosine hydroxylase, the enzyme that promotes dopamine synthesis (Larson and Wise, 1994; Freeman et al., 2000). Prado-Oviedo's (2013) study compared estrogenic levels between normal and hyperprolactinemic female elephants. The study expected to find high abundance of estrogen in hyperprolactinemic elephants, but found no direct correlation between hormone concentrations (Prado-Oviedo, 2013).

CHAPTER 2

Study Objectives

It has been long reported that elephants also exhibit issues with excess weight, infertility, and cardiovascular disease (Clubb and Mason, 2002; Morfeld et al., 2015). However, possible associations amongst hyperprolactinemia and these conditions are far less understood in African elephants than in humans. Morfeld and Brown (2014) investigated whether adiposity and metabolic hormones were associated with ovarian dysfunction in African elephants. They found significant relationships between reproductive function and circulating insulin and leptin concentrations. Female elephants that were acyclic had higher levels of insulin and leptin, but a lower glucose-to-insulin (G: I) ratio. However, prolactin was not assessed in the Morfeld and Brown (2014) study.

The aim of the study described here was to identify any potential associations between abnormal prolactin secretion (high and low) and metabolic disorders in elephants. We organized biomarkers into three health categories. The first category was thyroid hormones in which TSH, total and free triiodothyronine, and total and free thyroxine, were assessed. The second category was glucose and lipid metabolism. Health markers in this category included glucose, fructosamine, insulin, glucose: insulin ratio, and cortisol. Total cholesterol, LDL, HDL, triglycerides, and testosterone were health markers assessed under the third category, cardiovascular health.

Materials and Methods

Sample Collection

This study was conducted at Smithsonian Conservation Biology Institute (SCBI) in Front Royal, Virginia. SCBI has the largest elephant serum bank in North America. Bi-weekly sera samples were previously collected from African elephant females at Association of Zoos and Aquariums (AZA) accredited zoos in North America and used for this study. Samples were collected within a 12-month period from 2011- 2012. A total of n = 36 females was selected from our serum bank and separated into three status groups based on prolactin concentrations: Hyperprolactinemia, Normal (control), and Hypoprolactinemia. The hyperprolactinemic group (n = 12) was defined with individuals having prolactin concentrations ≥ 18 ng/ml over the 12-month sampling period; the normal/ control group (n = 12) had females that were cycling with prolactin concentrations < 18ng/ml; and the hypoprolactinemic group (n = 12) were non-cycling females with prolactin < 18 ng/ml and treated as the negative control group. Serum was analyzed for reproductive hormones: progestogerone (P_4), luteinizing hormone, and follicle-stimulating hormone. We then analyzed the following under the descriptor, thyroid function: TSH, total T4, free T4, and total T3. Cortisol, fructosamine, glucose, insulin, and calculated glucose-to-insulin ratio (G:I) were quantified to assess glucose and lipid metabolism. Finally, we examined the following under the category, cardiovascular health: triglycerides, testosterone, total cholesterol, direct HDL, and direct LDL. It should be noted that samples were collected regardless of last meal, and all results were interpreted assuming elephants were in a fed state.

Immunoassays

Serum progestogens were analyzed using a solid-phase ^{125}I radioimmunoassay (RIA) (Seimens Medical Solutions Diagnostics, Los Angeles, CA) and serum prolactin was analyzed by a heterologous RIA that utilized an anti-human prolactin antiserum (NIDDK-anti-hPRL-3) and ovine prolactin label and standards (NIDDK-oPRL-I-2) (Brown and Lehnhardt, 1995; Brown and Lehnhardt, 1997). Assay sensitivities (based on 90% maximum binding) were 0.05 and 5.0 ng/ml

for the progesterone and prolactin RIAs, respectively. The intra- and inter- assay coefficients of variation for all assays were <10% and <15%, respectively.

Testosterone, Cortisol, TSH, and Thyroid Hormones (Total T_3, Total and Free T_4)

Serum insulin concentrations were measured using a solid-phase, two-site bovine insulin enzyme immunoassay (EIA) (10-1201-01, Mercodia Inc., Uppsala, Sweden). TSH was measured via a heterologous ^{125}I RIA (Brown et al., 1997). Per Brown et al. (2007), cortisol and thyroid hormones (total T_3, total and free T_4) were quantified using a solid- phase ^{125}I RIA (Siemens Healthcare Diagnostics Inc., Los Angeles, CA).

Glucose, Apolipoprotein, Fructosamine, Triglycerides, and Cholesterol Analyses

Serum glucose, apolipoprotein A-1, fructosamine, triglycerides, cholesterol, direct LDL, were determined using a clinical chemistry analyzer (RX daytona, Randox Laboratories Ltd USA, Kearneysville, WV, USA). Glucose and triglycerides were tested with GOD-PAP and GPO-PAP enzymatic colorimetric methods with 4.42 and 11.9 mg/dl sensitivity, respectively. Total cholesterol, direct HDL, and direct LDL were analyzed with an enzymatic hydrolysis and oxidation process, with minimum standard sensitivities 33.4, 7.30, and 7.30 mg/dl respectively. Fructosamine was tested using an enzymatic assay, having a sensitivity of the minimum standard of 8.12 µmol/L. Results were converted to mg/dl for statistical analysis. Apolipoprotein A-1 was quantified with an immunoturbidimetric immunoassay (RX series). It should be noted that apolipoprotein tested unanimously low in all samples. Therefore, apolipoprotein was omitted from further analyses. All commercially available reagents, calibrators, and controls were purchased from Randox Laboratories Ltd.- USA (Kearneysville, WV, USA).

Glucose-to-insulin ratio (G: I) was calculated. The G: I was included to account for the non-fasting state of our study animals and is a common proxy for counteracting the effects of

changes in glucose and/or insulin due to feeding status. All samples were analyzed in duplicate; intra- and inter-assay CVs were <10% and <15%, respectively.

Statistical Analysis

Differences of variables amongst prolactin groups were tested using generalized linear mixed models (GLMMs) developed in R i386, version 3.5.3 (R Core Team. 2014. R Foundation for Statistical Computing, Vienna, Austria). Three binomial GLMMs were built to test differences amongst variables between PRL statuses (high prolactin, normal prolactin, and low prolactin). Model 1 tested for variable differences between high prolactin and the normal prolactin group; model 2 tested for variable differences between the high prolactin and low prolactin group; and model 3 tested for variable differences between high and low prolactin groups. Dependent variables in the models included: P_4, FSH, LH, TSH, total T_4, total T_3, free T_3, glucose, fructosamine, insulin, triglycerides, G:I, cholesterol, HDL, LDL, cortisol, and testosterone. The random effects in each model were zoos where samples were collected, and individuals nested within the zoos. All combinations of the dependent variables were collectively identified and assessed to find the best model. The lowest Akaike information criterion (AIC) determined the best fit model permutation. Any dependent variable removed from the model was singly reincorporated into the model to test for significance. The level of significance was set at $p < 0.05$.

Results

The mean, minimum value, maximum value, and standard deviation of all analyte concentrations were calculated for each prolactin status group (high prolactin, normal prolactin, and low prolactin), and presented in Table 1. Based on AIC values and biological significance three minimal models were identified from the statistical analyses (Table. 2). Model 1 (high v. normal prolactin) eliminated all biomarkers except progesterone and total T_3. Both models 2 and 3 (high v. low prolactin and low v. normal prolactin respectively) removed all biomarkers, except for testosterone, from the minimal model.

Table 1. Summary of average hormone concentrations (n= 36) by prolactin status and health group. Data includes the mean, minimum value (Min.), maximum value (Max.), and standard deviation (STD.) of each quantified variable.

Reproductive Hormones	Normal Prolactin (n=12)				High Prolactin (n=12)				Low Prolactin (n=12)			
	Mean	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**
Prolactin (ng/ml)	12.87	1.62	71.35	11.90	42.55	4.87	160.30	33.71	6.45	1.52	22.61	4.32
Progesterone (ng/ml)	0.35	0.05	1.63	0.33	0.10	0.05	0.89	0.11	0.09	0.05	0.31	0.05
Follicle- Stimulating Hormone (ng/ml)	3.22	0.50	6.46	1.24	2.89	0.50	32.40	3.12	3.39	0.50	9.12	1.59
Luteinizing Hormone (ng/ml)	1.11	0.46	6.86	0.77	0.99	0.37	2.00	0.38	1.13	0.25	4.49	0.81
Thyroid Hormones	**Normal Prolactin (n=12)**				**High Prolactin (n=12)**				**Low Prolactin (n=12)**			
	Mean	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**
Thyroid- Stimulating Hormone (ng/ml)	0.95	0.10	2.36	0.44	0.94	0.10	2.99	0.59	0.70	0.10	1.19	0.28
Total Thyroxine (µg/dl)	9.63	4.26	16.29	1.86	9.10	0.77	16.45	2.56	9.58	6.48	14.32	1.80
Total Triiodothyronine (ng/dl)	92.63	28.06	147.77	23.58	76.88	21.73	130.02	19.44	85.59	15.95	131.00	25.58
Free Thyroxine (ng/dl)	0.82	0.46	1.70	0.20	0.76	0.05	1.15	0.24	0.81	0.51	1.29	0.15
Glucose and Lipid Metabolism	**Normal Prolactin (n=12)**				**High Prolactin (n=12)**				**Low Prolactin (n=12)**			
	Mean	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**
Glucose (mg/dl)	79.08	24.32	108.82	11.90	72.66	4.42	113.14	15.43	77.13	12.97	110.08	11.82
Fructosamine (mg/dl)	11.69	0.31	17.11	2.71	10.83	6.98	16.60	1.89	11.31	1.46	16.17	2.33
Insulin (µg/L)	0.48	0.07	3.15	0.46	0.42	0.03	3.22	0.44	0.57	0.06	4.11	0.55
Glucose: Insulin Ratio	278.79	31.08	876.76	192.90	312.77	20.85	2088.69	281.63	263.24	5.94	1266.24	232.90
Triglycerides (mg/dl)	52.06	11.90	117.77	22.32	50.28	11.90	262.10	42.12	32.12	7.97	95.63	13.80
Cortisol (ng/ml)	16.42	3.30	49.60	10.55	18.38	5.30	83.80	11.10	20.86	3.90	68.70	11.93
Cardiovascular Health	**Normal Prolactin (n=12)**				**High Prolactin (n=12)**				**Low Prolactin (n=12)**			
	Mean	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**	**Mean**	**Min**	**Max**	**STD**
Cholesterol (mg/dl)	70.75	13.15	117.9.	16.78	71.00	33.40	125.28	14.37	67.79	6.96	119.87	16.63
High- Density Lipoprotein (mg/dl)	8.32	0.77	13.15	2.42	8.09	0.78	11.99	1.78	8.21	0.78	13.15	2.21
Low- Density Lipoprotein (mg/dL)	42.65	0.39	98.60	13.42	41.58	7.30	71.92	10.78	40.80	3.09	77.33	11.56
Testosterone (ng/ml)	0.63	0.02	3.83	0.52	0.53	0.06	1.99	0.28	13.54	0.05	196.53	38.18

Table 2. Summary of the minimal models determined by statistical analyses. Data includes the AIC, estimate value, standard error (SE) and the significance (p-value) of the predictors in each model. Each model tests the differences between two of the three groups: High (H), Normal (N), and Low (L) prolactin.

	Progesterone				**Total Triiodothyronine**			**Testosterone**		
	AIC	Estimate	SE	p-value	Estimate	SE	p-value	Estimate	SE	p- value
Model 1 (H v. N)	48.90	-0.03	<0.01	< 0.01	-0.04	< 0.01	< 0.01	--	--	--
Model 2 (H v. L)	41.10	--	--	--	--	--	--	-0.02	0.01	0.04
Model 3 (L v. N)	41.80	--	--	--	--	--	--	0.02	0.01	0.01

A summary of the results for each model is presented in Table 3. The table includes the estimate, standard error, and p-value of each biomarker assessed within a given health group.

Table 3. Summary of results by model and health group. Data includes the estimate value, standard error (SE), and p-value for each quantified variable.

	Model 1 (HvN)			Model 2 (HvL)			Model 3 (LvN)		
Thyroid Function	**Estimate**	**SE**	**p-value**	**Estimate**	**SE**	**p-value**	**Estimate**	**SE**	**p-value**
TSH (ng/ml)	0.006	0.002	0.003*	1.140	4.253	0.789	-2.862	0.006	0.642
Total T4 (µg/dl)	0.021	0.771	0.979	-0.089	0.765	0.908	0.074	1.008	0.942
Total T3 (ng/dl)	-0.036	0.002	< 0.001*	-0.012	0.002	< 0.001*	0.011	0.096	0.911
Free t4 (ng/dl)	-0.634	0.002	< 0.001*	-0.758	0.002	< 0.001*	-2.768	10.934	0.800
	Model 1 (HvN)			**Model 2 (HvL)**			**Model 3 (LvN)**		
Glucose and Lipid Metabolism	**Estimate**	**SE**	**p-value**	**Estimate**	**SE**	**p-value**	**Estimate**	**SE**	**p-value**
Glucose (mg/dl)	-0.020	0.101	0.841	-0.022	0.002	< 0.001*	0.002	0.151	0.991
Fructosamine (mg/dl)	-0.125	0.002	< 0.001	-0.227	0.925	0.807	0.053	0.869	0.951
Insulin (ug/L)	-0.173	3.350	0.959	-0.379	3.059	0.901	0.081	3.441	0.981
Glucose : Insulin Ratio	< -0.001	<0.001	0.963	< 0.001	0.006	0.961	< 0.001	< 0.001	0.930
Triglycerides (mg/dL)	-0.001	0.042	0.977	0.026	0.113	0.819	-0.140	0.125	0.261
Cortisol (ng/ml)	0.020	0.002	<0.001*	-0.023	0.002	< 0.001*	0.015	0.140	0.913
	Model 1 (HvN)			**Model 2 (HvL)**			**Model 3 (LvN)**		
Cardiovascular Health	**Estimate**	**SE**	**p-value**	**Estimate**	**SE**	**p-value**	**Estimate**	**SE**	**p-value**
Cholesterol (mg/dL)	0.012	0.002	< 0.001*	0.003	0.122	0.981	-0.033	0.118	0.783
Direct HDL (mg/dL)	0.003	0.002	0.157	0.028	0.988	0.977	-0.218	0.774	0.778
Direct LDL (mg/dL)	0.007	0.150	0.962	-0.004	0.161	0.982	-0.016	0.152	0.915
Testosterone (ng/ml)	-0.008	0.184	0.966	-0.017	0.009	0.048*	0.016	0.006	0.004*

Thyroid Hormones

High prolactin females showed significantly higher concentrations of thyroid stimulating hormone (p= 0.003) than the normal group but showed no difference from the low prolactin group (p= 0.789) (Figure 1.). TSH levels also had no significant differences between the normal and low prolactin groups (p= 0.642) (Table 3).

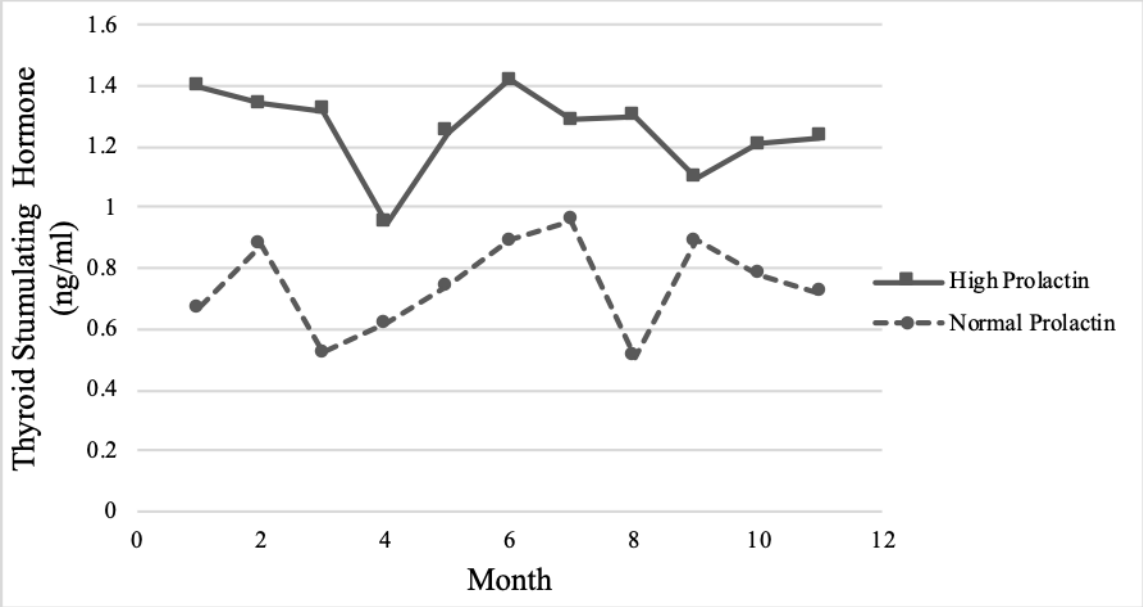

Figure 1. Representative TSH hormone profiles of a high prolactin and normal prolactin elephant.

Total triiodothyronine and free thyroxine were significantly lower in high prolactin females compared to normal and low prolactin females, with all p-values below 0.001 (figures 2. and 3.). There were no significant differences in total T3 and freeT4 between low and normal groups (p= 0.911 and 0.800, respectively). Total thyroxine showed no significant difference between high and normal (p= 0.979), high and low (p= 0.908), or low and normal groups (p= 0.942) (Table 3).

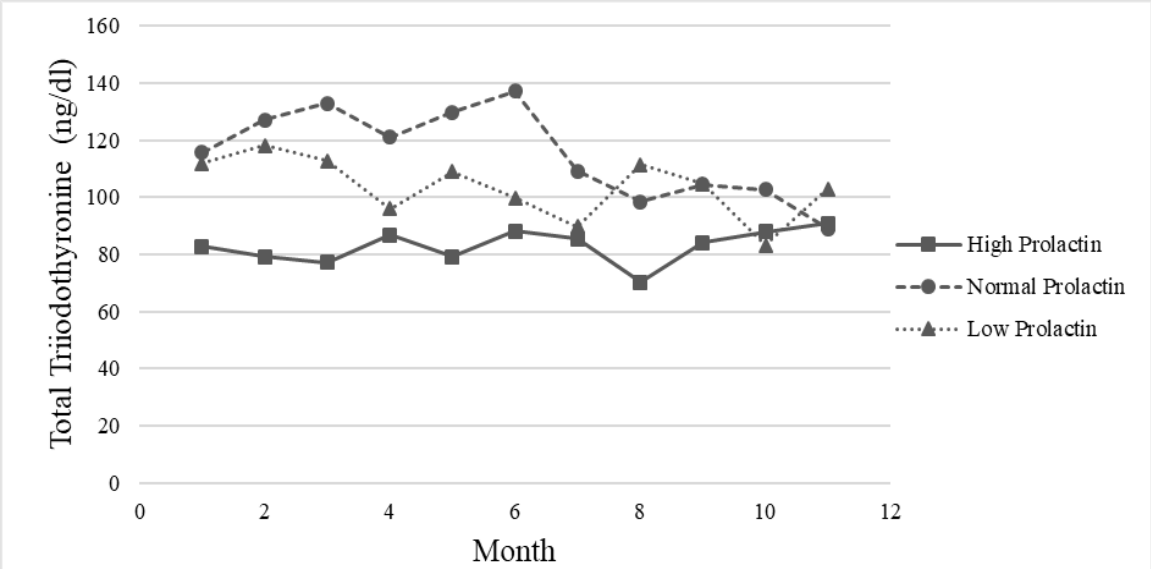

Figure 2. Representative total triiodothyronine (T_3) hormone profiles of a high, normal, and low prolactin elephant.

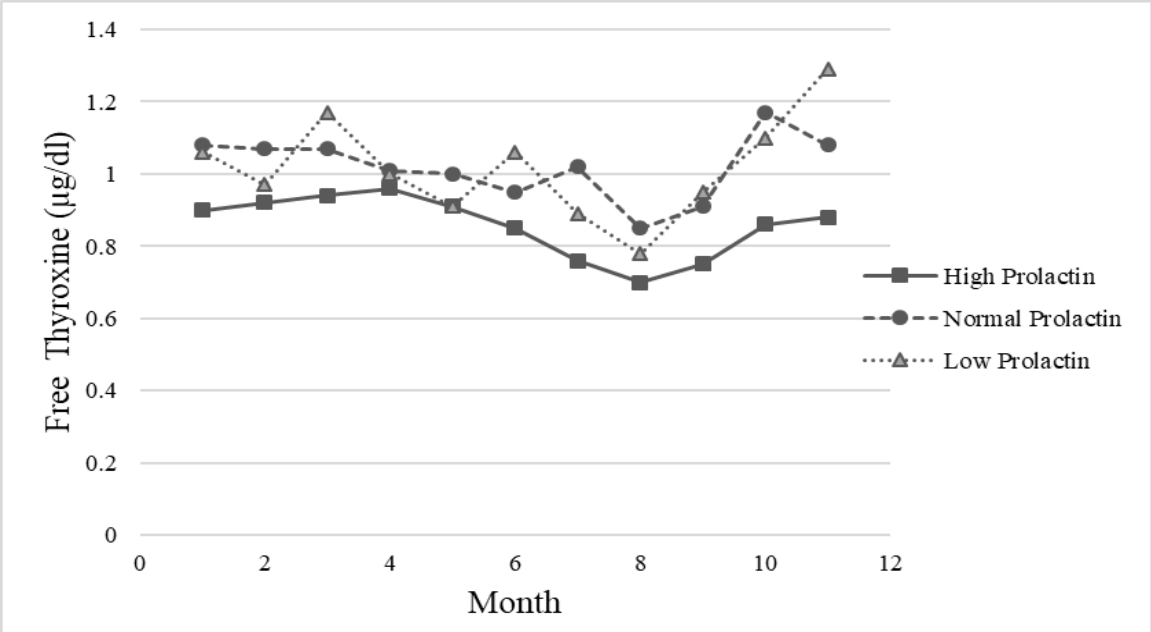

Figure 3. Representative free thyroxine (T_4) hormone profiles of a high, normal, and low prolactin elephant.

Glucose and Lipid Metabolism

Results indicated that highPRL females had significantly lower concentrations of fructosamine than both normal and lowPRL females ($p < 0.001$) (Figure 4.). HighPRL was observed with lower glucose levels than lowPRL elephants ($p < 0.001$) (Figure 5.). Fructosamine and glucose were otherwise insignificant. Insulin, glucose: insulin ratio, and triglycerides were not significantly different between any prolactin groups (Table 3).

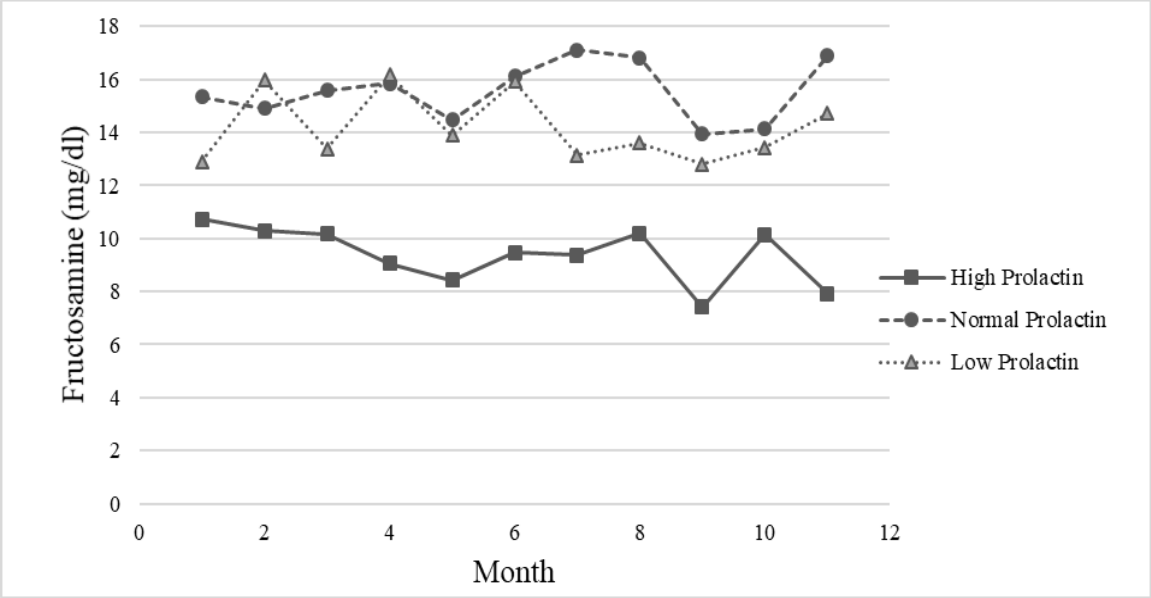

Figure 4. Representative fructosamine hormone profiles of a high, normal, and low prolactin elephant.

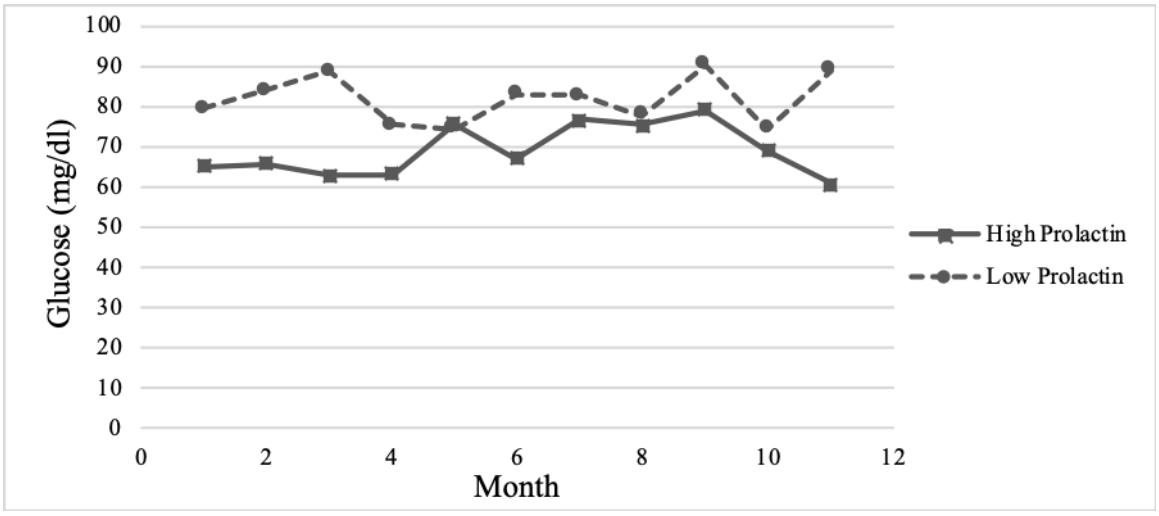

Figure 5. Representative glucose hormone profiles of a high and low prolactin elephant.

Cortisol was significantly higher in highPRL females when compared to normal females ($p < 0.001$) but had lower concentrations when compared to the low prolactin group ($p< 0.001$) (Figures 6.). Cortisol was insignificantly different between the normal and low prolactin group ($p = 0.913$) (Table 3).

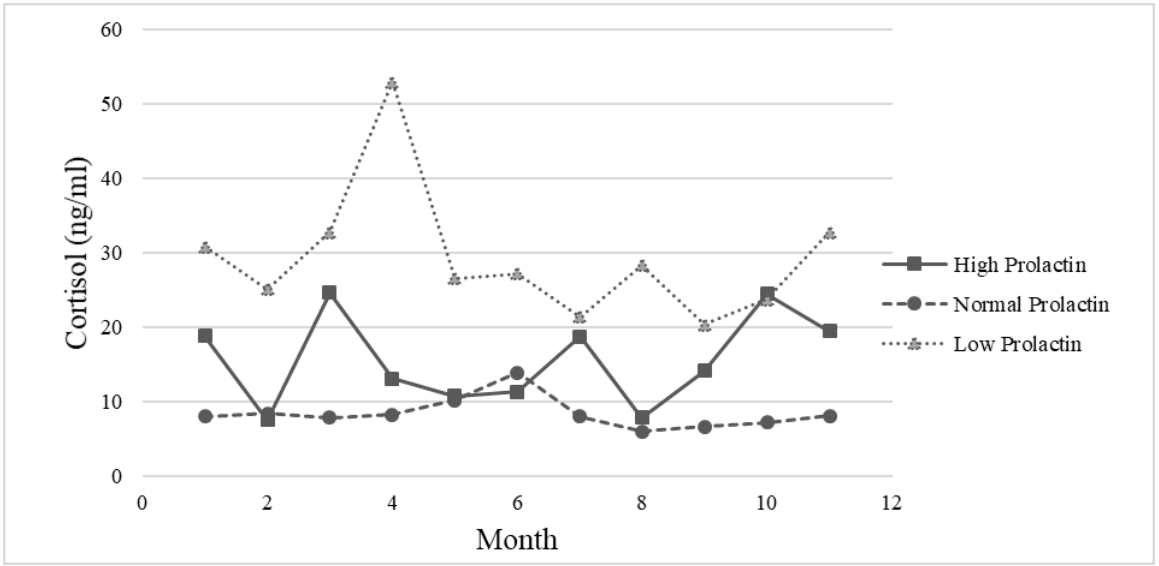

Figure 6. Representative cortisol hormone profiles of a high and normal prolactin elephant.

Cardiovascular Health

HDL and LDL were insignificantly different between all groups. Cholesterol was significantly higher in highPRL females than both normal and lowPRL females ($p < 0.001$) (Figure 7.). No differences were seen between the high and low prolactin groups ($p = 0.98$) or low and normal prolactin groups ($p = 0.78$) (Table 3).

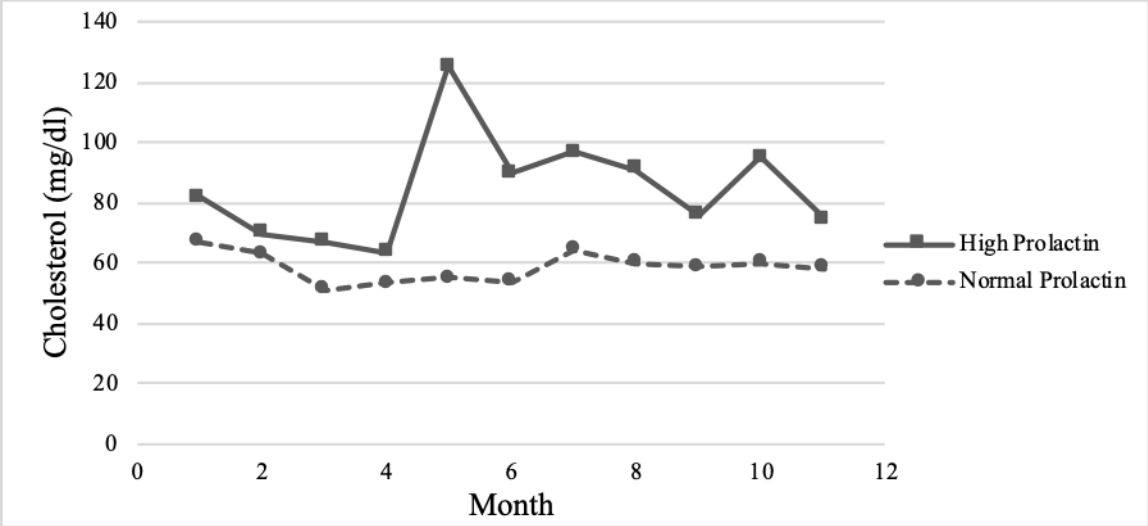

Figure 7. Representative cholesterol hormone profiles of a high and normal prolactin elephant.

Testosterone was not shown to be different between high and normal prolactin females ($p = 0.966$), but low prolactin females had significantly higher concentration than the high prolactin ($p=0.048$) and normal prolactin groups ($p = 0.004$) (Figures 8.) (Table 3).

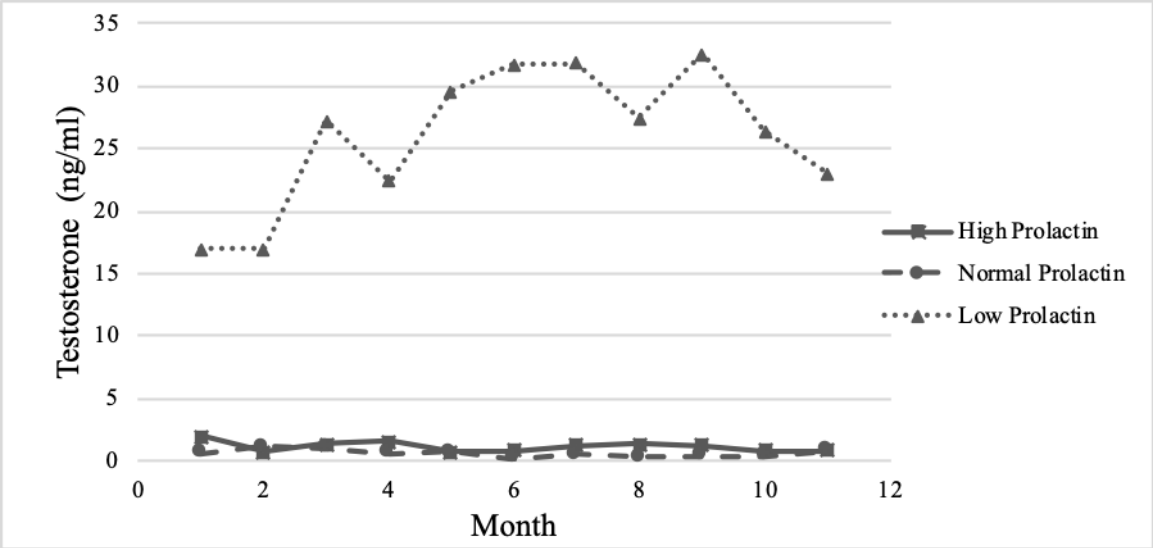

Figure 8. Representative testosterone hormone profiles of a high, normal, and low prolactin elephant.

Discussion

Hyperprolactinemia is a common disorder affecting fertility in women and has recently been associated with acyclicity in the African elephant population in North America as well (Serri et al., 2003; Wang et al., 2012; Brown and Lehnhardt, 1997; Dow and Brown, 2012). As of 2012, over 50% of acyclic female elephants in North America were hyperprolactinemic (Brown et al., 2016). Studies in both humans and animals found chronic hyperprolactinemia is associated with health and metabolic consequences (Shibli-Rahhal and Schlechte, 2009; Yavuz et al., 2003a; Yavuz et al., 2003b). In our study, we assessed the potential impact of hyperprolactinemia on circulating thyroid hormones, glucose metabolism, and cardiovascular health. From our results, we found that highPRL, when compared to normal cycling females, had reduced thyroid hormones (T_3 and T_4), and increased TSH, leading to possible associations with hypothyroidism. Overall, chronically abnormal prolactin elephants did not exhibit alterations in their glucose concentrations or lipid levels compared to normal cycling elephants. Cortisol, however, was shown to be higher in highPRL females, compared to normal cycling females, but was

significantly lower compared to lowPRL females. Lastly, cardiovascular health markers indicated highPRL females had increased cholesterol, while lowPRL females exhibited higher testosterone concentrations compared to the other two study groups.

Thyroid Hormones

Brown et al. (2004) were the first to describe thyroid hormone activity in Asian and African elephants. They compared hormone means of cyclic and acyclic of African and Asian female elephants and found no significant differences in TSH and thyroid hormones (free T_4 and total T_4, and free T_3 and total T_3). In our study, we found that hyperprolactinemic females had significantly higher concentrations of TSH than normal females accompanied with lower levels of total T_3 and free T_4 compared to both normal and lowPRL females. Low prolactin females showed no significant differences in TSH or thyroid hormones when compared to normal females. These results suggest that African elephants with chronically elevated prolactin states may exhibit tendencies for hypothyroidism.

Extensive evidence in humans and animals has shown that abnormal thyroid hormone levels can cause ovarian dysfunction (Meng et al., 2017; Armada-Dias et al., 2001). Armada-Dias et al. (2001) evaluated ovarian morphology and pituitary concentrations in adult female rats with hypothyroidism, normal, and T_4 treated groups. After 80 days, they found that 0% of hypothyroid females had regular ovarian cycles and significantly elevated levels of prolactin compared to the other two treatments. These are similar results as seen in the Shrestha et al. (2016) study that observed states of hyperprolactinemia in 75% of cases involving primary hypothyroidism (decreased T_3/T_4 and increased TSH) and 50% in primary hyperthyroidism (increased T_3/T_4 and decreased TSH). Additionally, subclinical hypothyroidism (elevated TSH and normal T_3/T_4) was significantly higher in the hyperprolactinemic cases than the controls. Raber et al. (2003) stated primary hypothyroidism to be a cause of hyperprolactinemia for patients with PRL concentrations

below 1920 mU/l. Based on the results of this study, thyroid function should be further investigated in relation to hyperprolactinemia in female African elephants.

Glucose and Lipid Metabolism

Obesity, hyperinsulinemia, and insulin resistance are conditions commonly seen in hyperprolactinemic women (Creemers et al., 1991; Atmaca et al., 2013; Yavuz et al., 2003a; Yavuz et al., 2003b). In our study, insulin, triglycerides and G: I ratio were not significantly different between the three prolactin study groups. However, hyperprolactinemic females exhibited decreased concentrations of fructosamine than normal females. Fructosamine is an estimator of glucose levels within the last 2-3 weeks. Elevated levels would indicate potential compromised glucose control and has also been associated with cardiovascular risk in humans (Selvin et al., 2015; Danese et al., 2015). However, low fructosamine may not be of concern in our study, especially as insulin was not observed to be elevated in hyperprolactinemic elephants. It should be noted that fructosamine is a glycation of a protein, typically albumin, but may also include other proteins such as globulins or lipoproteins (Danese et al., 2015). Low fructosamine could be due to decreased serum proteins. Future studies should investigate any potential association with fructosamine and serum protein levels in hyperprolactinemic elephants.

HighPRL females had lower glucose levels than the lowPRL females in this study. However, neither the highPRL nor lowPRL groups had significantly different glucose concentrations from the normalPRL group, which indicates neither hyper- nor hypoglycemia in abnormal prolactin elephants to be a concern.

In humans, prolactin has been documented to have a response to physiological stresses (Sobrinho et al., 1984). Sobrinho et al. (1984) described that prolactin, along with cortisol, can increase when stress levels rise in humans. These states have also been linked with depression and anxiety disorders (Sorbrinho et al., 1984; and Assies et al., 1992). In elephant studies,

cortisol is typically assessed to determine chronic stress. Brown et al. (2004b) looked at mean cortisol concentrations of cyclic and acyclic Asian and African female elephants and found no correlation. In this study, lowPRL females had higher cortisol than the hyperprolactinemic females, and highPRL had increased levels compared to the normal group. However, the low and normalPRL group showed no significant differences. It is interesting to note these results in relation to a study by Prado et al. (2015), which observed African female elephants with prolactin states and temperament. The authors found hypoprolactinemic females displayed more anxious and fearful behaviors, while hyperprolactinemic individuals were likely to exhibit a 'warm and nurturing temperament.' Future research should investigate possible associations with temperament and cortisol levels among elephants in different prolactin groups.

Cardiovascular Health

Both HDL and LDL showed no significant differences in this study. However, total cholesterol levels were significantly higher in hyperprolactinemic females compared to normal prolactin females. Chronically elevated cholesterol levels increases cardiovascular risk, including carotid intima thickening, arterial stiffness, and heart disease (Medic-Stojanoska et al., 2015; Jiang et al., 2014; Georgiopoulos et al., 2009). Previous studies have shown that dopamine agonist treatments with cabergoline in women with prolactinomas have reduced elevated prolactin levels accompanied by significant normalization of lipid and glucose profiles (Schwetz et al., 2017; Pala et al., 2015). Little is known about cardiovascular health in elephants. Morfeld et al. (2014) conducted a preliminary study treating hyperprolactinemic elephants with cabergoline. The authors observed reduction of prolactin concentrations in some individuals during the course of treatment, but cholesterol was not assessed during the study. If future exploration with dopamine agonist treatments concurrent lipid monitoring would give insight to the effect dopamine agonist has to total cholesterol in elephants. In humans, testosterone has been

found to have roles in breast cancer and cardiac protection, acts as a vasodilator, and aids in glucose metabolism and lipid profiles (Glaser and Dimitrakakis, 2013). Pre- and postmenopausal women with androgen deficiency experience symptoms of anxiety, depression, lack of well-being, sexual dysfunction, and are treated with testosterone therapy (Glaser and Dimitrakakis, 2013). Little is known about testosterone in female elephants. This is the first study which looked into testosterone levels in hyperprolactinemic female African elephants. Testosterone was raised in lowPRL females compared to both the normal and hyperPRL groups. These results were driven by two individuals in the study with lowPRL exhibiting heightened testosterone levels between 16 and 196 ng/ml. All other individuals in the study had testosterone levels from 2 ng/ml to below assay sensitivity (0.46 ng/ml). This new finding can provide a new avenue of research that deserves further exploration and, for the first time points to a possible difference in the underlying etiology for the acyclicity observed between low and high prolactin elephants. Possible associations should be further investigated among testosterone concentrations, acyclicity, health, and behavior. Improving sensitivity for the testosterone assay to detect concentrations under 0.46 ng/ml would improve further research.

Conclusions

Given the diverse physiological functions of prolactin, it is not surprising that chronically abnormal secretion has long-term health consequences in humans. This is the first study to investigate metabolic and cardiovascular health biomarkers in relation to abnormal prolactin secretion (high and low) in African elephant females.

We determined that high prolactin secretion is associated with abnormal TSH and thyroid hormone production, elevated cortisol and cholesterol, and reduced fructosamine. Low prolactin individuals were found to have heightened levels of testosterone. Taken together this study highlights several areas in need of further study. In particular, possible hypothyroidism in

hyperprolactinemic females and elevated testosterone in hypoprolactinemic females requires further investigation. Ideal ranges for TSH and thyroid hormones have yet been recorded, and requires comprehensive assessment for TSH, testosterone, and thyroid hormones is necessary for the North American population. This will allow for better insight into thyroid health. Testosterone is another hormone not typically tested in female elephants and needs to be assessed further.

Fructosamine is a glycation of serum protein, typically albumin. Fructosamine, total protein, and albumin should be assessed to further understand the reduced fructosamine levels observed in the hyperprolactinemic elephants. Advancing the understanding of African elephant physiology and the etiology of hyperprolactinemia will improve current medical management across the North American population. This work will aid in returning acyclic females to the reproductive population for conservation of the species

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CURRICULUM VITAE

Matthew D. Krcmarik was born in Flint, Michigan. After graduating from Powers Catholic High School in 2009, he attended Michigan State University to pursue a bachelor's degree in fisheries and wildlife management, achieved in 2013. Matthew went on to work as a wildlife ecology technician at the Wilds, a conservation center in Cumberland, Ohio. This position lead to an opportunity to research elephant reproduction as a veterinary medicine technician at The Ohio State University. His passion for the conservation of endangered species drove him to further pursue a graduate degree. Matthew moved to Fairfax, Virginia in 2016 to begin his master's work at Smithsonian Conservation Biology Institute (SCBI) and George Mason University (GMU). Throughout his degree, he worked at a graduate teaching assistant, teaching human anatomy and physiology. Currently, he works as the wildlife endocrinology technician at SCBI.